A PRELIMINARY STUDY OF THE LIGHT REACTION OF THE ROCK BARNACLE, *BALANUS BALANOIDES*

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The rock barnacle, *Balanus balanoides*, is extremely sensitive to decreases of illumination as noted by Cole '29. To an appropriate decrease of illumination active animals, viz. animals carrying on regular rhythmic movements of the cirri, respond by an immediate retraction of the cirri and closure of the valves. To an increase of illumination there is no such response. The length of the period of closure following a decrease of illumination depends upon the magnitude of the decrease. To a decrease from 24.5 to 23 f.c., seven animals given five trials each averaged 2.74 seconds of closure. To a decrease from 53 to 23 f.c. the same animals in the same number of trials averaged 9.91 seconds of closure. As expected, preliminary experiments also show a relation between the length of light adaptation and the magnitude of the decrease of illumination necessary to effect a response. Thus in animals continuously exposed to 23 f.c. an exposure for as brief an interval as 0.05 seconds to 53 f.c. sufficed to effect a response in some animals. Under similar conditions, however, an exposure of ca. 8 seconds to 24.5 f.c. was required to produce a closure in the most sensitive animals.

REFERENCE

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RENAL CLEARANCE STUDIES IN THE FRESH-WATER TURTLE, *PSEUDEMYS ELEGANS*

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Within the past few years considerable information has accumulated concerning renal clearances in the marine and terrestrial vertebrates, but with the exception of the Amphibians, little is known concerning renal function in the fresh-water vertebrates. Inulin has been established as a glomerular filtrate measuring substance for a wide variety of vertebrates (see Smith, 1937) and was used as such in this study of turtle excretion, its clearances being compared with those of glucose, xylose and creatinine in the normal and phlorizinized animal.

The exogenous substances, the elimination of which was studied, were injected intraperitoneally four to six hours before the first urine collection. During the experiment the animal was strapped to a turtle board and held ventral side up at about a sixty degree angle. Urine was collected by inserting a long forceps into the cloaca which kept the bladder sphincter open during the collection periods. Uretral urine which emptied into the bladder just anterior

to the sphincter then drained down the cloaca and was collected in graduated centrifuge tubes. Blood was obtained from the femoral vein, samples being taken at the beginning, middle and end of the experiment. Urine collection periods were of one-half or one hour duration, and from four to ten clearance periods were obtained from one animal. Plasma values to correspond to the urine collections were read from a curve relating the three blood collections. Urine flow was controlled by intraperitoneal injections of water. The chemical methods employed in this study were the same as those used with the frog (Forster, 1938).

The fresh-water turtle is capable of excreting large quantities of dilute urine. The highest urine flow obtained was 11 ml. per kg. per hr. (25 percent of the animal's body weight per day), when the inulin urine/plasma concentration dropped to 1.2. The highest inulin urine/plasma concentration obtained was 7.38 with a urine flow of 0.78 ml. per kg. per hr. Despite the turtle's ability to excrete a somewhat concentrated urine at low flows, the inulin clearance is not independent of the rate of urine production. The average inulin clearance value with a urine flow of 3.5 ml. per kg. per hr. was about 6 ml. per kg. per hr., while with a urine flow of 10 the average clearance value rose to about 12. This indicates that factors effecting filtration rate alter the urine flow. Although the uretral urine is dilute, the bladder urine of an animal freshly introduced into the laboratory is concentrated and usually possesses an almost sirupy consistency. These turtles always retain large quantities of bladder urine (200 to 350 ml. in animals weighing about 1.2 kg.). This bladder urine has a yellow color whereas uretral urine is always colorless.

In this study 54 clearance periods were obtained from 7 animals, and 12 periods from 2 phlorizinized animals. Despite wide ranges in plasma creatinine concentrations and urine flow, the average of the creatinine/inulin clearance ratios for the normal animals was 0.997, indicating that the elimination of exogenous creatinine in the turtle is solely by glomerular filtration without the intervention of tubular excretion or reabsorption. Disturbing individual variations in creatine/inulin clearance ratios were obtained, but inasmuch as these variations bore no relation to plasma concentration, and as the average of all clearance periods was so close to 1.0, these differences must have been due to some unknown random error in technique. The widest deviations were 0.90 and 1.18.

The xylose clearances in the normal turtle were always lower than those of inulin and creatinine, indicating that xylose is reabsorbed in this animal as in all other vertebrates studied. The xylose/inulin clearance ratio averaged 0.90.

