Table 2. Distribution of material with blood group A activity in influenza virus* inoculated allantoic fluid. The virus hemagglutinin titer was measured against chick erythrocytes. The blood group activity is expressed as in Table 1. Boiled aqueous suspensions of the nondialyzable part were fractionated with ethanol.

Ethanol fraction (%)	Yield (%)	Mg/ml of material which inhibits	
		Anti A_1	Anti A_2
a. Not absorbed, virus titer 1:16†			
0		5–10	2.5–5
40	57	±5	2.5
75	14	>5	1.2
90	3.5	5–10	2.5–5
>90	12	1.2	0.6
b. Absorbed with 1/3 vol. human group O erythrocytes, virus titer 1:2‡			
0		>10	5
40	67	±10	2.5–5
75	11	5	2.5
90	4	>10	5
>90	9	1.2–2.5	1.2
c. Eluate from human group O erythrocytes of b; virus titer 1:128§			
0		5	1.2–2.5
40	67	5	2.5
75	6.5	5	0.2
90	5.5	>5	±5
>90	~20	2.5–5	1.2–2.5

* Saline suspended. † Average of two experiments, 25 eggs total. ‡ Average of two experiments, 29 eggs total. § About 25 percent of nondialyzable material of part *a* was absorbed and eluted.

ments, however, it can be stated that there is no direct correlation between hemagglutinin titer of the virus and group A activity. These observations call to mind chemical findings that both influenza viruses and embryonated chicken eggs possess similar mucopolysaccharides which contain all sugars of blood group A, B and H(O) substances (*4*).

Crude in vitro active fractions of uninoculated membranes and allantoic fluid, 20 mg total for each rabbit, were given intravenously over a period of 8 days. Two of five rabbits given membrane fractions, and one of four given fluid fractions showed a significant rise in preexisting A_1 and A_2 agglutinins in sera which were obtained 1 week after the last injection. Whether the rise was true immunization or anamnestic response cannot be decided, as all responding animals had preexisting anti-A antibodies.

Of potential practical importance in relation to immunization of humans may be our finding, in the non-dialyzable portion (12 percent or less of total before the 48 hours' dialysis) of commercial influenza virus vaccines from six manufacturers, of material which inhibited anti-human blood group A agglutinins. This may be part of the virus or it may originate from embryonic structures or the diluting fluid or from all of these. The rather high activity of the unfractionated nondialyzable material (Table 1) suggests that much of the A activity may originate from the virus-diluting fluid.

Approximately 5 mg of the nondialyzable autoclaved part of commercial influenza virus vaccine was injected twice subcutaneously on two succeeding days into each of five healthy human volunteers of blood group B or H(O). These had received no injection of tissue-grown vaccines or other group A active materials during the previous 2 years. After 10 days, all subjects showed a four- to eightfold rise in anti-human blood group A_1 and A_2 titers; pre- and postimmunization sera were titrated simultaneously and a new pipette was used for each titration step dilution (*2*). The obvious potential hazards (*5*) inherent in a tissue-grown vaccine which contains even minute amounts (see also *6*) of substances similar to blood group antigens of extraneous and intrinsic origin may be significantly reduced if inoculations were performed with virus which had been suspended in fluid devoid of blood group activity (*7*), and if vaccines were made from more highly purified virus preparations. Complete elimination of blood group-like material from some tissue-grown vaccines, however, may be contingent on the antigenic composition of these tissues.

Note added in proof: Three additional volunteers of blood group O gave a similar increase of anti-A agglutinins when injected subcutaneously with one-fifth of the amount previously used.

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Partitioning of Body Fluids in the Lake Nicaragua Shark and Three Marine Sharks

Abstract. The relative volumes of major body fluids of freshwater and marine sharks are remarkably similar in spite of the differences in external medium and in osmotic pressure of body fluids. The small differences detected are in agreement with differences reported in comparisons of freshwater and marine teleosts: a slightly higher total water content and a smaller ratio of extracellular to intracellular fluids in freshwater forms.

