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mg/100cc early development is retarded and does not continue beyond the blastula stage; 20 - 30 mg/100cc produces a slight retardation of skeletal formation. Addition of 25 mg of tetracycline to 100cc of sea water lowers the pH to approximately 7.5. A similar reduction of the pH of sea water by addition of appropriate amounts of HCl does not affect the skeletal development. Lower concentrations permit gastrulation and skeletal formation to occur at a somewhat reduced rate. Examination of the skeletons of embryos grown in mixtures of 15 mg/100cc show a yellow fluorescence when examined with ultra violet light which indicates the incorporation of tetracycline or a derivative into the growing skeleton.

In view of the marked avidity which tetracyclines have for heavy metals, calcium and magnesium were added to the tetracycline-sea water mixtures. Magnesium had no effect; addition of calcium resulted in a slight improvement in development.

The Development of the Skeleton of *E. parma*

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Normal skeletal development in *E. parma* consists of the intra cellular formation of a large crystal, the triradiate spicule. Subsequent development occurs extra cellularly and is brought about by the incorporation of mineral salts into a protein matrix. The primary mesenchyme cells elaborate the matrix and also concentrate and transfer the mineral salts in the region of the growing skeleton.

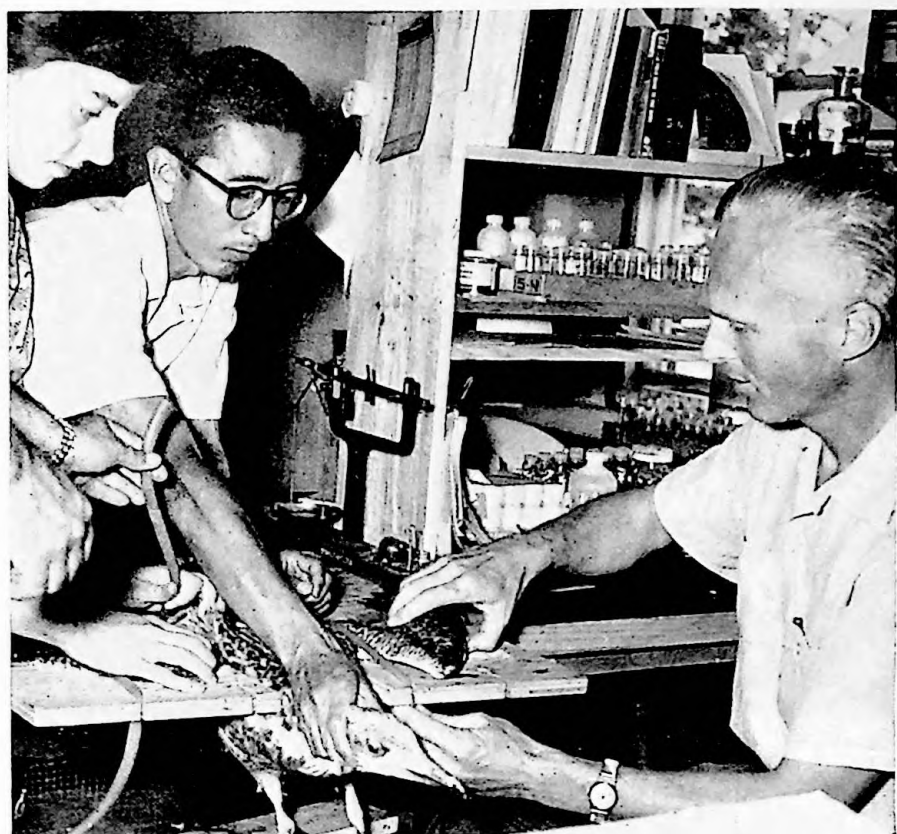
The accumulation and subsequent transfer of calcium carbonate is correlated with vacuole formation, crystal formation, and other cytological events in the mesenchymal cells. These phenomena are moreover dependent upon the osmotic conditions of the coelomic fluid.

The Bromination of Phenol Red by the Dogfish, *Squalus acanthias*

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Introduction of phenol red, determined as homogeneous by paper chromatography, into the uterus of the spiny dogfish in the later stages of pregnancy results within twenty-four hours in a visible different dye. Chromatographic comparison between the new dye and bromphenol blue in four solvent systems showed no significant differences, nor did a comparison of the light absorption characteristics. The quantity of purified dye did not permit complete chemical analysis, but it was determined that bromine not iodine was present. The evidence rather clearly indicates that the uterus of the dogfish can turn phenol red into bromphenol blue. The





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observation constitutes apparently the first example of biological bromination in a vertebrate.

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Hepatic Excretion of Inulin in the Dogfish, *Squalus acanthias*

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For flat and falling plasma levels of inulin over 540-260-mg./l. ranges, with bile flows of 0.26-0.64 ml./hr. following sodium dehydrochlorate injections, in experiments up to 37 hrs. in length, with female dogfish 4.5-7.5 kg., bile-plasma ratios were 0.6-0.21 most frequently around 0.1. Correcting the plasma and bile concentrations to concentration of inulin per 1000 g. water, does not alter greatly the ratios. The above ratios should be considered as for order of magnitude only. Imperfections in chemistry, lack of assurance of a uniform flow of bile and other factors prevented a thoroughly definitive quantitation. The data do show that inulin is in the dogfish, not a suitable base-line substance for the study of hepatic function, and that in the formation of the bile, inulin does not move equally with water.

Aided by the New York Heart Association.

Hepatic Metabolism of Some Dyes by the Dogfish, *Squalus acanthias*

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Exploratory paper chromatography of dogfish bile following the subcutaneous or intramuscular injection of some dyes showed that these dyes appeared in the bile in part in altered form. The solvent was 4 butanol: 1 acetic acid: 1 water. Initially homogeneous phenol red appeared in part as two yellow substances with no indicator properties, and with lower R_fs than the parent substance. One of these yellow substances was recovered in the urine. These alterations and those below did not appear with the stock dye, bile alone, bile incubated with the dye or blood incubated with the dye. Chlorphenol red which was not pure also gave two yellow non-indicator patterns in the bile, and bromphenol blue one. Sulfobromophthalein (BSP) resulted in two additional substances with the same color as the parent substance. While the conversion of BSP is known, the conversion of phenol red into non-indicator substances appears to be new. The results indicate that for precise quantitative work it can not be merely assumed that a substance passes through an organ unaltered.

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