Table 1. Reduced glutathione levels (GSH), packed cell volumes (PCV), and hemoglobins (Hb) of normal and glutathione-deficient sheep. The GSH was determined by DTNB method (*4*), PCV by microhematocrit, and the hemoglobin by the cyanmethemoglobin method. Values are means and, for normal sheep, ± standard deviation.

No. of sheep	GSH (milligrams per 100 ml of RBC)	PCV (%)	Hb (grams per 100 ml)
	Normal sheep		
101	104.0 ± 27.2	34.7 ± 5.8	12.6 ± 2.2
	Glutathione-deficient sheep		
	10.5	32.3	10.9
	19.3	34.2	11.8
	4.5	30.6	10.5

During investigations of the concentration of GSH in sheep blood, we recently found three ewes with a marked decrease in erythrocyte GSH (Table 1). The lowered GSH levels were determined by both the 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (*4*) and "Alloxan 305" (*5*) methods. This decrease has been seen for over 2 months and is unaccompanied by a readily apparent hemolytic disorder. The blood copper, glucose-6-phosphate dehydrogenase, glutathione reductase, serum glutamic pyruvic transaminase, blood urea nitrogen, sulfobromophthalein clearance, GSH stability, and bilirubin are all within the normal range for this species. Two animals have hemoglobin type A and one has type AB, and all have a low concentration of red cell potassium. The three ewes have delivered six lambs, one of which died before the GSH determination could be performed. Of the five remaining lambs, one has a GSH value (28.2 mg/100 ml of erythrocytes) that is approximately the same as his dam (19.3 mg), while his twin has a normal level. The other lambs have concentrations that are in the normal range for their age.

These limited genetic studies do not offer any definite evidence of the genetic transmission of this disorder. It is possible that all three ewes are homozygous and that the heterozygotes do not have a lowered erythrocyte GSH, as is the case in man (*4*). On the other hand, the three animals could be heterozygotes, and the lambs could be either homozygous or heterozygous for the normal GSH level. These animals should be useful in delineating the role of reduced glutathione in erythrocyte metabolism and drug sensitivity.

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Potamotrygon spp.: Elasmobranchs with Low Urea Content

Abstract. *All previously reported species of Chondrichthyes, from both marine and fresh water, have contained urea at concentrations ranging from about 300 to 1300 milligrams of urea nitrogen per 100 milliliters of fluid. Body fluids from two species of* Potamotrygon, *permanent residents of the Amazon basin, contained only 2 to 3 milligrams of urea nitrogen per 100 milliliters. Although they have abandoned the retention of urea exhibited by other chondrichthyans, the extent to which they have lost the mechanisms of retaining and tolerating urea in a hypertonic medium has not been determined.*

Staedeler and Frerichs (*1*) first demonstrated that the blood and other body fluids of chondrichthyans contain a concentration of urea far above that retained by most other vertebrates. Unusually high concentrations of urea have also been shown in a few other vertebrate species, notably the aestivating African lungfish, *Protopterus aethiopicus* (*2*), the crab-eating frog, *Rana cancrivora* (*3*), and the coelacanth, *Latimeria chalumnae* (*4*). However, the class Chondrichthyes is the only large vertebrate taxon in which retention of urea has, until now, appeared to be universal. All marine species studied to date, both elasmobranchs and chimaeroids, have contained urea nitrogen in the range of approximately 750 to 1300 mg/100 ml (*5*). It has also been shown (*6*) that trimethylamine oxide (TMAO) is present in unusually high concentration in the blood and tissues of elasmobranchs (about 80 to 120 mmole/liter).

