

**WATER UPTAKE
BY THE CRAB-EATING FROG *RANA CANCRIVORA*, AS
AFFECTED BY OSMOTIC GRADIENTS AND BY
NEUROHYPOPHYSIAL HORMONES**

BY S. E. DICKER AND ANNIE B. ELLIOTT

*From the Department of Physiology of Chelsea College,
University of London, and the Department of Physiology,
University of Singapore*

(Received 30 July 1969)

SUMMARY

1. The rate of water uptake across the skin was investigated in live *Rana cancrivora*, an euryhaline frog which has been reported to tolerate sea water. When they were exposed to distilled water at 29° C, the rate of water uptake was $8.4 \pm 0.4 \mu\text{l./cm}^2 \cdot \text{hr}$; when bathed in solutions ranging from 30 to 570 m-osmole/l., irrespective of whether the solute was sucrose, urea or NaCl, the rate of fluid uptake during the first day was inversely related to the osmolarity of the solution. No appreciable fluid movement was observed when the bathing solution had an osmolar concentration of 270 m-osmole/l.

2. The rate of fluid uptake was not affected by injections of vasopressin, oxytocin or of extracts of amphibian or rat pituitary glands, irrespective of whether *R. cancrivora* were bathed in distilled water or in solutions of NaCl or sucrose.

3. In *Bufo melanostictus*, in contrast with *R. cancrivora*, injections of neurohypophysial extracts produced a marked increase of the rate of fluid uptake.

4. In the laboratory, *R. cancrivora* could be acclimatized stepwise to tolerate NaCl solutions up to 700 m-osmole/l. for 7 days.

5. After 24 hr exposure either to distilled water or to NaCl solutions from 100 to 670 m-osmole/l., the osmolar concentration of the plasma of *R. cancrivora* was always higher than that of the bathing fluid. In *R. pipiens* or *R. temporaria* plasma osmolar concentration was higher than that of the bathing fluid only when the latter did not exceed 300 m-osmole/l.

6. Under all conditions investigated, the osmolar concentration of the urine of *R. cancrivora* was always lower than that of the plasma.

7. The amounts of pressor and oxytocic activities of pituitary glands of

R. cancrivora kept in distilled water or in NaCl solutions up to 300 m-osmole/l. were 8.9 ± 0.8 and 1.8 ± 0.3 m-u./gland, irrespective of sex or body weight within the range 30–50 g. After 3 days exposure to hypertonic NaCl solutions, the amounts of pressor and oxytocic activities were 14.7 ± 1.2 and 3.1 ± 0.3 m-u./gland. In both instances the pressor/oxytocic ratio was 4.9. Pituitary glands of *R. temporaria* similarly showed increased pressor and oxytocic activities after exposure to NaCl solutions of 300–360 m-osmole/l.

INTRODUCTION

The water balance of anuran Amphibia appears to be regulated by a series of factors among which movements of water across the skin, renal excretion and controlled re-absorption of water and/or of ions from the urinary bladder are the most important (Deyrup, 1964).

While most Amphibia are unable to survive more than brief exposure to media of about 330 m-osmole/l., some euryhaline anurans such as *Bufo viridis* (Gordon, 1962), *Rana cancrivora* and *R. tigerina* (Gordon, Schmidt-Nielsen & Kelly, 1961; Schoffeniels & Tercafs, 1966) have been reported to tolerate for several months external salinities varying from 650 to 950 m-osmole/l. This resistance to concentrated solutions does not appear to be based on skin impermeability (Gordon *et al.* 1961).

Rana cancrivora, usually found in mangrove swamps along the coast of the Gulf of Thailand (Van Kampen, 1923; Gordon *et al.* 1961), occurs also in Singapore. It has been reported to seek its food in full-strength sea water of a concentration of about 1000–1100 m-osmole/l. Since according to Gordon *et al.* (1961) and Schmidt-Nielsen & Lee (1962) the osmolar concentration of the plasma of *R. cancrivora* is always greater than that of the external fluid in which it lives, it was of interest to investigate some characteristics of its skin permeability and to see whether its rate of water uptake would be affected by neurohypophysial hormones.

METHODS

The frogs were identified by Dr Pek-Yong Berry of the school of Biological Sciences, University of Malaya, Kuala Lumpur, as *R. cancrivora* Gravenhorst. They were collected in Singapore, where there is little seasonal or diurnal variation in temperature (28–29° C) or in humidity (80–100 %). The pond water in which the animals were caught had an osmolar concentration of 7 m-osmole/l. containing Na^+ 3.0 and K^+ 0.8 m-equiv/l. respectively. Frogs of both sexes with weights of 30–80 g were used.

