## 1962 #13

STUDIES ON THE RELATIONSHIP OF THE THYROID TO BLOOD SUGAR LEVELS IN THE SKATE

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Blood sugar was determined in 0.1 ml samples of whole blood by the glucose oxidase method. Forty-nine females of the little skate, <u>Raja erinacea</u>, averaged 56.25 mg% glucose with a range from 85 to 39 mg%. Values for freshly caught skates were higher at 75.28 mg% with an average drop of about 30 mg% during the first three days following capture. Recovery was usually good and in individuals starved for six weeks blood sugar levels remained relatively constant. Average values of 62.9 mg% and 48.2 mg% glucose were recorded for a few speciments of the big skate, Raja diaphanes and the barn door skate, Raja stabuliforis respectively.

Thyroidectomized skates showed an average rise in blood sugar levels of 81% over an 8 day period following operation and remained in a much healthier condition than sham-operated controls which showed a post-operative raise in blood sugar of over 100% in some cases. Intramuscular injection of 3.7 to 5 U.S.P. units of ovine TSH (NIH-TSH-S-1) produced a slight rise then an average drop of 24.3 mg% glucose in 48 hours. However, repeated injections over a five day period had no additional effect. Injections of L-thyroxin (400  $\mu$ g/kilo/day for five days) and thiourea (80 mg/kilo/day for two weeks) failed to produce any significant shift in running blood sugar levels over those of controls. Although histological studies may yield further information of endogenous activity it appears that the thyroid-pituitary axis of the skate has little direct effect on carbohydrate metabolism. This is in general agreemtn with the work of Matty (1954) and Olivereau (1954) on the dogfish.

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## 1962 #14

## THE ELECTRICAL CHARACTERISTICS OF THE DOGFISH STOMACH

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The absence of a transmucosal potential difference across the isolated dogfish gastric mucosa provided a unique opportunity to evaluate the interdependence of active transport of  $H^+$  and  $Cl^-$ . Augmentation of  $H^+$  secretion by carbachol shifted the potential difference from -1.2 to -3.0 mV (mucosal surface considered zero) and the resistance from 303 to 236 ohm.cm<sup>-2</sup>. After  $H^+$  inhibition by SCN, these values changed to +1.9 mV and 260 ohm.cm<sup>-2</sup>.

An apparent driving potential for  $H^{\dagger}$  transport of more than 75 mV was indicated by continued  $H^{\dagger}$  secretion against an adverse potential difference of -75 mV. The Cl<sup>-</sup> secretory potential (assuming a reversible process) calculated from the Cl<sup>-</sup> flux ratio, after correction for exchange diffusion, is about 40 mV.

Given these values and the very small changes in the transmucosal potential differences after carbachol and SCN, the active transport of  $H^+$  and  $Cl^-$  must be tightly coupled and they are not appreciably independent as might be predicted by a hypothesis of two separate transport mechanisms.

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  $20\gamma/10cc$  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).