Urea concentrations have been reported for elasmobranchs occurring in freshwater by Smith (*7*), Urist (*8*), and Thorson (*9*). In general, all three papers reported a reduction to approximately 25 to 35 percent of the urea concentrations in marine elasmobranchs. In all cases, the freshwater animals studied were probably of marine or brackish-water origin, or, in any case, had access to the sea. Smith's work dealt with the sawfish *Pristis microdon*; the shark *Carcharhinus melanopterus*; and two rays, *Dasyatis warnak* and *Hypolophus sephen*, about 40 miles up the Perak River in the Federated Malay States (now Malaysia). Urist studied the shark *Carcharhinus leucas* of Lake Nicaragua, and Thorson studied *C. leucas* and the sawfish *Pristis perotteti*, both from Lake Nicaragua. This lake is drained into the Caribbean Sea by a large, broad river, the Río San Juan, whose rapids probably do not, as often claimed, restrict the passage of sharks and sawfish (*10*). These elasmobranchs have probably come from the sea, or at least are of very recent marine ancestry. The fate of the urea following return of the freshwater animals to the sea has not yet been determined, but it is reasonable to assume that it would return to the marine concentration, probably quite rapidly.

Since the only study of osmoregulation in freshwater elasmobranchs has involved species whose length of residence in freshwater was unknown, but probably brief, it appeared pertinent to study elasmobranch species known to live permanently in freshwater a long distance from the sea. These species would, therefore, have experienced a protracted absence from the hypertonic medium which apparently elicited the retention of urea in the Chondrichthyes. Such animals are the sting rays of the family Potamotrygonidae (the genera *Potamotrygon, Elipesurus*, and *Disceus*), which occur commonly in South America, particularly in the Orinoco and Amazon drainage systems, as much as 4000 or 4500 km from the sea. They have most likely been there for at least many

thousands of years, although the history of their occurrence appears to be unknown.

In June 1967, we procured seven living river rays of the genus *Potamotrygon* (*11*). Five, identified as *P. motoro*, were taken near the town of Atalaya on the Yavarí River; two, tentatively identified as *P. circularis*, were taken in the Tichito River in extreme western Brazil. Both locations are approximately 3200 km from the mouth of the Amazon River (*12*). That the rays breed here is attested to by the occurrence in one female *P. circularis* (?) of four embryos with disks about 1 inch in diameter. Four other females (*P. motoro*) contained eggs which apparently were not yet fertilized. Only one of the seven (*P. motoro*) was a male. The disks of five specimens of *P. motoro* averaged 46 cm long and 42.5 cm wide and had an average weight of 5.4 kg. The disks of two specimens of *P. circularis* (?) averaged 60.0 cm long and 55.6 cm wide. The only one whose weight was determined weighed 12.27 kg.

Samples of serum, perivisceral fluid, cranial fluid, and pericardial fluid were kept frozen in a freezer or on dry ice until analyzed, except during initial processing, which required several hours at ambient temperature for clot separation and centrifugation, and during the 6-hour trip from Leticia to Bogotá, Colombia, when they were kept on ice in a styrofoam box. Total time from the first sampling to the beginning of analysis in our Lincoln laboratory was 8 days. Sodium, potassium, and calcium were determined by flame photometry; magnesium by the titan yellow method (*13*); chloride by mercuric nitrate titration (*14*); inorganic phosphorus by the method of Fiske and Subbarow (*15*); total protein by the biuret method (*16*); trimethylamine by the method of Dyer *et al.* (*17*); and urea nitrogen by the diacetylmonoxime method (*18*). The procedure for the determination of urea was checked several times with the Nessler reaction, which would also include the breakdown products of hydrolyzed urea. The results were virtually identical. The urea method was further checked with known quantities of urea as well as with fluid samples from other elasmobranchs having high concentrations of urea, and we have full confidence in the reasonable accuracy of our data. As there appeared to be no appreciable difference between the two species, the data are combined in Table 1.

Detailed comparisons with marine and freshwater rays can be made by consulting Bernard, Wynn, and Wynn (*19*) and Smith (*7*), respectively. In general, the osmolality and inorganic ion content are lower than those of other freshwater rays and are in approximately the range for most teleosts. Total protein is likewise somewhat lower than values reported for other elasmobranchs, and trimethylamine oxide was not found in the single determination attempted.