In experiments of some duration, or when the frogs were being acclimatized to solutions of different concentrations and compositions, the animals were kept in dishes covered with wire netting. The depth of the fluid in each dish was about 2.5 cm. The frogs, which took up a crouching position on the bottom of the dish, were

always at least half submerged. For the estimation of the rate of water uptake, frogs with the cloaca ligatured were placed in 100 ml. jars containing 50 ml. of the bathing fluid. The rate of fluid uptake was estimated by weighing as previously described (Dicker & Elliott, 1967). For the investigation of the effect of neurohypophysial hormones, the frogs were injected intramuscularly, and the rate of fluid uptake during the hour immediately after the injection was compared with that of the hour preceding it. The area of skin exposed to the bathing fluid was found to be related to weight by the formula $A = W^{2/3} \times 6.3$, where A is area in cm^2 and W weight in g. The same formula is applicable to *B. melanostictus* (Dicker & Elliott, 1967).

Collection of blood and urine. The animals were killed by opening the body wall and cutting the tip of the heart, so that the blood could drain out. It was collected in heparinized tubes and centrifuged immediately to obtain the plasma. The lower abdomen was then opened and the bladder punctured for collection of the urine.

Preparation and assay of neurohypophysial extracts. For injections pituitary glands were taken from decapitated animals, ground in 0.6% (w/v) NaCl solution, acidified with 1% (w/v) acetic acid, boiled for 3 min and filtered. For the estimation of hormone activities, glands were first placed in acetone. After drying *in vacuo* extracts were prepared in the usual way. The pressor activity was assayed using the blood pressure of a rat anaesthetized with urethane (1 ml./100 g of a 10% solution) injected intraperitoneally, while the oxytocic activity was assayed on an isolated rat uterus preparation, using vasopressin (Pitressin, Parke Davis & Co., containing presumably lysine-vasopressin) and oxytocin (Pitocin, Parke Davis & Co.) respectively as standard preparations. The same preparations were used for injections into frogs and toads.

The osmolar concentration of plasma, urine and various bathing fluids were estimated with an osmometer (Advanced Instruments Inc.).

RESULTS

Frogs kept without access to water usually died within 48 hr. After being exposed to room air (29° C) for 24 hr they lost about 20% of their original weight. No urine was found in their bladders. The osmolarity of their plasma was 494 ± 19 (ten) m-osmole/l. When frogs were kept for several days in distilled water, the osmolarity of their plasma was 264 ± 4 m-osmole/l. (thirty), while that of their urine ranged from 35 to 105 m-osmole/l.

Of twenty-four frogs placed into NaCl solutions of 400 m-osmole/l. for 7 days, all survived. Although not all frogs survived for 7 days when placed directly in NaCl solutions of greater concentrations than this, they could become acclimatized. For instance, after exposure for 3 days to NaCl solution of 330 m-osmole/l., they all survived for 7 days in solutions up to 470 m-osmole/l., and could stepwise be made to tolerate concentrations up to 700 m-osmole/l. for 7 days.

Rate of water uptake across the skin. The rate of fluid uptake across the skin of frogs kept in distilled water at 29° C was 8.41 ± 0.39 $\mu\text{l.}/\text{cm}^2$.hr (ten). This remained very much the same for several days. After 7 days it was 7.91 ± 1.36 $\mu\text{l.}/\text{cm}^2$.hr.

When frogs were exposed to NaCl solutions of low osmolarity (up to

270 m-osmole/l.) the rate of water uptake fell but then stabilized at the decreased level. For instance, for external osmolar concentration of 190 m-osmole/l., the rate of water uptake was $2.4 \mu\text{l./cm}^2 \cdot \text{hr}$ and was still the same after 7 days. With external osmolar concentration above 270 m-osmole/l., there was first a loss of weight, which then was recovered after a few days. As an example, when animals were exposed to NaCl solutions of 390 m-osmole/l., the rate of fluid movement during the first 24 hr was $-3.4 \mu\text{l./cm}^2 \cdot \text{hr}$ (fluid moving out of the animal), but after 7 days it was $+0.8 \mu\text{l./cm}^2 \cdot \text{hr}$. (fluid moving into the frog).

The rate of water uptake during the first day of exposure was inversely proportional to the osmolar concentration of the medium in which the animals were bathed, irrespective of whether the solute was NaCl, urea or sucrose. Thus, in contrast with the results in *B. melanostictus* (Dicker & Elliott, 1967), in *R. cancrivora* there was no difference between the rate of water uptake when the osmotic gradients were made up with an electrolyte (NaCl) or a non-electrolytic solute (urea, glucose). When frogs were immersed in solutions of 270 m-osmole/l., no appreciable change in body weight was observed (Fig. 1).

Plasma and urine osmolar concentrations. Batches of ten *R. cancrivora* were kept in various solutions for 3 days and were then killed. The osmolar concentrations of plasma and urine were measured. Similar experiments were also performed on *R. pipiens* and *R. temporaria* as control. Whereas *R. cancrivora* could live for several days in bathing solutions of concentrations up to 700 m-osmole/l., *R. pipiens* and *R. temporaria* died within 48 hr when in solutions above 300 and 370 m-osmole/l. respectively. In contrast with the concentration of the plasma of *R. pipiens* and *R. temporaria* which on exposure to solution of higher osmolarity became equal to that of the bathing fluid, the osmolar concentration of the plasma of *R. cancrivora* was always greater (Table 1). The urine of *R. pipiens* and *R. temporaria*, though more dilute than the plasma when the frogs were in solutions below 200 m-osmole/l., tended to become iso-osmotic with both plasma and external medium when the osmolar concentration of the latter was raised. In contrast to *R. pipiens* and *R. temporaria*, the urine of *R. cancrivora* was always less concentrated than the plasma and the bathing fluid, confirming the observation by Gordon *et al.* (1961). The fact that the difference between the osmolarity of plasma and urine increased when the frogs were kept in more concentrated solutions suggests the possibility that the permeability characteristics of the bladder of *R. cancrivora* may be different from that of other amphibians (Fig. 2).

