109, pp. 532-541; 542-549, 1934; Ann. Rep. Tortugas Lab., Carnegie Inst. Wash., 1935-36, pp. 90-91) that in dog and man the excretion of exogenous creatine may be adequately accounted for on the basis of a mechanism of filtration and perhaps reabsorption, while in the marine teleost, *Epinephilus morio,* in addition to the glomerular route, a tubular secretory mechanism must be invoked to account for the large amount of creatine appearing in the urine. A further study was undertaken of the mechanism of excretion of creatine in the dogfish, *Squalus acanthias.*

Simultaneous clearances of inulin and creatine were determined in 22 normal dogfish and in 4 after phlorizin, following the intravenous or intramuscular administration of creatine and inulin. The clearance of creatine at all plasma levels was found to be higher than the simultaneously determined glomerular (inulin) clearance. Below a plasma level of creatine of 10 mgm. per 100 cc. the creatine clearance is some 6 times the glomerular clearance while at plasma levels above 150 mgm. creatine per 100 cc. it drops off to 1.5 times the glomerular clearance. Thus at all plasma levels tubular secretion of creatine accounts for a considerable fraction of the creatine excreted. Calculation of the amount of creatine secreted by the tubule indicates that at low plasma levels there is some diminution in tubular secretory ability while at moderate plasma concentrations a secretory maximum of 50 to 60 mgm. per kgm. body weight per day is reached which is not exceeded though the plasma level be increased many fold. The secretory ability of the tubule for creatine is not influenced specifically by doses of phlorizin which are sufficient to paralyze the ability of the tubule to reabsorb glucose. The above evidence for a highly developed tubular secretory mechanism for creatine correlates with the known fact that a considerable fraction of total nitrogenous excretion in the dogfish is in the form of creatine.

THE EXCRETION OF PHOSPHATE IN THE DOGFISH, *SQUALUS ACANTHIAS*

WILLIE W. SMITH, HELEN KIEGHER AND RUDOLPH NAUMANN

Department of Physiology, New York University College of Medicine

The excretion of inorganic phosphate in the dogfish was examined with two objects in view: to determine whether or not endogenous and exogenous phosphate are secreted by the tubules, and to what extent the pH of the urine is controlled by selective secretion of acid phosphate. Unfortunately the nature of the problem is such that, with the methods at our disposal, neither question can be answered definitely. We believe, however, that the data accumulated have some value with regard to the general problem of phosphate excretion.

Methods: Inulin (2 to 3 gm. per kg.) was injected intramuscularly or intravenously, and after two or three control periods phosphate (0.15 to 1.5 gm. per kg. at pH 7.4 to 7.7) was injected intramuscularly or intravenously. Intravenous injections of both phosphate and bicarbonate are preferable to intramuscular injections. The condition of the fish was invariably poor when the plasma phosphate was increased to 10 mM per liter or more. Inulin was determined by the method described by Smith, Goldring, and Chasis (J. Clin. Invest., *17*, 263, 1938) and phosphate by the method of Fisk and Subbarow (J. Biol. Chem., *66*, 375, 1925).

Results: The PO_4/inulin clearance ratio in normal dogfish ranges from 4 to 27. About 70 per cent of the normal plasma phosphate is filtrable from plasma, and at 7 mM per liter 85 per cent of the phosphate is filtrable. The magnitude of the PO_4/inulin ratio shows that phosphate is excreted by the renal tubules of the dogfish, but from the data collected it is not possible to determine the origin of this phosphate, which may be derived from some organic precursor. There is no regular relationship between plasma level and PO_4/inulin clearance ratio.

When plasma phosphate is raised to 9 mM per liter, the absolute quantity excreted by the tubules (the total excretion minus the quantity filtered, the latter being calculated as inulin clearance corrected for per cent of filtrable phosphate times the plasma concentration) is almost doubled. In 87 control periods the average phosphate secreted is 1.17 mM per kg. per day, and in 27 periods in which the plasma level was increased to 2 to 9 mM per liter, 2.22 mM per kg. per day.

