This value exceeds the filtration rate by a factor of about 3. Therefore if these preliminary results are valid more fluid is excreted than that which is filtered. This is quite possible since many nephridia have no nephridiostome and presumably must function by tubular secretion.

Techniques for micropuncture studies in vivo of the nephridia were developed. Analysis of nephridial fluid will be made in later experiments.

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1968 #35

THE MISSING MILLIEQUIVALENT–H^+ BALANCE DURING METABOLIC ALKALOSIS IN THE DOGFISH, Squalus acanthias

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Previous studies have shown that following a bolus intravascular injection of $NaHCO_3$ in Squalus acanthias there is a rapid increase in blood pH and HCO_3^- concentration followed by a rapid progressive return to normal values over several hours (Bull. MDIBL 4-4:75, 1962). This return to normal is not accompanied by changes in urinary pH nor is it quantitatively accounted for by intracellular buffering. The present studies were designed to define the quantitative kinetic processes responsible for the return to normal acid-base values and to attempt to define the anatomical sites of buffering and HCO_3^- excretion. Six animals were studied. The rate of restoration of normal acid-base values was estimated by sequential measurements of arterial pH, pCO_2 and HCO_3^- concentrations. Gill net H^+ excretion and renal net H^+ excretion were measured as previously described.

Restoration toward normal values occurs at a rate consistent with approximately 1.0 to 1.5 mEq/Kg/hour HCO_3^- loss. Urinary pH remained unchanged and preliminary studies of urinary titratable acid and ammonia excretion do not account for the observed HCO_3^- loss. Direct penetration of coelomic fluid by HCO_3^- occurred, but buffering in this compartment accounted for less than 0.25 mEq. Preliminary studies of gill HCO_3^- excretion do not indicate substantial changes during control versus HCO_3^- loading periods. Measurements of renal and gill TMA and TMAO are now in process.

Although gill excretion is possible, the precise locus of this regulation has not been established and approximately 1 mEq of HCO_3^-/hour is still missing.

1968 #36

THE DEVELOPMENT OF APOLAR EMBRYOS OF Fucus vesiculosus L. IN SUCROSE-SEAWATER

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Using cytological methods developed over the past several years, it has been possible to prepare squash preparations of Fucus embryos in which accurate nuclear counts can be made of developmental stages up to about ten days following fertilization. These data provide a quan-

titative description of embryo development not previously available. In embryos grown in seawater at 15°C in fluorescent light nuclear number increases from one (the fertilized egg) to a mean value of 42 by 4 days and to 155 by 10 days. At this stage a single rhizoid has developed and a multicellular thallus body with one to several apical hairs has formed.

When fertilized eggs are placed in seawater supplemented with 0.6M sucrose, there occurs an initial rapid plasmolysis of the cell but the embryos soon deplasmolyze and proceed to develop, lacking, however, normal polarization or rhizoid formation. Embryos can be maintained for up to 10 days in sucrose-seawater as apolar structures, developing as slowly growing, symmetrical, multicellular spheres. Mitoses continue, but at a much reduced rate compared to the seawater controls; embryos in seawater containing 0.6M sucrose showed a nuclear count of about 10-11 at 4 days. If such embryos were transferred after 4 days to seawater, they resumed the normal rate of cell division. Instead of forming a single rhizoid, the released spherical embryos developed multiple rhizoids, each rhizoid arising from one of the small peripheral cells of the sphere, usually all located together in one quadrant of the embryo. As many as 7 rhizoids were observed developing from sucrose-treated embryos, instead of the single rhizoid typical of normal embryos.

In normal embryo development in seawater, the single rhizoid cell typically develops in that embryo-half away from low-intensity unilateral illumination. A preliminary analysis of the influence of unilateral illumination on sucrose-treated apolar embryos indicated that polarization could be imposed on the multicellular spherical embryo either early or late in its development before release from sucrose inhibition of rhizoid formation. The implications of these results on the concept of a rhizoid-forming stimulus subject to environmental orientation are still being explored.

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1968 #37

STUDIES ON THE CLEAVAGE CYCLE IN THE SAND DOLLAR EMBRYO: EFFECTS OF PROTEIN SYNTHESIS INHIBITION AND OF ADENINE DERIVATIVES

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We have previously observed that proteins required for S_2 and for the first cleavage of fertilized ova of Echinarachinius parma were synthesized between 20 and 35 minutes following fertilization (Fed. Proc. 27:366, 1968). Therefore, synthesis of these proteins was coincident with the initial DNA replication or S phase. Protein synthesis was not required for extensive replication of DNA present in pronuclei but was necessary for a second replication. To determine the time of synthesis of the proteins which mediate S_3 and the second cleavage (Cl_2) similar studies have been undertaken. Aliquots from a population of simultaneously fertilized ova were added to cycloheximide (1μg/ml) at 10 minute intervals from 60 to 130 minutes following