Effects of neurohypophysial hormones on rate of water uptake. Neurohypophysial hormones are known to increase the permeability to water of the skin of most Amphibians, and especially anurans (Heller, 1965). In

Bufo melanostictus, a dose of vasopressin (4 m-u./g) injected intramuscularly reduces the activation energy of water uptake by about 4000 cal, whether in the absence or the presence of NaCl; this has been interpreted as due either to an increase of the size or of the number of pores of the skin through which water enters (Dicker & Elliott, 1967). Since it would appear that *Rana cancrivora* can tolerate exposure to sea water it was of interest to see whether its water balance was controlled by hormones from the neurohypophysis. Batches of ten *R. cancrivora* were injected with vasopressin or oxytocin. The doses of vasopressin were 40 and 100 m-u./g; those

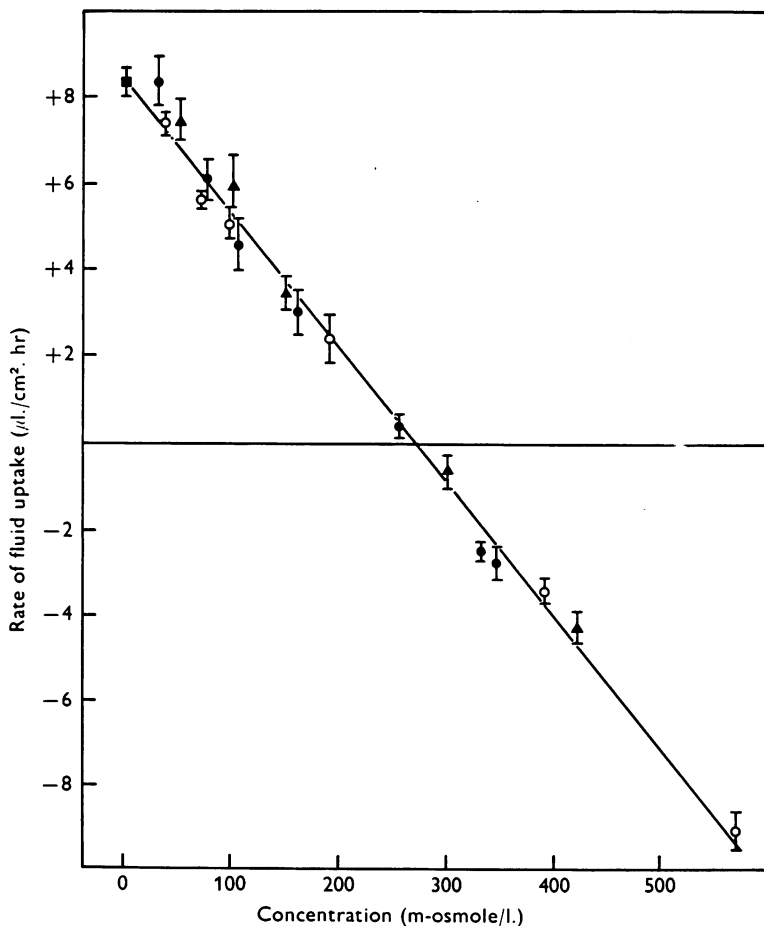


Fig. 1. Initial rate of water uptake in *Rana cancrivora* as related to concentration of the bathing fluid. The bathing fluid was either distilled water (■), NaCl solutions (○), urea solutions (▲) or sucrose solutions (●). Abscissa, osmolar concentrations (m-osmole/l.). Ordinate: rate of water uptake ($\mu\text{l.}/\text{cm}^2\cdot\text{hr}$). Each point is the mean of at least five estimations, and \pm s.e.

of oxytocin were 10, 40 and 100 m-u./g. The animals were kept either in distilled water, in 5% sucrose or in 0.1, 0.6 or 1.2% NaCl solutions. In no case did the injections of vasopressin or oxytocin produce a significant change in the rate of water uptake, the lowest *P* value for any of the twelve tests with different injections and bathing solutions and for four controls being over 0.2. Likewise extracts of rat pituitary gland (80 m-u. oxytocic and 80 m-u. pressor activity per frog) did not alter the rate of water uptake in *R. cancrivora*, although the same dose increased the rate of water uptake in *B. melanostictus* (Table 2).

