infectivity were not demonstrable for whole virus.

*Summary.* Evidence presented shows that the influence of salt concentration of the environment on RNA infectivity for mammalian cells is due at least in part to alteration of the state of susceptibility of the host cell. The 3 different cell lines tested simultaneously responded differently when exposed to the same RNA preparation diluted in varying salt concentrations. Other factors which alter host cell susceptibility, sucrose concentration, age of host cell, nutritional environment of host cell and temperature during the adsorption interval, emphasize the importance of the physiological state of the cell at time of exposure to RNA to the degree of infectivity expressed.

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# Failure of Reserpine to Cause Depletion of Gastrin in Canine Antral Mucosa.‡ (27058)

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Although the chemical composition of the hormone gastrin is not known, it is thought to be a protein-like substance of low molecular weight, or possibly a more simple substance attached to a protein molecule. Gastric mucosa is known to contain both histamine(1) and serotonin(2) and these have been considered as possible constituents of gastrin.

Reserpine has the property of stimulating acid secretion from the stomach(3). One possible mechanism for this action is the release of gastrin from the antral mucosa. Reserpine is known to deplete tissue of catechol amines(4), serotonin(5) and histamine(6), and if gastrin were bound in the tissue by the same mechanism as those amines, then reserpine might be expected to release gastrin also.

If the effect of reserpine upon gastric secretion is exerted through release of gastrin, it might be expected that the gastrin content of the antral mucosa would be reduced following its administration. Indirect evidence concerning the storage and binding of gastrin might also be established in this manner.

*Methods.* Reserpine was administered by mouth in a dose of 5 mg per kg body weight to 13 adult mongrel dogs. Twenty-four hours later the animals were killed and the stomachs immediately removed. Each stomach was opened, washed and mucosa of the antral region dissected from the underlying muscle. The mucosa was stored at $-70°C$ until required.

*Extraction.* The mucosa was thawed and minced and extraction was performed by the

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method of Gregory and Tracy(7). This involves preliminary treatment with picric acid, followed by extraction with acidified acetone and repeated precipitation and washing with trichloracetic acid. The final product does not contain free histamine. Gastrin was also prepared by this method from the antral mucosa of normal dogs. Linear dose response curves for normal hog gastrin prepared by the Gregory method have been established in this laboratory(8).

*Testing.* The extracts were tested by subcutaneous injection in conscious dogs with gastric fistula or Heidenhain pouches. The dogs were fasted for 24 hours before the tests. Secretion in response to gastrin normally starts within 30 minutes of the injection and has practically ceased after 3 hours. Accordingly, secretions were collected over a 3-hour period following injection and total output of HCl during this period was determined by titration with 0.1N NaOH using phenol red indicator.

*Results.* The extract prepared from the reserpinized dogs was divided into 2 portions, each equivalent to 70 g antral mucosa. In the Heidenhain pouch dogs this dose of gastrin caused an output of 2.6 mEq. HCl over a period of 3 hours. The same doses of gastrin prepared from the antral mucosa of normal dogs caused an average output of 2.2 mEq. HCl from the same Heidenhain pouch dogs. In another dog with a fistula from an intact stomach, the gastrin prepared from 70 g of mucosa after reserpine produced a response of 17.8 mEq. HCl over a 3 hour period. It was not possible to establish the response of gastric fistula dogs to gastrin prepared from normal dog mucosa but the response to the gastrin prepared from reserpinized dogs was larger than that obtained from comparable amounts of hog gastrin tested on several occasions.

*Discussion.* Several methods have been described for preparing gastrin(7,9-15). In all of them a biologically active substance has been obtained from the protein fraction ot mucosal extracts, but the products have differed in their chemical properties. Thus, the isoelectric point has ranged from pH 4.5(10) to pH 7(12); gastrin has been described as dialysable(10,14) and non-dialysable(11,12, 13); and as soluble(9) or insoluble(16) in 80% ethanol. These facts suggest that gastrin itself may be a relatively simple substance attached to a protein molecule and that the precise properties of the "gastrin" in mucosal extracts may depend on method of extraction.