The most striking finding was the nearly complete absence of urea, which ranged from 2 to 3 mg of urea nitrogen per 100 ml in all fluids examined. In no individual sample did it exceed 5 mg/100 ml, a value even lower than that for mammals and many other vertebrates. A low concentration of urea might have been predicted in elasmobranchs that appear to have lived in freshwater for at least thousands of generations. However, such an extreme reduction would hardly have been anticipated in a group that has so universally and for such a long time depended upon urea retention for a major part of its osmoregulation. Apparently every feature of the metabolism of marine elasmobranchs has become adapted to functioning in the presence of high concentrations of urea in the body fluids. This tolerance to urea has been retained by the species of freshwater elasmobranchs previously studied. Indeed, the presence of urea is probably essential to the normal functioning of most physiological systems of marine elasmobranchs. It would appear then that basic changes in many facets of elasmobranch physiology would be involved in reverting to the virtual absence of urea in the internal milieu, particularly those related to the permeability of the gill epithelium to urea, the secretory activity of the kidney tubules, and enzymatic activities throughout the body.

The possibility exists that *Potamotrygon* has not actually lost its ability to tolerate and function in the presence of high urea levels. Although it seems unlikely, this genus may simply have a very wide range of tolerance to urea, and, if exposed to a high external salt concentration, would call on its ancestral osmoregulatory mechanism and increase its internal osmotic concentration by the accumulation of urea.

The evolutionary history of the batoid elasmobranchs is not well documented, and, in particular, the history of the occurrence of sting rays in freshwater is virtually unknown. It is therefore impossible to state positively that the rays under study were derived from marine ancestors that possessed mechanisms for retaining urea. The possibility exists, although remotely, that they descended from ancestors that did not leave freshwater, as the class Chondrichthyes as a whole

Table 1. Summary of data on body fluid chemistry of *Potamotrygon* spp. Number refers to the number of samples that were analyzed.

Value	Ions (meq/liter)					Concentration (mg/100 ml of fluid)				Osmolality (mosmole)
	Na	K	Ca	Mg	Cl	P	Total protein	Urea nitrogen	TMAO	
					Serum					
Average	150.1	5.9	7.2	3.55	148.8	6.7	1800	3.0	0	308.2
Number	6	6	6	6	6	5	6	6	1	5
High	161.5	8.3	11.4	4.86	162.0	8.5	2300	5.0	0	320.0
Low	140.5	4.6	5.1	2.83	131.0	5.5	1100	2.0	0	301.0
					Perivisceral fluid					
Average	102.8	6.2	2.9	3.08	136.5	1.7	*	2.0		293.0
Number	4	4	3	2	4	2		3		1
High	114.5	12.1	3.5	3.22	183.0	2.4		4.0		
Low	88.0	3.8	2.61	2.94	69.0	1.0		0.0		
					Cranial fluid					
Average	147.0	4.6	4.1	2.61	147.0	5.9	~100	2.0		289.0
Number	2	2	3	3	3	2	1	3		1
High	150.0	5.0	5.1	3.42	162.0	6.0		2.0		
Low	144.0	4.1	3.4	1.94	133.0	5.7		2.0		
					Pericardial fluid					
Average	113.8	3.0	0.2	0.54	179.5	0	*	2.5	0	323.5
Number	2	2	2	2	2	2		2		2
High	116.0	3.6	0.2	1.08	180.0	0		4.0		338.0
Low	111.5	2.5	0.2	trace	179.0	0		1.0		309.0

* No precipitate with trichloroacetic acid.

did, and thus never developed urea retention.

In either case, freshwater river rays now provide excellent subjects for the study of urea metabolism and elasmobranch osmoregulation in general.

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11. We acknowledge the cooperation of M. Tsalickis and G. Tsalickis, Leticia, Colombia; Dr. W. Colwell, University of Nebraska Agricultural Mission in Colombia; Dr. S. Fonseca, National University of Colombia; and Dr. G. S. Myers, Stanford University.
12. The first location is on the border between Peru and Brazil about 40 km from the confluence of the Yavarí (Javary) and the Amazon; the second is about 152 to 160 km south-southwest of Leticia, Colombia (by river), about 8 km from the confluence of the Tichito and the Itacuai. The latter flows into the Yavarí, which in turn joins the Amazon.
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Virus-Induced Peliosis Hepatis in Rats

Abstract. *Inoculation of newborn Fischer, W/Fu, or Lewis rats with the 9H virus recently isolated from leukemic rat tissues resulted in the development of peliosis hepatis. The virus was recoverable from the peliotic livers. The cystic lesions were of the parenchymal peliosis type and appeared to be the result of focal hepatic necrosis followed by hemorrhage.*