TABLE 1. Comparison of plasma and urine osmolar concentration when frogs were bathed in fluids of different NaCl concentrations

| Bathing fluid (m-osmole/l.) | <i>R. cancrivora</i> | | <i>R. temporaria</i> | | <i>R. pipiens</i> | |
|--------------------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|------------------------|
| | Plasma (m-osmole/l.) | Urine (m-osmole/l.) | Plasma (m-osmole/l.) | Urine (m-osmole/l.) | Plasma (m-osmole/l.) | Urine (m-osmole/l.) |
| 0 | 264 | 62 | 204 | 22 | 203 | 28 |
| 100 | 300 | 223 | — | — | — | — |
| 200 | 329 | 277 | 232 | 190 | 210 | 123 |
| 303 | 333 | 298 | 303 | 305 | 300 | 303 |
| 320 | 352 | 315 | — | — | — | — |
| 340 | 375 | 320 | — | — | — | — |
| 370 | — | — | 370 | 368 | — | — |
| 450 | 480 | 410 | — | — | — | — |
| 530 | 570 | 420 | — | — | — | — |
| 606 | 646 | 510 | — | — | — | — |
| 673 | 700 | 520 | — | — | — | — |

All data are averages. Each figure represents an average for groups of twelve *R. cancrivora*, twelve *R. temporaria* and ten *R. pipiens*.

Since the posterior pituitary glands of Amphibians do not contain vasopressin, *R. cancrivora* were injected with extracts of pituitary glands of *B. melanostictus* and of *R. cancrivora*, and the results compared with those obtained in *B. melanostictus*. Again no effect on the rate of water uptake by *R. cancrivora* was observed (Table 3).

Pressor and oxytocic activities of the pituitary gland of R. cancrivora. The finding that the skin of these frogs was insensitive to the administration of neurohypophysial hormones raised the question of whether the neural lobe of *R. cancrivora* contained the usual hormone activities and, if so, whether they were released. Frogs were kept in distilled water for 3 days, after which a control batch of five animals were killed. The remainder were put in a solution of NaCl of 286 m-osmole/l., and batches of five animals were killed after 8, 24, 48, 54, 72, and 126 hr. Plasma was collected for osmolarity estimation. The pituitary glands were extracted in the usual

way, and assayed for their pressor and oxytocic activities. After 8 hr of exposure to NaCl solutions (286 m-osmole/l.) the plasma concentration had risen from 264 to 334 m-osmole/l. and remained at this level for the whole of the period of observation. The mean pressor activity of pituitaries was 7.0 ± 0.41 (six), and the mean oxytocic activity 2.0 ± 0.16 (six) m-u./gland.

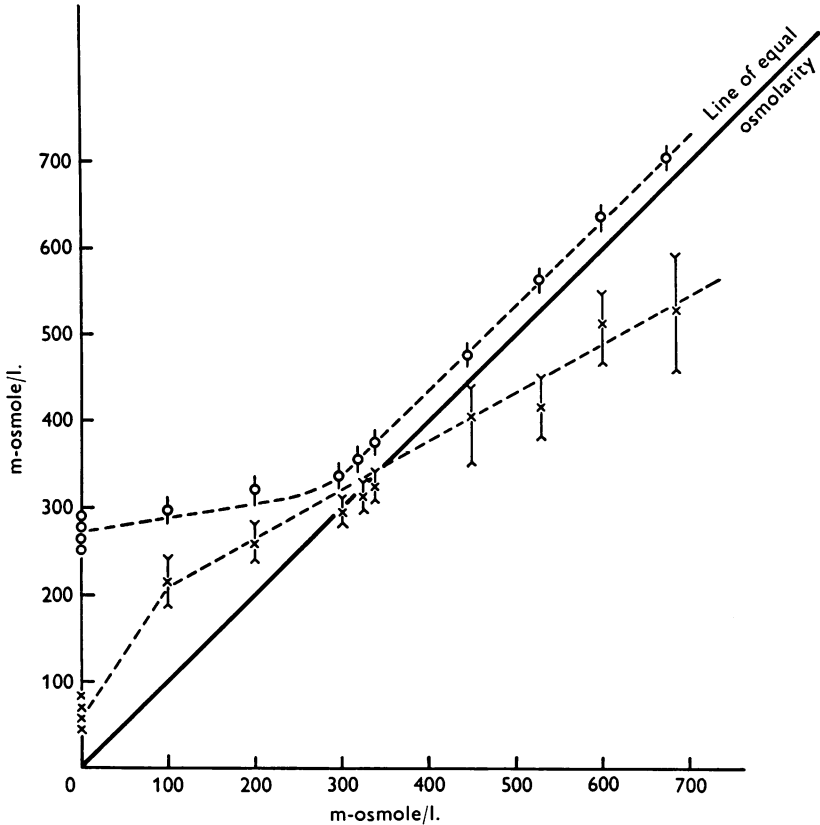


Fig. 2. Relation between osmolar concentration of bathing fluid and that of plasma and urine, in *Rana cancrivora*. Frogs were exposed for at least 3 days to the different concentrations of NaCl solutions before being killed. Each point represents the mean \pm s.e. of at least five experiments. Plasma osmolarity \circ ; urine osmolarity \times . Abscissa: concentrations of NaCl in bathing fluid solutions. Ordinate: concentrations of plasma and urine. Note that the plasma osmolar concentration is always above the line of equal osmolarity.