Whereas there is a somewhat limited literature on the physiology of marine elasmobranchs, very little has been reported on the physiology of forms adapted to fresh water. Since the work of Homer Smith on freshwater elasmobranchs in the 1920's and early 1930's (*1*), the physiology of these animals has apparently been completely neglected. The ready availability of the Lake Nicaragua shark, *Carcharhinus nicaraguensis* (*2*), makes it a good subject for studies on the peculiar osmoregulatory phenomena encountered in elasmobranchs, yet, not a single reference is available on the physiology of this widely known freshwater selachian (*2a*). Most of the reports on the osmoregulation of elasmobranchs have concerned the urea retained in high concentrations, trimethylamine oxide, and the ionic concentration of body fluids. There are appreciable differences between marine and freshwater species in the concentrations of these solutes (*1*). It was thought that further clarification of the osmoregulation of elasmobranchs might be attained by comparing the body-water content and its apportionment among the various ma-

jor fluid-containing compartments in marine and freshwater sharks.

The freshwater species of choice was *C. nicaraguensis* (family Carcharhinidae) because this is the only shark known to occur relatively permanently in a freshwater environment. Claims and counterclaims notwithstanding, it is not known whether these sharks remain in Lake Nicaragua throughout life or whether they move up and down the Rio San Juan between the lake and the Caribbean Sea (*3*). In any case, my work was carried on at Granada (*4*) at the northeast end of Lake Nicaragua, about 100 miles from the point where the river drains the lake. This, in turn, is well over 100 miles (by river channel) from the Caribbean. Therefore, sharks occurring at the northeast end of the lake, even if relatively newly arrived from the sea, must be quite well adapted to the freshwater situation.

The ideal marine species for comparison with the lake population would be *Carcharhinus leucas,* the closest relative of *C. nicaraguensis* if not the same species. To date comparison with *C. leucas* has not been possible, but specimens of the lemon shark, *Negaprion brevirostris* (also a member of the Carcharhinidae), and the nurse shark, *Ginglymostoma cirratum* (family Orectolobidae), were obtained at the Lerner Marine Laboratory, Bimini, Bahamas (*5*). In addition, data on the spiny dogfish, *Squalus acanthias* (family Squalidae), are available from previous work for comparison (*6*).

Details of the investigational methods used were reported in 1958 (*6*). In brief, the sharks were anesthetized with a narcotic known commercially as M.S. 222 (*7*). The total water content was determined by complete desiccation of representative portions of the animals at 105°C after other measurements had been completed. The volume of plasma was measured by dye dilution (T-1824), the volume of extracellular fluid by sucrose dilution. The volume of whole blood was determined from the volume of plasma and the hematocrit reading. The value derived by subtracting the T-1824 space from the sucrose space was taken as an approximation of the volume of interstitial fluid, including lymph; the value for sucrose space was subtracted from that for total body water to arrive at an estimate for the volume of intracellular fluid. Admittedly these methods of measurement are crude, especially that for estimating the volume of intracellular fluid, but in the absence of better methods, they serve for purposes of comparison. Since in the time employed (3 or 4 hours) sucrose does not penetrate the minor compartments, such as those containing coelomic, ocular, and cerebrospinal fluid, these fluids are included in the volumes given for intracellular fluid rather than in those for extracellular fluid, where they belong. However, none of these fluids is plentiful in the species of sharks studied, and in no case would they normally account for as much as 0.5 percent of the body weight.