The turtle releases endogenous glucose into the blood stream during the course of an experiment and its plasma concentration rises to unusually high levels. In a typical experiment the glucose plasma concentration at the time of the first blood collection was 147 mg. percent, and this rose to 313 mg. percent after 7 hours. It was found that a turtle could be "trained" by handling it several times in a manner simulating the experimental procedure. After this treatment the plasma glucose level did not rise above 50 mg.

percent. At low plasma concentrations all the glucose was reabsorbed, and at 160 mg. percent the glucose/inulin clearance ratio was about 0.50, indicating that at this level about 50 percent of the glucose filtered at the glomerulus was reabsorbed. At very high glucose plasma levels (1300 mg. percent) the glucose/inulin clearance ratio rose to 0.82.

Two turtles were phlorizinized by injecting 500 mg. per kg. body weight of recrystallized phlorizin intraperitoneally. The simultaneous excretion of inulin, glucose, xylose and creatinine was studied in 12 clearance periods on these animals. The results were similar to those obtained with the frog. The creatinine/inulin clearance ratio which averaged 0.997 in the normal animal was depressed to 0.80, the xylose/inulin ratio was lowered from 0.90 to 0.85 and the glucose/inulin ratio was raised to 0.86. That all these substances are reabsorbed to approximately the same extent indicates that phlorizin not only blocks the active reabsorption of the renal tubule, but also renders it partially permeable to these low molecular weight substances, thereby permitting a non-selective back diffusion from lumen to peritubular fluid. The failure of phlorizin to elevate the clearances of glucose and xylose to that of inulin has not been observed in any of the marine and terrestrial kidneys studied, but has been demonstrated in the frog by renal clearance studies (Forster, 1938) and by direct analysis of tubular urine (Walker and Hudson, 1937).

Summary:

1. A method is described for renal clearance studies in the fresh-water turtle.
2. The fresh-water turtle is capable of excreting large quantities of very dilute urine.
3. The inulin clearance varies directly with the urine flow, indicating that changes in filtration rate account, at least in part, for the rate of urine production.
4. Exogenous creatinine is excreted solely by the process of filtration at the glomerulus, with neither tubular excretion nor reabsorption playing a part in its elimination by the turtle kidney.
5. Xylose is reabsorbed to the extent of about 10 percent.
6. High endogenous glucose plasma levels are attained during experimental manipulation. At low plasma levels this glucose is completely reabsorbed, and as the plasma concentration increases the percentage reabsorbed diminishes.
7. In the phlorizinized turtle the data indicate that active reabsorption of glucose is blocked and that the tubule cells are rendered non-selectively permeable, thereby permitting the passive back diffusion of glucose, xylose and creatinine to the extent of about 15 percent under the conditions of this study.

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THE EXCRETION OF PHENOL RED BY THE FROG, *RANA CATESBIANA*

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In an earlier paper it was demonstrated that inulin and creatinine are excreted by the frog kidney solely by the process of ultrafiltration at the glomerulus, neither substance being secreted nor reabsorbed by the renal tubule cells (Forster, 1938). In this study of 92 clearance periods in 14 animals, simultaneous inulin and phenol red determinations are made in order to analyze the processes involved in the renal elimination of the latter. In all marine and terrestrial animals investigated, in which quantitative examination has been possible, it has been shown that tubular excretion plays a part in the elimination of phenol red, and that this process is limited by a maximal rate (see Shannon, 1939). However, on the basis of direct analysis of the glomerular filtrate in the frog, Richards and Walker (1935) concluded that all the phenol red excreted might have been contained in the glomerular filtrate. In this clearance study of phenol red elimination the difference between the total amount of phenol red excreted and the calculated amount filtered at the glomerulus indicates that a large fraction of the phenol red is excreted by the renal tubule cells, and that this tubular fraction has a maximal value such as has been demonstrated in the marine and terrestrial animals.

The methods used in this study for inulin determination and the collection of blood and urine were the same as those employed in an earlier investigation (Forster, 1938). Usually 2 grams of inulin and varying amounts of phenol red, depending upon the desired plasma concentration, were injected into the dorsal lymph sac 3 to 4 hours before the first urine collection. Hourly clearances of inulin and phenol red were then determined on a falling plasma curve obtained by analyses of blood collections taken at the beginning, middle and end of a 5 to 12 hour experiment. A photoelectric colorimeter was used for many of the low phenol red plasma and urine concentrations, and a Duboscq-type microcolorimeter with a 74 Wratten filter, for the others. An average plasma blank determination was obtained (0.167 mg. percent) and this value was subtracted from all plasma phenol red readings.

The free phenol red fractions at various total concentrations in the plasma were determined from pooled samples of plasma taken from 3 animals after the method of Shannon (1935). These values vary from 45 percent free at very low plasma total phenol red concentrations, to 74 percent free at high levels (112 mg. percent). The filtration rate of phenol red elimination was determined as the product of the inulin clearance and the percent free of plasma phenol red,