These results were not statistically different from those obtained from control frogs kept in distilled water: mean pressor activity of 8.8 ± 0.70 (five) and oxytocic activity of 1.7 ± 0.17 (five) m-u./gland.

To see whether the content of the glands was affected by the weight, and

therefore presumably by age, of the animals, extracts from glands taken from frogs weighing 30, 35, 45 and 50 g were assayed (Table 3). Though the five frogs with a body weight of 30 ± 5 g had a smaller amount of activity, it was not significantly different from that in the other groups when exposed to distilled water (Table 4).

TABLE 2. Effect of neurohypophysial extracts on the rate of water uptake across the skin of *Rana cancrivora* and of *Bufo melanostictus* when placed in distilled water

| Treatment Injection i.m. of | % increase in rate of water uptake | |
|---|------------------------------------|-------------------------|
| | <i>R. cancrivora</i> | <i>B. melanostictus</i> |
| Extract of 1 pituitary gland of <i>B. melanostictus</i> | None ($P > 0.8$) | 90 ± 17 |
| Extract of 1 pituitary gland of <i>R. cancrivora</i> | None ($P > 0.9$) | 166 ± 17 |
| Extract of $\frac{1}{2}$ pituitary of rat (80 m-u. vasopressin + 80 m-u. oxytocin/animal) | None ($P > 0.9$) | 142 ± 16 |
| Acidified saline as control | None ($P > 0.8$) | None ($P > 0.8$) |

The effects are expressed as % increase of rate of water uptake during the hour immediately following the injection, the basal rate of water uptake being for *R. cancrivora* $8.4 \pm 0.4 \mu\text{l./cm}^2 \cdot \text{hr}$ and for *B. melanostictus* $16.9 \pm 1.3 \mu\text{l./cm}^2 \cdot \text{hr}$. Data are means and \pm s.e., each determination being made on five animals.

TABLE 3. Comparison between body weight and neurohypophysial activity

| Body wt. (g) | Pressor activity (m-u./gland) | Oxytocic activity (m-u./gland) | P/O |
|-----------------|-------------------------------------|--------------------------------------|-----|
| 50 + 2 | 8.3 | 1.9 | 4.3 |
| 43 ± 3 | 8.6 | 2.0 | 4.3 |
| 40 ± 5 | 8.6 | 2.1 | 4.1 |
| 35 ± 5 | 8.4 | 2.0 | 4.2 |
| 30 ± 2 | 7.4 | 1.8 | 4.1 |
| Average | 8.2 | 1.9 | 4.2 |

Number of frogs in each group was five. The glands of each group were pooled for assays.

The pressor and oxytocic activities of pituitary glands of female and male frogs were also assayed and compared. No significant differences were found. For instance, in frogs kept for 3 days in 0.3% NaCl solution (100 m-osmole/l.), two groups of females had a pressor activity of 8.3 and 6.3 m-u./gland, and that of male frogs was 9.6 m-u./gland. The oxytocic activity in male frogs was 1.4 m-u./gland, while the two batches of females averaged 1.9 and 1.6 m-u./gland. The variations are well within those encountered in other batches. To check that all the peptide-containing tissue was being

extracted, the pituitary activity from ten frogs kept in distilled water was compared with that from a similar group, the only difference being that in the first instance the pituitaries were carefully dissected, whereas in the second instance a generous amount of adjacent hypothalamic tissue was included. No statistical difference in pressor or oxytocic activity was found.

To see whether the amounts of pressor and oxytocic activities of the

TABLE 4. Correlation between osmolar concentration of bathing fluid and pressor and oxytocic activities in neurohypophysial glands of *R. cancrivora* and *R. temporaria*