The entity peliosis hepatis is characterized by blood-containing cystic lesions in the liver. A number of cases occurring in man, most frequently in patients with pulmonary tuberculosis, have been described. It also has been observed in patients with other chronic wasting diseases, such as cancer, and in patients receiving androgens therapeutically (*1*). Several investigators have observed what appear to be peliotic lesions of the liver, spleen, and adrenal glands in mice after transplantation of ovarian or testicular tumors (*2*). A similar lesion also occurs spontaneously in animals, particularly cattle, and is referred to as "telangiectasis of the liver" (*3*).

The etiology and mechanisms of development of peliosis hepatis are unknown, although several theories have been proposed (*1*, *4*). To our knowledge, a viral cause of this disease has neither been previously demonstrated nor proposed in animals or humans. We now describe the induction of peliosis hepatis in rats with the 9H virus recently isolated from the same species (*5*).

The virus strain used in our experiments was extracted from leukemic liver and spleen cells maintained in vitro. The cells and supernatant of the culture were frozen and thawed three times and then centrifuged at 10,000*g* for 10 minutes. The resulting supernatant was filtered through a Millipore filter impermeable to bacteria (0.45 μ). The filtrate was centrifuged at 73,000*g* for 1 hour. The resulting pellet was again suspended in an amount of Hanks' salt solution containing 2 percent calf serum (HCS) which represented one-tenth of the original volume of the culture fluid. This material was negative when tested (*6*) for mycoplasma. Upon inoculation of a line of rat embryo cell cultures (REL) (*5*), hemagglutinins appeared in the supernatant, and cytopathic effects became evident within 5 to 7 days. Intraperitoneal inoculation into 15 newborn Fischer rats produced disease in four animals, with clinical symptoms as described below. Three more consecutive passages were made in Fischer rats with pooled liver-spleen extracts of the diseased rats.

To compare the incidence and pathology of the disease in different strains, as well as to attempt to recover the virus in vitro, we injected Fischer, W/Fu, and Lewis rats with liver extracts of the fourth Fischer rat passage. The extracts were prepared as follows: The tissue was weighed and homogenized with alundum and HCS, as a 20 percent (by weight) suspension. Coarse debris and alundum were removed by low-speed centrifugation. The extract was subsequently treated with high-frequency sound by means of a Raytheon 10-kc oscillator for 1 minute. It was partially purified by centrifugation at 2400*g* for 20 minutes and then centrifuged at 105,000*g* for 1 hour. The resulting pellet was again suspended in an amount of HCS equivalent to 0.5 g per milliliter of the original weight of tissue. This concentrated extract did not agglutinate guinea pig erythrocytes; however, the presence of the virus was ascertained when eight REL cell cultures, inoculated with the extract, developed typical cytopathology. Hemagglutinins also appeared in the culture supernatant within 7 days. Other samples of the extract were injected intraperitoneally (0.1 ml) into newborn rats. Thirty W/Fu rats (100 percent), 30 of the 36 Fischer rats (83 percent), and 11 of the 13 Lewis rats (85 percent) inoculated with the extract became ill and moribund within 12 to 30 days. Thirty control rats, consisting of untreated animals and rats inoculated with heated extract (86°C for 30

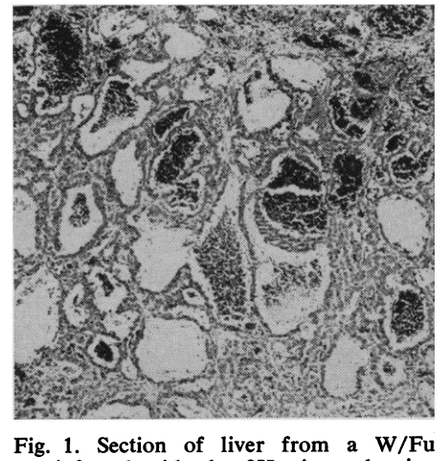

Fig. 1. Section of liver from a W/Fu rat infected with the 9H virus, showing multiple blood-containing cysts of various sizes lined by hepatic cells; hematoxylin-eosin stain. (× 48)