The second part of this study was designed to test the effect of toludin blue on the development of the skeleton in this form. In mammals it has been reported that toludin blue inhibits or prevents in vitro calcification when in combination with a polysaccharide by competing for available calcium. Our observations reveal that the dye has an over all inhibiting effect on the growth of the embryo which in no sense can be construed as specific for the skeletal system. A similar effect was observed with a dissimilar dye neutral red and other substances.

In another group of experiments, embryos were reared at reduced temperatures, 2 degrees C. Untreated specimens developed normally but at much reduced rates. When these embryos were subjected to either Toludin blue or protamine at reduced temperatures, embryos developed with a completely invaginated gut yet showing no skeletal elements at all.

The observations reported above show that the embryo is separated from its aqueous environment by a cellular membrane rich in sulphated acid polysaccharides. The combination of Toludin blue with this substance cannot be interpreted as a calcium competing mechanism. Several factors such as reduced ion availability, reduced temperatures, blocking of cell pores, as well as a depolymerizing effect resulting from a modification of the electric charge on the membrane are suggested as the possible mechanisms whereby ionic transfer may be modified.

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Electrophoretic Patterns of Sera From Various Animals As Contrasted With Patterns of Human Sera.

Elmer D. Bueker and Gerrit Bevelander
College of Dentistry and Graduate School of Arts and Science, New York University

Paper electrophoretic patterns of sera were prepared from four or more individuals from each of the following species: (1) Squalus acanthias, (2) Lophius piscatorius, (3) Ameriurus melas, (4) Rana pipiens, (5) garter snake (Thamnophis), cormorant (Phalacrocorax), human. Buffers used varied in pH from 5. 4-8.6, potentials 100 - 110 volts, current 6-12 milliamperes; duration 8-20 hours.

In striking contrast to the five protein components of human sera only three or four were observed in the other animals. Linear migrations of protein components of sera from these animals were only approximately in the range of alpha, beta, and gamma globulins and albumen of human sera. The relative proportions were usually very different. Sera from the shark (Squalus acanthias), for example, contained three protein components: (1) a small component (1% - 5%) which migrates to the cathode beyond the range of human gamma globulin; (2) a large component

(70% - 90%) with a mobility corresponding to the range of gamma and beta; (3) a smaller amount (3% - 30% within the range of the alpha complex; an albumen component comparable to that in human sera was missing; observations are based on thirty animals.

Mobilities of serum proteins from each animal group were observed in buffers of pH 5.4 to 8.6. Data were plotted (distance of components at given pH ranges). Each group presented a characteristic pattern of its own.

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Effects of 6 - Diazo - Oxo - 1 - Norleucine (Don) and O - Diazoacetyl - 1 - Serine (Azaserine) On The Fertilized Sand-Dollar Egg. (E. parma)

Gerrit Bevelander and D. A. Karnofsky, New York University College of Dentistry and the Sloan Kettering Institute

Sand-dollar eggs were fertilized and after 20 minutes aliquots containing about 200 fertilized eggs were transferred to Syracuse dishes containing 10 cc fresh, filtered sea water. Solutions of test compounds were added at various intervals, and their effects on the development of the fertilized eggs observed.

The normal sand-dollar eggs cleaved within one to two hours after fertilization, blastula developed at 12 hours, movement began at 14 hours, early gastrulation at 22 hours, and the plutei stage was reached at 36 hours. DON at a dose of 10 to 100 milligamma/10cc., added shortly after fertilization permitted normal development through the blastula stage, but gastrulation was largely prevented. Doses up to 1,000,000 milligama/10cc. did not interfere appreciatively with cleavage, but the embryo began to disintegrate in the late blastulae stage. If DON were added later in the development, larger doses were necessary to prevent gastrulation. DON added during the period of gastrulation did not interfere with continued development, but the embryo disintegrated on reaching the pluteus stage. Doses in the order of 10 milligama /10cc. of sea water were effective in arresting pluteus formation. Similar effects were produced with azaserine at approximately 100 times the concentration of DON.

A number of purine analogues were tested for protective action against the effects of DON. Guanine, guanosine, and adenine were effective at a dose of 20 gamma/10cc. of sea water, and some protection was obtained with DPN, xanthine, and hypoxanthine. 4-aminoimidazole carboxamide was ineffective. Further studies on the effects of DON, and a more precise exploration of the effects of physiological purines on DON toxicity will be investigated.