| Bathing solutions | | | Pressor activity (m-u./gland) | Oxytocic activity (m-u./gland) | P/O |
|-----------------------------|----------------------------|------|----------------------------------|-----------------------------------|--------------|
| Osmolarity (m-osmole/l.) | Composition (g/100 ml.) | | | | |
| <i>A. Rana cancrivora</i> | | | | | |
| 0 | Distilled water | (5) | 8.8 | 1.7 | 5.17 |
| | | (10) | 12.5 | 2.3 | 5.43 |
| | | (20) | 8.2 | 1.7 | 4.82 |
| 100 | 0.3 g NaCl | (5) | 5.6 | 1.9 | 2.95 |
| | | (5) | 8.3 | 1.4 | 5.93 |
| | | (4) | 6.3 | 1.9 | 3.32 |
| 200 | 0.6 g NaCl | (3) | 9.5 | 1.6 | 5.94 |
| | | (5) | 10.4 | 2.1 | 4.95 |
| | | (5) | 11.0 | 2.1 | 5.24 |
| | | Mean | 8.9 ± 0.77 | 1.8 ± 0.26 | 4.86 ± 0.37 |
| 300 | 0.9 g NaCl | (5) | 16.0 | 2.5 | 6.40 |
| | | (5) | 16.8 | 2.8 | 6.00 |
| 330 | 1.0 g NaCl | (10) | 14.9 | 2.6 | 5.73 |
| 467 | 1.4 g NaCl | (10) | 6.8 | 2.5 | 2.72 |
| | | (5) | 14.8 | 3.1 | 4.77 |
| | | (5) | 16.4 | 3.1 | 5.29 |
| 530 | 1.6 g NaCl | (10) | 20.3 | 4.4 | 4.61 |
| 600 | 1.8 g NaCl | (10) | 12.1 | 2.4 | 5.04 |
| 670 | 2.0 g NaCl | (10) | 16.7 | 5.1 | 3.27 |
| | | Mean | 14.7 ± 1.18 | 3.1 ± 0.30 | 4.87 ± 0.33 |
| <i>B. Rana temporaria</i> | | | | | |
| 0 | Distilled water | (5) | 7.2 | 1.7 | 4.24 |
| | | (10) | 7.2 | 1.6 | 4.50 |
| | | (5) | 7.3 | 1.8 | 4.06 |
| 300 | 0.6 g NaCl | (5) | 7.8 | 1.8 | 4.33 |
| | | Mean | 7.3 ± 0.04 | 1.7 ± 0.03 | 4.30 ± 0.025 |
| | | (5) | 10.8 | 2.6 | 4.15 |
| 360 | 1.1 g NaCl | (5) | 10.4 | 2.2 | 4.64 |
| | | (7) | 11.2 | 2.4 | 4.67 |
| | | (5) | 12.2 | 2.5 | 4.88 |
| | | Mean | 11.1 ± 0.42 | 2.4 ± 0.03 | 4.58 ± 0.14 |

In parentheses, number of glands pooled for each assay.
Results are given as means and s.e.

pituitary glands varied with the concentration of the bathing fluid, and hence with the osmolar concentration of their plasma, frogs kept initially for 3 days in distilled water were put in various solutions of NaCl of increasing concentrations. They remained in each solution for 3 days, before being put in the next one, and so on. Batches of five or ten frogs were killed every third day and their pituitary glands assayed. From the results (Table 4) it will be seen that there was some variation in the neurohypophysial activities of frogs kept in the same solutions, for which there is no explanation. While in distilled water or in NaCl solutions of up to 200 m-osmole/l., the mean pressor activity was 9.1 ± 0.77 m-u. and the oxytocic activity 1.8 ± 0.26 m-u./gland. When kept in more concentrated NaCl solutions ranging from 300 to 670 m-osmole/l., both pressor and oxytocic activities increased to 14.7 ± 1.18 and 3.1 ± 0.30 m-u./gland respectively, but the ratio pressor/oxytocic (*P/O*) activity remained the same as in the controls. Similarly an increase of the amounts of both pressor and oxytocic activities of the pituitary gland obtained when *R. temporaria* were immersed in solutions of 300 and 260 m-osmole/l., for 24 hr (Table 4). When after 2 days in solutions of 600 m-osmole/l., *R. cancrivora* were returned into distilled water for 48 hr, the amount of pressor and oxytocic activities of their pituitary glands was comparable to that of control animals: 9.2 m-u. pressor activity and 1.8 m-u. oxytocic per gland.

When *R. cancrivora* were kept without water at 29° C until they had lost about 20 % of their body weight and their plasma osmolar concentration was 494 ± 19 (ten) m-osmole/l., the amounts of pressor and oxytocic activities of their pituitary glands were 12.6 ± 1.2 and 2.5 ± 0.5 m-u./gland, values which are of the same order as those of frogs bathed in 1.4 % NaCl solution (Table 4).

DISCUSSION

According to the opinion of palaeontologists, the earliest forms of Amphibians appeared some 300,000,000 years ago and two different sub-groups of rhipidistid crossopterygians gave rise independently to the present groups of urodeles and anurans, and from palaeo-ecological evidence it would appear that this transition for both groups took place in fresh water environments (Gordon *et al.* 1961; Bentley & Heller, 1964) and that on the whole stenohaline amphibians have lived since the Devonian age in fresh water. Though amphibians in general cannot survive for more than a few hours in external media more concentrated than 300–350 m-osmole/l., at least two anurans, *Bufo viridus* and *Rana cancrivora*, are known to have developed a considerable tolerance to salinity. The crab-eating frog of South-East Asia, *R. cancrivora*, is found in mangrove swamps where alternate flooding and evaporation occur. It can be collected from fresh-

water pools and has been reported to seek its food in full-strength sea water. These frogs must have evolved means by which they can in a few hours adapt themselves to widely different osmolar environments. In some aspects the situation which they face is similar to that of teleost fishes. When the eel is transferred from fresh water to sea water a series of changes in its metabolism occur, among which a gradual increase of the colloid osmotic pressure of the plasma, a reduction of the glomerular filtration rate and some shut-down of the glomeruli have been noted (Chester Jones, Chan & Rankin, 1969).

The rate of water movement through the skin of *R. cancrivora* was found to be the same at any given osmolarity whether the external medium consisted of NaCl, sucrose or urea solutions, suggesting that the skin was virtually impermeable to these solutes. When the concentration of the bathing fluid was below 170 m-osmole/l., water moved into the animal; when it was higher, water initially moved out.