Examination of the data in Table 1 discloses a high degree of consistency for the three marine species with respect to the values for volume of fluid, even though the three species represent three quite divergent families. Indeed, they represent two suborders, the family Squalidae belonging to the suborder Squaloidea and the families Orectolobidae and Carcharhinidae, to the suborder Galeoidea (*8*). More remarkable than the uniformity among taxonomic groups is the similarity between findings for the marine species and the freshwater *Carcharhinus nicaraguensis.* It has long been known that marine elasmobranchs retain sufficient urea to bring the internal osmotic pressure up to, or slightly above, the pressure of the external medium. On movement into fresh water the internal osmotic pressure drops, due to a reduction of salts and especially of urea, but the body fluids are still greatly hypertonic relative to the external medium (*1*). Consequently, the maintenance of water balance would appear to be a more acute problem in the freshwater environment. With the drop in the concentration of solutes in the body fluids of freshwater elasmobranchs, one might look for marked shifts in the total water content and in the distribution of water among the fluid-containing compartments. However, no such shifts are clearly evident. The freshwater shark has a slightly higher water content than the marine species, and the relative volumes of intracellular and extracellular fluid are a little higher

Table 1. Comparison of data on body characteristics and distribution of fluids in fresh-water Lake Nicaragua shark and in three species of marine sharks. All fluid volumes are expressed as mean percentage of body weight (italic type), followed in parentheses by the number or animals. Ranges are given in parentheses in the second line of each entry. Standard deviations (s.d.) are given for primary measurements of fluid parameters.

Parameter	Fresh-water Carcharhinidae, *Carcharhinus nicaraguensis*	Marine species			
		Carcharhinidae, *Negaprion brevirostris*	Orectolobidae, *Ginglymostoma cirratum*	Squalidae, *Squalus acanthias*	Combined marine sp.
Weight (kg)	*48.07* (10) (27.7–57.2)	*6.40* (9) (3.18–12.27)	*16.03* (5) (11.36–22.70)	*2.63* (33) (1.12–6.35)	
Length (in.)	*74* (10) (63–81)	*41* (9) (33.5–51.0)	*56.3* (5) (53–61)	no data	
Pulse (beats/min)	*12.2* (10) (8–18)	*26* (9) (20–32)	*22* (5) (16–24)	*31* (14) (18–40)	
Hematocrit (% cells)	*22.8* (10) (15–30) s.d., 4.46	*21.5* (9) (18–25) s.d., 1.12	*17.4* (5) (12–22) s.d., 2.93	*18.2* (25) (14–24) s.d., 2.76	
Spec. grav., plasma	*1.026* (3) (1.025–1.027)	*1.027* (3) (1.025–1.028)	*1.028* (3) (1.027–1.029)	no data	
Spec. grav., blood	*1.044* (3) (1.043–1.045)	*1.045* (3) (1.043–1.047)	*1.046* (3) (1.044–1.048)	no data	
Plasma volume (T-1824 space)	*5.1* (10) (4.3–6.8) s.d., .71	*5.4* (9) (5.0–6.0) s.d., .34	*5.7* (5) (4.7–6.6) s.d., .6	*5.5* (24) (2.5–9.0) s.d., 1.4	*5.53*
Blood volume	*6.8* (10) (5.4–9.1) s.d., 1.12	*7.0* (9) (6.5–7.9) s.d., .42	*6.8* (5) (5.8–7.6) s.d., .73	*6.8* (24) (3.1–10.9) s.d., 1.79	*6.86*
Extracellular fluid (sucrose space)	*19.7* (8) (17.1–21.8) s.d., 1.54	*21.2* (8) (18.8–23.5) s.d., 1.34	*21.9* (4) (17.8–24.5) s.d., 2.48	*21.2* (3) (18.5–24.3) s.d., 2.38	*21.43*
Interstitial fluid (sucrose space minus plasma)	*14.6*	*15.8*	*16.2*	*15.7*	*15.9*
Total body water	*72.1* (4) (71.0–73.1) s.d., .75	*71.1* (6) (70.9–71.9) s.d., .34	*71.7* (3) (71.2–72.1) s.d., .38	*71.7* (16) (68.4–75.4) s.d., 1.9	*71.5*
Intracellular fluid (total water minus sucrose space)	*52.4*	*49.9*	*49.8*	*50.5*	*50.06*

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-