Secondary observations included: 1) a failure to reverse the membrane potential by substituting SO_4 for Cl of the bathing saline; 2) a question of a SCN diffusion potential developed when there was a SCN concentrated gradient; 3) ten isolated mucosae of the skate (Raja erinacea) unlike those of vertebrates other than elasmobranches only developed a spontaneous potential difference of +2.7 ± 0.5 mV.

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1962 #15

EFFECTS OF ANTIMETABOLITES AND GROWTH INHIBITORS ON THE DEVELOPMENT OF THE SAND-DOLLAR EMBRYO

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The developing sand-dollar embryo has continued to be used as a system for examining the mechanism of action of a number of drugs inhibiting cellular mechanisms involving growth and differentiation. A review of sand-dollar embryology has recently been published by our group.*

During the summer of 1962 we have concentrated on the following drugs:

1. The 5 chloro,-bromo-and iodo analogues of deoxyuridine. These drugs appear to act in the same manner, and BrUdR was shown to be incorporated into DNA as rapidly as thymidine. These compounds, when added immediately after fertilization, do not interfere with fusion of the pronuclei, but during cleavage nuclear bridging occurs, with incomplete division of the nuclear masses. The cells may divide several times and non-nucleated cells appear. Development usually steps at the early morula stage. The effect can be prevented if thymidine or almost any purine or pyrimidine riboside is added within 30 minutes after fertilization, or if the halogenated pyrimidine is washed out within 30 minutes. Once the BUDR, as the prototype compound, is incorporated into DNA, its effects are irreversible. BUDR added up to 3 hours after fertilization causes a similar interruption in development. After 4 hours, although BUDR is incorporated into the DNA of each cell, the effects on the development of the organism is less immediate or severe, and some embryos develop to the pluteus stage. It may be postulated that the damage to BUDR incorporated prior to 4 hours after fertilization severely interferes with DNA function, but after this period the DNA may have conveyed sufficient information to the cytoplasm so that differentiation can proceed. Investigation of this interesting problem will continue.

2. Actinomycin D also blocks the embryo at the early morula stage. If it blocks DNA directed RNA synthesis, as it does in other types of cells, its effect at the early morula stage may be due to failure of the embryo to form informational RNA. The sand-dollar provides the possibility of studying the effect of drugs on specific cellular events in relation to the onset of differentiation.

3. Hydroxyurea is highly active on the sand-dollar embryo, interfering with development at the concentration of 20γ/10cc of sea-water. At all effective concentrations the embryo devel-

*Karnofsky, D. A., and E. B. Simmel: Effects of Growth-Inhibiting Chemicals on the Sand-Dollar Embryo, Echinarachnius Parma, Progress in Experimental Tumor Research, 3:254-96 (1962).

ops for 4 hours, and then further cell division is blocked. The nuclei incorporate tritiated thymidine, but after further division is interrupted, the nuclei become vesicular and increase in size. Hydroxyurea can be removed from the solution up to 3 hours after fertilization and development proceeds. It thus may interfere with RNA production or protein synthesis. Urethane, which is somewhat similar to hydroxyurea in structure is far less active in the range of 20-30 mg/10cc and it acts to inhibit cleavage.

4. Whereas drugs which act later on development probably influence certain pathways for cell synthesis, others act in inhibiting the cleavage. Vincristine and vinblastine are very effective, in the range of 10-20 γ/10cc of sea-water. Another compound, 8-mercapto-2-piperidino adenine was also an extremely active cleavage inhibitor, at doses in the range of 2 γ/10cc of sea-water. This is one of the most active cleavage inhibitors known; analogues of 8-mercapto-2-piperidino adenine were also tested, but none approached it in activity.

1962 #16

OXYGEN MOVEMENT ACROSS THE EEL GILL EPITHELIUM

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Measurements of the oxygen tension in fluid bathing the inside and outside surfaces of the isolated perfused eel gill epithelium were made by means of an oxygen electrode. It was found that under all conditions the oxygen tension was the same in both fluids. It would appear that oxygen is not actively secreted across the isolated perfused eel gill epithelium.

1962 #17

SODIUM TRANSPORT BY THE EEL GILL in vivo

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Previous work in this laboratory with isolated perfused eel gill has demonstrated the importance of such factors as the rate of blood flow, the capillary pressure, and the colloid osmotic pressure of the fluid perfusing the blood vessels of the gill in determining the rate of salt and water movement across the gill epithelium. These earlier results also indicated that salt water adapted eel gills actively transport sodium and chloride ions outward but that fresh water adapted eel gills apparently do not actively transport salt inward. It seemed of interest to see if similar results would be obtained with intact eel. The U-tube technique of Krogh was used. Lightly anesthetized (MS-222) fresh water or salt water adapted eels were placed in a Tygon tube in such fashion that the anal papilla drained to one side of the tube while the mouth was in the other side of the U. Under proper conditions, the eel remained quiet and breathed regularly in this apparatus. Influx and outflux of sodium through the mouth end of the fish were estimated with the use of Na^{22}. Control experiments in which the gut was blocked showed that the fluxes occurred primarily across the gill epithelium. It was found that fresh water adapted fish actively took up salt whereas salt water adapted eels actively excreted salt through the gills.