4 - day embryo, it has been shown that the prospective skeletal parts of the wing (Saunders, '48) and leg (Plouff, '51; Hampé, '56) arise in proximo - distal sequence with the future long axes in essentially their definitive orientation (i. e., femur and humerus adducted; forearm and shank flexed).

An earlier study (Saunders, '53) showed that the contours of the posterior side of the upper arm and elbow are shaped by a furrow which proceeds antero-medially from the posterior junction of wing bud and body wall. The presence of degenerating cells in this area suggested that cell deaths may be a major factor in the shaping of the limb. Accordingly, an extensive study of the spatial distribution of degenerating cells has been made in wing and leg buds of embryos of stages 22 to 32 (3½ to 8 days), employing chiefly three-dimensional glass-plate reconstructions of the limbs, and supravital straining with Nile Blue, a dye which is selectively taken up by the degenerating cells.

Study of the reconstructions and of the stained embryos discloses that the anterior and posterior contours of wing and leg buds, and to a lesser extent the dorsal and ventral sides, are carved out by a wave of cellular degeneration which passes distally along the borders of the limb. The most striking effects are noted in connection with the formation of the elbow and knee. This process is essentially complete by stage 32, or shortly thereafter, when separation of the toes is accomplished by degeneration of the intervening tissues.

These results reveal a hitherto undisclosed role of cell deaths in the morphogenesis of the limb. Furthermore, because of the favorable nature of the material, excellent opportunity is offered for the causal analysis of this phenomenon.

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Notes on the In Vitro Procedure Used For The Study of Cellular Transport Kinetics In Isolated Renal Tubules Of The Flounder, Pseudopleuronectes americanus.

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Current procedures were generally the same as those described earlier (Forster, '48, Science, 108:65) except that chlorphenol red was used instead of phenol red in control experiments. The bluish-red color of the former is more readily perceived, and its color stability within the range of pH encountered in these preparations is also a distinct advantage. Of the various fishes tested for these *in vitro* studies the readily available

flounder was found best; its kidney composed exclusively of proximal (brush border) tubules and almost completely devoid of lymphoid tissue presents a uniform appearance when teased and viewed under the microscope, and its tissues are most viable under *in vitro* conditions.

Several modifications of the basic technique introduced by others to improve quantitative determination of phenol red uptake were found of no value when applied to chlorphenol red, especially elevated temperature and high bicarbonate concentration in the sustaining medium. Comparators may be used to ascribe absolute values to dye concentrations within cells or lumina, but questionable assumptions need then be made concerning such factors which may interfere with light absorption as depth of standard, depth of structure in kidney containing dye, and the presence of extraneous cellular factors which modify color. In our experience the most satisfactory procedure for semi-quantitative evaluation of dye concentration ratings ranging from + (definitely detectable) to ++++ (maximal). Surprising uniformity in interpretation can be achieved by several observers after some practice, and actually eight useful ratings can be made between zero and maximum concentration.

Freshly captured flounder were broken open after an incision was made through the vertebral column in the area of the gills, and within several seconds small fragments of renal tissue were transferred to an oxygenated, balanced, isotonic salt solution which had been empirically devised for its ability to sustain secretory activity in renal tubules. This solution contained the following concentrations of salts in millimoles per liter: NaCl 135, KCl 2.5, $CaCl_2$ 1.5, $MgCl_2$ 1.0, NaH_2PO_4 0.5, and $NaHCO_3$ 10. It is best to add sodium bicarbonate as dry salt while stirring, after water has been added to the mixture. A phosphate buffered solution without bicarbonate ion does not sustain maximal transport of dye. If 5% CO_2 - 95% O_2 is used rather than pure oxygen the mixture stabilizes within the pH range 7.0 - 7.4, whereas with 100% O_2 the pH climbs in time as high as 8.5. The dye transport process, however, is not affected by the variations in pH obtained with either oxygenation procedure.

Petri dishes (1.2 x 4 cm.) containing 5 ml. of the sustaining solution were used to observe dye transport in teased kidney fragments under 100x magnification. Usually 7 such petri dishes, containing 3 x 10 - 5M concentration of the various dyes in the medium described above, were oxygenated simultaneously via 22 gauge hypodermic needles at laboratory temperatures. Oxygenation was interrupted momentarily when the dishes were transferred to the microscope stage from time to time for evaluation of dye concentration in cells or lumina.