The Effect of Some Ecological Factors on Renal Function in the Frog*

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These studies were undertaken to examine alterations in renal function which accompany exposure to cold and dryness, two ecological states which the bullfrog encounters in its normal habitat. Precautions were taken to make these observations under conditions resembling those of the natural environment. Endogenous urea clearances were studied to determine whether these were maximal, and, as such, commensurate with renal plasma flow. It was disclosed that reductions in glomerular activity accompanied both chilling and dehydration. Endogenous urea clearances were found to be significantly lower than those which measure renal plasma flow (diodrast and p-aminohippurate).

Methods

Creatinine clearances were used to measure glomerular filtration rates[1] and p-aminohippurate clearances for renal plasma flow determinations. PAH clearances have been found to be identical with diodrast clearances[2]. The bullfrogs were taken from local ponds and used for experiments within a day or two of capture. Body weights (BW) varied from 100 to 170 grams.

When PAH and creatinine clearances were studied simultanously, a priming dose of 4 to 6 mg of PAH and 10 mg of creatinine per 100g BW was injected into the dorsal lymph sac at the start of the experiment. Blood levels were sustained by hourly maintenance doses of 0.6 mg PAH and 1 mg creatinine per 100g BW. The urine samples were collected every hour by means of a specially designed glass catheter[1].

Blood was withdrawn directly from the heart. To test the effect of a dry environment, frogs, after being kept in water for 4 control clearance periods, were then placed in an open wire cage for varying periods of time and after this again returned to water.

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Only filtration rates and urea clearances were determined in the cold experiments. Creatinine was administered in a single injection the night before the start of the experiment. The frogs were acclimatized to low temperature by being kept in water at 2 to 5°C for 24 hours; then the cloaca was closed with a ligature. This was necessary because the urine would otherwise leak out, due to the pressure from the enormously increased amount of fluid in the peritoneal cavity of these "cold" frogs. Five hours later the urine was collected, and a blood sample was taken from the heart.

Creatinine was determined using Clark and Thompsons' modification of the Folin and Wu method[3]. Urea was determined by Conway's microdiffusion method[4], and PAH by Bratton and Marshall's method[5].

Results and Discussion

Urea excretion

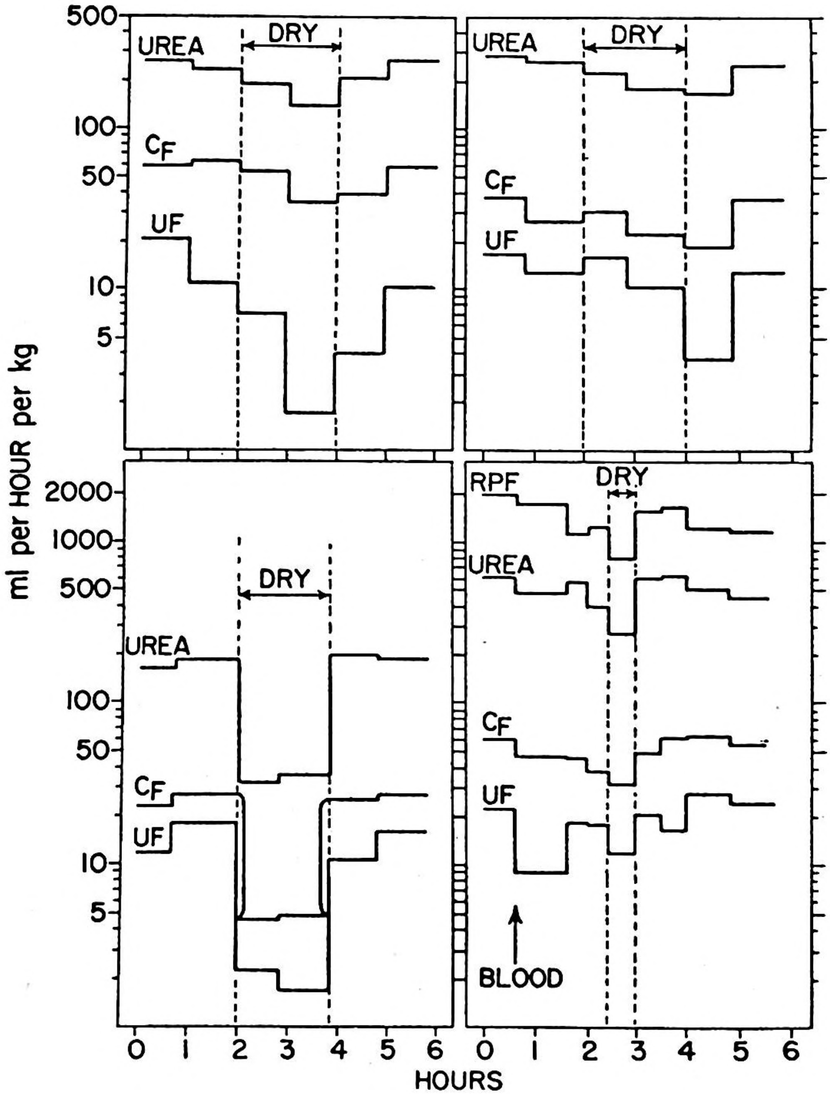
The urea clearances were consistently 4 to 10 times higher than the simultanous creatinine clearances in 112 clearance periods. This confirms the previous view that urea is actively excreted by the renal tubules in the frog (Marshall[6]). The PAH clearances were approximately three times higher than the simultanous urea clearances when the plasma concentration of PAH was low enough to measure renal plasma flow. The problem of determining whether urea clearances are lower than PAH clearances because of back diffusion of urea subsequent to secretion, or because its transfer process is relatively sluggish has not been resolved.

