volved in β-alanine metabolism was very close to the actual concentration of the amino acid in the liver. It therefore seems likely that the overall oxidation of β-alanine in the liver would be very sensitive to changes in the intracellular concentration of the amino acid, at least partly determined by the uptake rate which would itself be very sensitive to changes in the concentration of β-alanine at the transport site. In the incubated liver slices used by King et al, the medium β-alanine concentration was held constant at 0.1 mmol $\ell^{-1}$. In this case, the much expanded extracellular space in tissue from skates adapted to 50% seawater may be expected to pose less of a restriction on the access of β-alanine in the incubation medium to all the available transport sites as compared to that in the tissue from fish kept in normal seawater. Hence the physical characteristics of the tissue, when incubated as a slice, may have led to changes in the actual concentration of β-alanine at the transport site and hence, for the reasons described above, to oxidation rate. In vivo small increases in plasma β-alanine concentration may be expected to produce relatively large increases in the oxidation rate of this amino acid in the liver and may thus form the basis of an increased metabolism of β-alanine by the liver of skate on exposure to a dilute environment. This work was supported by grants from the Nuffield Foundation (to T.J.S.) and Whitehall plus National Science Foundations (to L.G.).

## CRYOGENIC ACTIVITY IN THE URINE OF DOGFISH SHARK, SQUALUS ACANTHIAS

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The urine of human beings, rabbits, and dogs contains a substance that lowers body temperature (Herringham, J. Pathol. Bacteriol. 6:158, 1900; Kluger et al, Am. J. Physiol. : R271, 1981). This endogenously produced cryogenic substance, "endogenous cryogen" (EC), results in a regulated fall in body temperature. Injection of EC into rabbits results in peripheral vasodilation, a decrease in metabolic heat production, and an increase in respiratory rate. During heat stress the amount of EC that is excreted by resting human subjects decreases (Kluger et al, Fed. Proc. 41:976, 1982). We hypothesized that this decreased excretion results in an increased plasma concentration of EC, and as a result, part of the thermoregulatory responses designed to lower body temperature during heat stress could be triggered by this increase in EC. It is also possible that many cyclical changes in body temperature (e.g. circadian changes, cyclical fevers, etc.) are regulated by the internal concentration of EC.

Other than the appearance of EC in the urine of the mammals listed above, nothing is known about the comparative biology of EC. For example, do nonmammalian vertebrates produce and excrete a cryogen in their urine? In this report we present preliminary data indicating that the urine of the dogfish shark contains a substance that lowers deep body temperature and induces peripheral vasodilation in rabbits.

MATERIALS AND METHODS--Experimental Animals. Female sharks, Squalus acanthias, weighing 3.4 to 6.8 kg were collected off the coast of Maine and maintained in fresh seawater at 14 to 16°C at the Mount Desert Island Biological Laboratory. To bioassay for the presence of EC, male New Zealand white rabbits weighing approximately 2.8 to 3.3 kg were used in these studies. The rabbits were maintained on a diet of Teklad rabbit chow and water ad libitum.

Urine Collection.--Large balloons (Smurf) were washed 10 times with tap water and then 3 times with sterile pyrogen-free 0.9% sodium chloride. A piece of polyethylene tubing (ca. 15 cm) was inserted approximately 3 cm into the opening of the balloon and tied in place with cotton thread. To insert the free end of the catheter into the urinary tract each shark was held out of the water by its tail with the anterior 1/3 still in the holding tank. The distal 3 mm of the renal papilla was cut and the catheter inserted about 3 cm and tied in place. The catheter was also sutured to the tail of the shark about 5 cm posterior to the renal papilla, and the shark released into the holding

tank. The balloons were emptied once/day by insertion of an 18 ga. sterile needle connected to a 50 ml sterile syringe through the distal end of the balloon wall. Daily collections of urine varied between 16 and 132 ml/shark. The needle was then removed and the needle hole closed with a cotton ligature. All urine was stored at -20°C until used in experiments.

To confirm that the urine collected was sterile, a small sample was plated onto sheep blood agar. This confirmed that no viable bacteria were present.

Chloroform Extraction of Urine.--Urine was mixed and shaken with an equal volume of chloroform in a separatory funnel and then allowed to stand until the aqueous fraction could be easily collected. This aqueous fraction was then either put in a dessicator or had $N_2$ bubbled through it for several hours to get rid of residual chloroform, and then stored at -20°C until tested for cryogenicity.

Bioassay of Urine for Cryogen.--Rabbits previously implanted with catheters in their jugular veins (Kluger et al, Am. J. Physiol. 241:R271-R276, 1981) were placed in standard rabbit stocks in a temperature controlled chamber set at 17°C. Rectal and ear skin temperature were monitored and recorded using copper-constantan thermocouples connected to a Digistrip-III 32 channel datalogger (Kay Instruments). The intravenous catheters were cleared with sterile pyrogen-free saline and after at least a 1 hour period to allow each rabbit to reach steady-state body temperature, injections of various solutions were made through the intravenous lines. Rectal and ear skin temperature were recorded for at least 90 minutes post-injection.

RESULTS--Injection of 10 ml and 20 ml of pooled shark urine into 6 rabbits resulted in a small but statistically insignificant fall in rectal temperature ($0.05 < p < 0.10$). Since urine contains significant amounts of lipid soluble substances known to be fever inducers, we next attempted to remove these pyrogens by chloroform extraction. We hypothesized that by removing these pyrogens we would unmask any possible cyrogenic component. Injection of the water soluble fraction of chloroform extracted shark urine into 4 rabbits resulted in a fall in rectal temperature of 0.38°C and a rise in ear skin temperature of 8.56°C within 5 minutes ($P < 0.01$). Injection of rabbits with chloroform extracted saline, (controls) resulted in no change in rectal or ear skin temperature.

To ensure that all the cryogenic activity was not coming from one shark, chloroform extracted urine from 4 individual sharks was injected into groups of 3 to 4 rabbits. The results of these experiments indicated that cryogenic activity was present in the urine of 3 of the 4 sharks.

We have begun experiments to determine the molecular weight of the cryogens present in the urine of sharks. Based on hollow fiber dialysis, preliminary data indicate that the cryogen is greater than 5,000 daltons.

DISCUSSION--Shark urine contains a substance that when injected into rabbits results in both a fall in rectal temperature and a rise in ear skin temperature. These are identical responses to those observed after injection of human urine into rabbits. Whether the cryogenic activity is attributable to the same or similar molecule in both groups of vertebrates is currently being investigated. It is not known whether there is any biological significance to the presence of cryogens in fish urine; however, the presence of cryogens in both mammals and fishes would support the hypothesis that these substances have some biological function, perhaps in regulating body temperature.

In future experiments we hope to determine whether injection of fish cryogens into fish would result in their behavioral selection of a cooler habitat. In addition, we plan to determine whether changing the temperature of fish (e.g. placing fish into a warm or cool tank) alters the urinary excretion of these cryogens. We thank Dr. Richard Malvin for his many helpful suggestions and Sharon and Hilary Kluger for their assistance in collecting shark urine. This research was supported in part by NIH GM 28242.