One of the most interesting characteristics which seems to be shared by *B. viridis* and *R. cancrivora* (Gordon, 1962) is that whatever the osmolar concentration of the bathing fluid, the osmotic pressure of the plasma is always higher. Thus within the limits of the experiments, for external solutions of osmolar concentrations between 0 and 700 m-osmole/l., there was never a maintained osmotic equilibrium between bathing fluid and body fluid, and water must always have moved inwards, though at different rates. The observation, also reported by Gordon *et al.* (1961), Schmidt-Nielsen & Lee (1962) and Schoffeniels & Tercafs (1966), that *R. cancrivora* adapts itself to increasing external concentrations by raising the osmotic pressure of its plasma not only goes against the principle of the constancy of the 'milieu intérieur', but raises important questions as to its mechanism and to the function of other physiological systems, such as muscle contraction and/or renal excretion. The existence of a hypertonic plasma, and hence of hypertonic extracellular fluid, would normally produce a decrease of the intracellular fluid phase which would be deleterious to the functioning of organs. This, however, could be partially averted if, for instance, the increased osmotic pressure of the extracellular fluid could be accounted for by an enhanced concentration of a diffusible component such as urea. According to Gordon *et al.* (1961) and to Schmidt-Nielsen & Lee (1962) part of the high osmotic pressure of the body fluids of these amphibians is due to a salt concentration about twice the level found in freshwater amphibians, and to an exceptionally high concentration of urea, which increases tenfold from 40 mM/l. when *R. cancrivora* is in distilled water to about 400 mM/l. when it is acclimatized to solutions of 800 m-osmole/l. If urea diffused into the cells, this would afford them some protection against dehydration.

The permeability of skins and bladders of amphibians is usually increased by neurohypophysial hormones, though not to the same extent (Heller, 1965). There is, however, evidence that oxytocin and its derivatives may stimulate sodium but not water transfer across the skin of *Xenopus* (Maetz, 1963) or of *Triturus* (Bentley & Heller, 1964). Since the rate of water uptake by *R. cancrivora* was not affected by administration of oxytocin, vasopressin or of extracts of its own neurohypophysial gland or of neurohypophysis of a toad, it is possible that in that respect the crab-eating frog behaves like urodeles (Heller, 1965).

Examination of the hypophysial gland of *R. cancrivora* reveals that both pressor and oxytocic activities are present; since 8-arginine vasotocin, which has been shown to be present in some amphibian neurohypophyses (Pickering & Heller, 1959; Sawyer, Munsick & van Dyke, 1959), has a *P/O* ratio of 2.1 (Berde & Boissonnas, 1966), and since the hypophysis of *R. cancrivora* is now seen to have a *P/O* ratio of 4.9, the question arises whether the difference could be due to the presence of other peptides. Although it has been suggested that mesotocin (Follett & Heller, 1964; Acher, Chauvet, Chauvet & Crepy, 1964) and oxytocin (Munsick, 1966) occur in *R. esculenta* and *R. pipiens*, these two peptides have little pressor activity, and their presence would not seem to be indicated by the present results. Recalling the findings of Morel & Jard (1963), who showed competitive inhibition between various peptides, it would be unwise to hypothesize about the additive effects of such peptides as may be present in the gland of *R. cancrivora*. It seems safe to say, however, in view of the identity of the *P/O* ratio found in *R. cancrivora* and in *R. temporaria*, that the neurohypophysis of *R. cancrivora* probably has peptides which are similar at least to those of the non-euryhaline *R. temporaria*. If the assumption is made that the main hormone is 8-arginine vasotocin then, using the range of potencies quoted by Schwartz & Livingston (1964), it can be calculated that one pituitary of *R. cancrivora* has an oxytocic activity equivalent to from 0.01 to 0.07 μg vasotocin, and a pressor activity equivalent to from 0.05 to 0.2 μg vasotocin. Such calculations can however be tentative only.

When *R. cancrivora* were exposed to environments of different salinities, there were some variations in the amount of the pressor and oxytocic activities from batch to batch but no statistically significant correlation could be found between them and loss of body water. When bathed in solutions of an osmolar concentration higher than 270 m-osmole/l., however, there was an increase of both pressor and oxytocic activities of about 70% above that of controls. Though it was not possible to determine whether the hormones were released, the increase of the neurohypophysial activities could be explained by an increase of the synthesis and transport of the hormones to the neurohypophysis. An increase of the hormones in

the neurohypophysis has also been observed during periods of dehydration in rats, though only at the beginning (Dicker & Nunn, 1957). It may well be that rates of synthesis and transport of these hormones can be influenced by the osmolar concentration of the plasma, though how this could be effected is not known.

The existence and maintenance of a plasma osmolar concentration higher than that of the bathing fluid, especially when the latter was above 270 m-osmole/l., is clearly advantageous to the animal, since it provides the frog with a constant inflow of water, which may account for the normally large amounts of urine excreted by the kidneys. In frogs kept without water, no urine was found in their bladders. When urine was present in the bladder it was always more dilute than the plasma and the bathing fluid. The existence of urine which is always more dilute than the plasma, even when the latter is highly concentrated, suggests that either there is little release of neurohypophysial hormones in the circulating blood or else that the bladder is not very responsive to their presence.

