and a little lower, respectively, in the freshwater species. The latter difference is reflected in the volumes of plasma and interstitial fluid, both subcompartments of the extracellular fluid. The differences in pulse rate appear to be related to the size of the animals. With the exception of the values for whole blood, where differences in the hematocrit reading influence the figures, these small differences in compartment volumes prevail for all measurements, whether the freshwater shark is being compared with individual species of marine sharks or with averages for all marine species. That these are valid differences cannot be positively stated, but they are in close agreement with similarly slight differences observed between freshwater and marine teleost fishes (*9*). It would appear that in elasmobranchs as well as in teleosts the body-water content and the apportionment of the body water are maintained within fairly close limits, whether in a marine or a freshwater environment and in spite of differences in osmotic pressure of the body fluids. But the small differences that are evident suggest that marine sharks as well as marine teleosts function with a slightly larger fraction of the total water in the circulating or mediating compartments, and with a smaller fraction of intracellular or protoplasmic water, than their freshwater counterparts (*10*).

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2a. Since this report was submitted, two papers have appeared, one by me [*Am. Zoologist* **2**, 452 (1962)] and one by M. R. Urist [*Science* **137**, 984 (1962)].
3. A tagging program is being planned to determine the extent of movement of sharks both up and down the Rio San Juan between the Caribbean Sea and Lake Nicaragua, as well as within the lake itself.
4. The work on *C. nicaraguensis* was done in July and August 1960, at Colegio Centro America, Granada, Nicaragua. I thank the Rev. José V. Aranguren, at that time rector of the school, for his generous provision of living accommodations, laboratory space and facilities, and transportation, and the many members of the staff and student body who aided me in every phase of my work.
5. The work on *N. brevirostris* and *G. cirratum* was completed in April 1962. I am deeply indebted to Dr. James A. Oliver (director of the American Museum of Natural History), to Dr. Robert F. Mathewson (resident director of the Lerner Marine Laboratory), and to other staff-members and investigators at the Lerner Marine Laboratory who helped make completion of the work possible.
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10. This paper is study No. 345 from the Department of Zoology and Physiology, University of Nebraska. This research was supported by the U.S. Public Health Service (grant No. H-3134) and by the University Research Council of the University of Nebraska. I wish to acknowledge my appreciation for the stimulating interest and enthusiasm for research on sharks of Robert C. Dorion of Guatemala City.

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Guttation Fluid: Effects on Growth of Claviceps purpurea in vitro

Abstract. In laboratory inoculation experiments, Rosen rye seedlings were more susceptible, Genesee wheat seedlings were less susceptible, and Trail barley seedlings were not susceptible to *Claviceps purpurea*. Guttation fluids from these plants were used as media for the growth of germinating spores of *C. purpurea*. Growth experiments consisted of spores which were germinated in thin agar films on microscope slides. Germ tube growth was determined by counting the tubes that crossed the perimeter of a square in the microscope field, counting the conidia in the square, and computing the number of crosses per conidium. Rye guttation fluid produced the most growth and barley fluid the least. Thus the degree of susceptibility is correlated with the amount of growth of the parasite in vitro.

Hale and Roane found that the in vitro growth of *Helminthosporium carbonum*, a parasite of corn, "was inhibited by . . . extracts of diseased susceptible plants, but not by extracts of diseased resistant plants or by extracts of uninfected resistant and susceptible plants" (*1*). These results are quite typical of most experiments of this sort. There is no correlation between in vitro growth of the parasite and the degrees of susceptibility of the hosts when extracts or press juices are used as the growth medium for the parasite. This lack of correlation is not surprising since an extract is probably very different from the fluid a parasite meets after it penetrates its host.

The experiments reported herein were based on the assumption that the guttation fluid from a host might be more similar to the fluid a parasite meets when the host is first penetrated, than to an extract or a press juice which has been prepared from the host. The results have in part supported this assumption.

Claviceps purpurea is a fungus parasite of rye and other cereals. Seedlings of three varieties from three host genera were tested for susceptibility to this fungus in laboratory inoculation experiments (*2*). Under these conditions 39 percent of the Rosen rye, 11 percent of the Genesee wheat, and none of the Trail barley seedlings became infected.

Guttation fluids were collected, drop by drop, from the tips of seedlings which had been grown on moist paper in metal boxes. Since guttation fluid cannot be gathered easily in large quantities, a special technique was developed for growth experiments. Generally, microscope slides, cut in half lengthwise, were dipped into a melted agar suspension of conidia. The slides were hung until the agar gelled; then they were placed in test tubes containing 0.2 ml of guttation fluid and incubated. From a set of ten slides a growth curve was obtained. Two slides were removed from the incubator every 24 hours. They were dried and counted.

Guttation fluid was collected and filtered immediately through a 0.30 Millipore filter in a Swinny hypodermic adapter. It was then stored at 4°C in Pyrex screw-top tubes. The filter and all glassware, with the exception of that used for growing cultures and for preparing the suspension of sugar and conidia, were rinsed before use in glass-distilled water. All suspensions and solutions were made with this water.

In order to produce chemically clean, sterile, hydrophilic surfaces, the slides were soaked for 2 hours in a strong solution of detergent (*3*), rinsed eight times in tap water, soaked for 10 minutes in strong NaOH, rinsed eight times in tap water, soaked 10 minutes in 50 percent H_2SO_4, rinsed 15 times in tap water, five times in sterile distilled water, and finally twice in sterile glass-distilled water. They were then removed with sterile forceps, placed with the frosted end downward in a slotted block, and used as soon as they were dry.

The conidial suspension was prepared from potato-dextrose-agar-slant cultures. The conidia were placed in a sucrose solution (2 g cp sucrose per 3 ml of 0.1 percent KH_2PO_4) and filtered through sterile cotton. If stored at 4°C these conidia remained viable for months (*2*).