Repeated intravenous injections of $NaHCO_3$, totaling in one case 36 cc and in another 10 cc of 10 per cent solution, gave rise to a slight increase in secreted phosphate, the latter rising from the control level of 0.715 mM to 0.99 mM per kg. per day. Hippuran intravenously (500 to 700 mgm. per kg.) slightly depressed secretion (from 0.88 mM to 0.60 mM per kg. per day) and phlorizin did not significantly affect it.

The pH of the urine as determined by the glass* and quinhydrone electrodes is remarkably constant. In a series of 97 determinations on 32 normal fish the average pH was 5.75 with a standard deviation of 0.12 pH units. Immediately after phosphate injection the average pH was 5.63, but in the series in which the plasma phosphate level had been raised to 2 to 9 mM per liter (32 determinations on 9 fish) the pH was 5.76. After intravenous administration of $NaHCO_3$ (2 fish, 15 periods) the pH remained normal (5.74) as was the case with fish injected with hippuran and with phloridzin. It is appar-

---

*We wish to thank Mrs. Samuel Fels whose gift of a Beckman pH meter to the laboratory made these determinations possible.

ently impossible to move the pH of the urine nearer to neutrality than about pH 6.

By titrating H_3PO_4 in urine and in plasma, pK_2 was found to be 6.5 for urine and 6.4 for plasma. Using the Henderson-Hasselbach equation the distribution between $HPO_4^{=}$ and $H_2PO_4^{-}$ was calculated for each urine sample. In control fish and after intramuscular injection of PO_4 or intravenous injection of $NaHCO_3$ the basic phosphate fraction of the secreted phosphate remained fairly constant (0.13) for 50 to 60 hours. However, in the first period following intravenous injection of phosphate sufficient to give a plasma level of 5 to 9 mM. per liter, the total basic phosphate is less than the amount filtered (indicating reabsorption of basic phosphate or conversion of basic phosphate to acid phosphate) with return to the normal relationship in about 20 hours. The following table illustrates the relationship between acid and basic phosphate following intravenous injection of 4 cc. of 10 per cent PO_4 (pH 7.7) in 19 cc. of blood.

	Plasma PO_4 mM. per liter	Filtered mM. per kgm. per day		Secreted mM. per kgm. per day	
		$HPO_4^{=}$	$H_2PO_4^{-}$	$HPO_4^{=}$	$H_2PO_4^{-}$
1.	1.10	0.059	0.004	+0.100	0.706
2.	1.10	0.021	0.001	+0.194	0.852
3.	8.70	0.518	0.036	−0.134	1.884
4.	7.20	0.405	0.028	+0.114	2.136
5.	4.85	0.290	0.020	+0.179	2.561
6.	2.33	0.131	0.009	+0.281	2.149

Because of the high Mg content of dogfish urine, at about pH 6 $Mg(OH)_2$ begins to precipitate. The physiological necessity of maintaining the pH below this value is apparent. It seems probable that the changes observed in both the $HPO_4^{=}$ and $H_2PO_4^{-}$ excreted in excess of filtration are a part of the mechanism by which the constant pH of the urine is maintained, but the significance of changes in the secretion of these ions is not clear. A more complete presentation of the data will be made at a later time.

THE EXCRETION OF PHENOL RED IN THE DOGFISH, *SQUALUS ACANTHIAS*

WILLIE W. SMITH, KATHARINE TILSON AND SAUL FARBER

Department of Physiology, New York University College of Medicine

In all instances in which adequate examination has been possible, it has been found that the process of tubular excretion is limited by a maximal rate (See Shannon, Physiol. Rev., 1939. In press). It is here demonstrated that phenol red is excreted in great measure by tubular excretion in the dogfish, and that this process is limited by a maximal rate as in other animals.