R. cancrivora appears thus to have solved its problem of survival by allowing its 'milieu intérieur' to change according to the concentration of the fluid in which it lives: while in freshwater ponds its plasma osmolarity is of the order of 260–270 m-osmole/l., when temporarily exposed to solutions of high osmolarity its plasma concentration rises and water uptake and urine production are maintained. How it manages to adapt the different functions of its organs in these conditions of high osmolarity is now under investigation.

We would like to thank Miss C. Morris for her technical help in the assays of the neurohypophysial glands, and Dr Pek-Yong Berry for the identification of the frogs.

One of us (S. E. D.) would like to express his thanks to Professor G. R. Wadsworth for the hospitality he received in his Department.

REFERENCES

- ACHER, R., CHAUVET, J., CHAUVET, M. T. & CREPY, D. (1964). Phylogénie des peptides neurohypophysaires: Isolement de la mésotocine (Ileu₈-ocytocine) de la grenouille, intermédiaire entre la Ser₄-Ileu₈-ocytocine des poissons osseux et l'ocytocine des mammifères. *Biochim. biophys. Acta* **90**, 613–615.
- BENTLEY, P. J. & HELLER, H. (1964). The action of neurohypophysial hormones on the water and sodium metabolism of urodele amphibians. *J. Physiol.* **171**, 434–453.
- BERDE, B. & BOISSONNAS, R. A. (1966). Synthetic analogues and homologues of the posterior pituitary hormones. In *The Pituitary Gland*, ed. HARRIS, G. W. & DONOVAN, B. T. London: Butterworths.
- CHESTER JONES, I., CHAN, D. K. O. & RANKIN, J. C. (1969). Renal function in the European eel (*Anguilla anguilla* L.): changes in blood pressure and renal function of the freshwater eel transferred to sea-water. *J. Endocr.* **43**, 9–19.
- DEYRUP, I. J. (1964). Water balance and kidney. In *Physiology of the Amphibia*, ed. MOORE, J. A. New York and London: Academic Press.

- DICKER, S. E. & ELLIOTT, A. B. (1967). Water uptake by *Bufo melanostictus*, as affected by osmotic gradients, vasopressin and temperature. *J. Physiol.* **190**, 359-370.
- DICKER, S. E. & NUNN, J. (1957). The role of the antidiuretic hormone during water deprivation in rats. *J. Physiol.* **136**, 235-248.
- FOLLETT, B. K. & HELLER, H. (1964). The neurohypophysial hormones of lungfishes and amphibians. *J. Physiol.* **172**, 92-106.
- GORDON, M. S. (1962). Osmotic regulation in the green toad (*Bufo viridis*). *J. exp. Biol.* **39**, 261-270.
- GORDON, M. S., SCHMIDT-NEILSEN, K. & KELLY, H. M. (1961). Osmotic regulation in the crab-eating frog (*Rana cancrivora*). *J. exp. Biol.* **38**, 659-678.
- HELLER, H. (1965). Osmoregulation in Amphibia. *Archs Anat. microsc.* **54**, 471-490.
- MAETZ, J. (1963). Physiological aspects of neurohypophysial function in fishes with some reference to the amphibia. *Symp. zool. Soc. Lond.* **9**, 107-140.
- MOREL, F. & JARD, S. (1963). Inhibition of frog (*Rana esculenta*) antidiuretic action of vasotocin by some analogues. *Am. J. Physiol.* **204**, 227-232.
- MUNSICK, R. A. (1966). Chromatographic and pharmacologic characterization of the neurohypophysial hormones of an amphibian and a reptile. *Endocrinology* **78**, 591-599.
- PICKERING, B. T. & HELLER, H. (1959). Chromatographic and biological characteristics of fish and frog neurohypophysial extracts. *Nature, Lond.* **184**, 1463-1464.
- SAWYER, W. H., MUNSICK, R. A. & VAN DYKE, H. B. (1959). Pharmacological evidence for the presence of arginine vasotocin and oxytocin in neurohypophysial extracts from cold-blooded vertebrates. *Nature, Lond.* **184**, 1464-1465.
- SCHMIDT-NIELSEN, K. & LEE, P. (1962). Kidney function in the crab-eating frog (*Rana Cancrivora*). *J. exp. Biol.* **39**, 167-177.
- SCHOFFENIELS, E. & TERCAFS, R. R. (1966). L'osmorégulation chez les Batraciens. *Annls Soc. r. zool. Belg.* **96**, 23-40.
- SCHWARTZ, I. L. & LIVINGSTON, L. M. (1964). Cellular and molecular aspects of the antidiuretic action of vasopressins and related peptides. *Vitams Horm.* **22**, 261-358.
- VAN KAMPEN, P. N. (1923). *The Amphibia of the Indo-Australian Archipelago*. Leiden: Brill.