Gastrin prepared by any of the methods referred to does not contain significant amounts of free histamine. Recently, Wojciech and Ivy(15) described a new method of preparing gastrin. This gastrin contained no free histamine on biological assay; and the active principle was of large molecular size, or attached to a substance of large molecular size (17). When treated chemically, this gastrin yielded histamine in sufficient amount for a supra-minimal secretory response(18). It was, therefore, suggested that gastrin is histamine or a histamine-like compound bound to another substance. Confirmation of these findings has not been reported.

In the present investigation administration of a large dose of reserpine to dogs did not deplete the antral mucosa of gastrin. Histamine and serotonin are liberated from tissues by reserpine, and the results, therefore, suggest that these substances are not constituents of gastrin, and that the known secretagogue effect of reserpine cannot be attributed to release of gastrin.

*Summary.* A large dose of reserpine was given to dogs. The dogs were subsequently killed and gastrin was extracted from the antral mucosa. The activity of this gastrin was similar to that of gastrin prepared from normal dogs. This finding is thought to be evidence that histamine and serotonin are not constituents of gastrin, and that reserpine does not stimulate gastric secretion through release of gastrin.

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# Studies with Cells From Marine Fish in Tissue Culture.* (27059)

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*In vitro* cultivation of normal and abnormal mammalian and fowl cells has proven a valuable tool for biochemical, physiological and virological studies. The comparatively sparse work on *in vitro* cultivation of cells from fresh water fish is adequately summarized by Wolf and Dunbar(1) as is their recent work with explants of trout and goldfish tissues. More recently both Grutzner(2) and Wolf *et al.*(3) have utilized slight modifications of the technic of dispersion of cells by primary trypsinization for preparation of monolayer cultures of cells from fresh water fish. Wolf *et al.* (4) have used tissue cultures of trout cells for studying the virus causing infectious pancreatic necrosis in the young of that species. To our knowledge, there are no published reports on cultivation of tissues from marine fish. This paper describes the successful *in vitro* cultivation of cells from some marine teleost fish.

*Materials and Methods.* Tissues from the yellow-striped grunt (*Haemulon flavolineatum*) were used for most of this study due to their abundance in South Florida Waters, although the black angelfish (*Pomacanthus* sp.), porgy (*Calamus* sp.), pork fish (*Anisotremus virginicus*), red snapper (*Lutjanus* sp.), and sand perch (*Diplectrum formosum*) were also used at various times. These fish were all trapped in the Atlantic Ocean or in Biscayne Bay near Miami, Fla. and were kindly furnished for this study by the Miami Seaquarium. All fish were of a size to be considered sexually mature. Fish were killed just before use by severing the spinal column immediately posterior to the head. The tissues to be used were removed with sterile instruments and received three 10 minute washes at room temperature in Hanks' balanced salt solution (Hanks' BSS) containing penicillin (300 units /ml), streptomycin (150 mg/ml), and mycostatin (300 units/ml). Three to 4 explants (1-3 $mm^3$) per tube were cultured in stationary Bellco pyrex brand culture tubes or Leighton tubes with coverslips, usually on a coagulum of equivalent amounts of 50% chick embryo extract (prepared from 9-day-old chick embryos in Hanks' BSS) and chick plasma (Microbiological Assoc.).

Primary trypsinization was accomplished by mincing the washed tissue into small fragments which were then added to about 10 volumes of 0.25% trypsin (Difco) prepared in $Mg^{++}$ free Hanks' BSS containing penicillin (100 units/ml) and streptomycin (50 mg/ml). In later experiments the trypsin solution contained 0.206M NaCl instead of the usual 0.137M used with mammalian systems. The trypsin was allowed to act on the tissue fragments in an Erlenmeyer flask over a magnamix at room temperature for 30 minutes after

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