Effect of dry environment

When frogs were taken out of water, urine flows usually decreased within ½ hour. In most instances the reduction in urine volume was related primarily to a reduced filtration rate, but such reductions were also accompanied by increased creatinine U/P ratios. In 12 such experiments the creatinine U/P ratios increased two to ten-fold when the animals were removed from water. The reduction in filtration rate varied similarly between individual frogs. Fig. 1 shows some of the responses. If the reduction in filtration rate is due to a fraction of the glomeruli closing down completely (Forster[7]), while others maintain normal filtration, the increased creatinine U/P ratio must indicate that the amount of water reabsorbed in the functioning nephrons is increased. It seems likely that the sites of antidiuretic action include the afferent glomerular arterioles as well as the renal tubules (see also Sawyer[8]).

After the frogs were returned to water, urine flows rose to hydration values within 1 to 2 hours. Changes in renal plasma flow and urea clearance, in most instances, paralleled changes in filtration rate.

Figure I. The effect of dryness in four different frogs.



Effect of cold environment

Bullfrogs transferred suddenly to cold water (2 to 5°C) submerge, arrest their respiratory movements and remain motionless at the bottom. If the bladder is emptied just before the frog is placed in the cold water, no more urine can be obtained for the next 12 to 18 hours. These findings are similar to those noted in Rana esculenta by Barker-Jorgensen[1]. The weight of the frog increases during that period by about 8%. After this quiescent period, the animals rise to the surface and urine formation again commences but at a slower rate than before. What happens to the renal circulation in the initial period when no urine is formed we do not know, but after that period the glomerular filtration rate can be determined. The results are given in Table 1. It is seen that the glomerular filtration rate is reduced to approximately one-tenth that at room temperature. The tubular reabsorption of water is low, most of the creatinine U/P ratios being under 2. Maximal PAH clearance could not be measured in these experiments but the urea/ creatinine clearance ratios were somewhat lower at this temperature than at higher temperatures.

Table I

The Effects of Cold

Frog#	Urine flow ml/h/kg	Creatinine U/P	C_F ml/h/kg	Urea clearance / Creatinine-clearance
1	2.89	1.13	3.42	3.16
2	1.53	2.53	3.86	8.1
3	5.00	1.38	6.90	5.9
4	2.03	1.63	3.30	7.1
5	4.10	1.02	4.15	4.1
6	2.90	1.86	5.40	3.4
7	3.66	1.15	4.20	3.4
Average	3.16	1.54	4.47	5.02

Summary

1. Urea clearances invariably exceed those of glomerular filtrate measuring substances in the frog, but are only 1/3 of the renal plasma flow as measured by p-aminohippurate clearance.

2. Exposure to a dry atmosphere results in decreased urine flow, which is associated with both a decrease in glomerular filtration rate and an increase in tubular water reabsorption.

3. When placed in cold water (2 to 5°C), frogs are initially anuric. Subsequently they exhibit low urine flows which are accompanied by markedly depressed rates of glomerular filtration.

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