Dipping vials (6.5- by 1.5-cm Pyrex tubes) containing 2.5 ml of 4 percent agar were autoclaved and transferred to a 44°C water bath. The stock co-

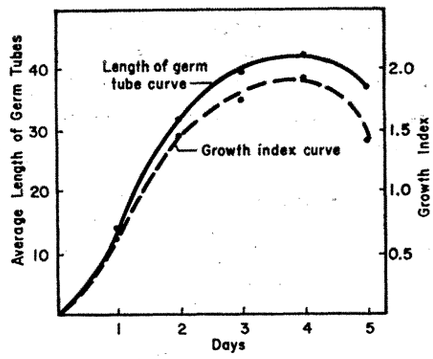

Fig. 1. Growth curves from one set of slides on which the conidia were germinated in water. The upper curve was obtained by actual measurement of the lengths of germ tubes (in micrometer units) and the lower curve by the growth index method.

nidial suspension (0.2 ml) was added to 2.5 ml of water in a screw-top tube, shaken thoroughly, warmed 20 seconds with gentle shaking in the bath, and poured into the vial which contained the agar. The agar and suspension were mixed for 20 seconds. The slides were then immediately dipped 3 cm into the suspension and hung with the dipped end downward to gel. As soon as dipping was completed the agar was scraped from the lower surface and the slides were labeled and slipped into sterile tubes containing 0.2 ml of guttation fluid. A loose roll of sterile moist filter paper was placed in the upper part of the tube above the slide, the tube was closed with a metal cap and the set of tubes was placed in an oven at 28°C. The slides were handled rapidly to prevent drying of the agar film.

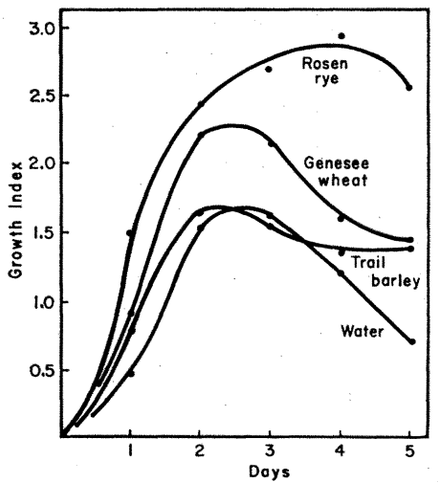

Fig. 2. Growth of *Claviceps purpurea* germ tubes in the presence of guttation fluids. Differences between treatments are: rye and wheat at 5 percent level, wheat and barley at 1 percent, barley and control at 1 percent. Susceptibility of hosts: rye, 39 percent; wheat, 11 percent; barley, none.

Growth was determined by examining 10 fields on each slide. The fields were chosen 1 cm from the lower end of the slide and at least 2 mm away from the edges. A square whose side equaled about one-third the diameter of the field was placed in the field as an ocular insert. The number of germ tubes which crossed the perimeter of the square and the number of conidia within the square were counted. Each crossing was counted regardless of the location of the conidium in the field, even though a single germ tube might be counted more than once because of position, curved growth, or branching. The growth index was computed by dividing the total number of crosses in all the fields by the total number of conidia inside the squares.

Figure 1 compares this method of determining growth with the actual measurement of the length of germ tubes in the same number of fields on the same set of slides. The curves are very similar. Since the growth index method required less than one-tenth the time, it was used in the comparative growth studies.

The guttation fluids caused a pronounced difference in growth of the germ tubes of the parasite (Fig. 2). The fluid from Rosen rye, the more susceptible host, produced the most growth; the fluid from Genesee wheat, a less susceptible host, produced less growth; and the fluid from Trail barley, the insusceptible host, produced about the same amount of growth as the water controls. Other experiments on different collections of guttation fluids and different preparations of conidia confirmed these results.

If similar results should be obtained for many other host-parasite combinations, the kind of experiment described here would be useful for testing available hypotheses (*5*) and for extending the general understanding of the host-parasite relationship. After further analyses have been completed and synthetic guttation fluids have been prepared, we may have approximated the parasite's "definitive nutrition"—that is, the complex nutritive environment which the parasite encounters once it penetrates into diffusion contact with host cells (*6, 7*).

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Cholinergic Tracing of a Central Neural Circuit Underlying the Thirst Drive

Abstract. Cholinergic stimulation of any of a number of interrelated limbic and diencephalic structures in the rat elicits a rapid and marked increase in water intake. We postulate that a generalized Papez circuit mediates the thirst drive, that the circuit is specifically and functionally sensitive to cholinergic action, and that other primary drives depend on closely parallel neural circuits partitioned both structurally and biochemically.

Recent work with chemical stimulation of the brain (*1*) has correlated application of cholinergic drugs to the perifornical region with drinking behavior, and application of adrenergic drugs to the perifornical and far lateral hypothalamic areas with eating. The data suggest that the thirst drive is partly regulated by cholinergic action, and the hunger drive, by adrenergic action.

In early investigations in this area we were concerned with the possible central action of insulin or glucagon, or both, on brain systems related to hunger and satiety. No evidence for such action was uncovered, and early work with adrenergic drugs proved inconclusive.

A series of experiments with cholinergic drugs, however, are providing evidence that the perifornical region is only one of many brain areas in which localized application of minute quantities of cholinergic agents is followed by a marked increase in drinking.

The basic experimental procedures are as follows. Adult albino or hooded rats are prepared for experimentation by stereotaxic implantation of hypodermic guide shafts. The animals are then returned to special cages that contain feeding and drinking receptacles designed to permit continual and accurate measurement of food and water