current theory, which is sustained by considerable evidence, this lake has been formed out of an open bay through upheaval of this part of New Guinea by tectonic forces. Volcanism is not known to have occurred in this region.

During our recent visit to Netherlands New Guinea (October 1954–May 1955), we succeeded in obtaining from Lake Santani two large sawfishes that measured 9½ and 11 feet. With the invaluable help of the Royal Netherlands Navy, we were able to ship these voluminous specimens to the Rijksmuseum van Natuurlijke Historie at Leiden. Because only small specimens or just saws have generally been collected and preserved, this was a very rare and valuable addition to our collections, much more remarkable than the occurrence of these specimens in fresh water.

After a provisional examination, we decided that both specimens seem to belong to *Pristis microdon* Latham, a species known to be at home in fresh water as well as in brackish and salt water. Specimens may occur far upstream in large rivers, and probably not only as occasional stragglers. For some species, there are strong indications of breeding in fresh water, a well-known habit of the specimens in Lake Nicaragua. For further data I refer to a previous rectification by A. W. C. T. Herre [*Science* **122**, 417 (1955)].

Because we did not have the opportunity to investigate closely the conditions in the upper reaches of the effluent river, it still remains uncertain whether sawfishes may venture so far upstream as to reach Lake Sentani. However, although conditions may prevent the intrusion of specimens of the size we collected, it seems likely that small examples can make the journey, at least under favorable circumstances—for example, in the rainy season.

Reconsidering the adaptability to salt and fresh water, and the probable existence of a passable connection between Lake Sentani and the sea, the occurrence of sawfishes in this lake can easily be explained without using the theory of gradual upheaval and gradual replacement of salt water by fresh water. I should be more inclined to adopt this theory for the explanation of the occurrence of various other species that belong to essentially marine groups—for example, jacks (Carangidae)—which do not usually invade fresh water by free will. On the other hand, fishes from marine groups with little adaptability for fresh water were found—for example, in the Digoel River near Tanah Merah, about 450 miles from the sea, in a region where obviously no gradual upheaval and consecutive gradual replacement of previously salt water has taken place.

Small sawfishes are also said to occur in the rivers near Genjem, about 15 miles west of Lake Sentani, but none could be obtained. Further specimens belonging to a different species were collected in the Digoel River near Tanah Merah.

Sharks were not collected in, or reported from, Lake Sentani, and they probably do not exist there. The only fresh-water sharks we obtained were found in Lake Jamoer, a rather large and almost circular lake (diameter approximately 5 miles) situated on the narrow neck of the Vogelkop Peninsula (longitude 135°E). The altitude is about 200 feet. The physical characteristics of the effluent river Omba are insufficiently known, which makes it at present impossible to establish with certainty whether the species is landlocked. The collected examples measure up to 5 feet and, according to a superficial examination, are closely related to the landlocked shark from Lake Nicaragua and to the Ganges shark.

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Inhibition of Audiogenic Seizures by Carbon Dioxide

In previous publications, it has been shown that the acid-base balance plays a role in the susceptibility of rats and mice to audiogenic seizure (*1*, *2*). Acidosis, as determined by blood *p*H measurements (*1*) was produced by a carbonic anhydrase inhibitor (acetazoleamide). This effect was correlated with diminished seizure susceptibility. Alkalosis, produced by injections of subtetanic doses of sodium bicarbonate, conversely increased the number of seizures. Presumably, the observed inhibition of audiogenic seizures was due to the accumulation of carbon dioxide and the presence of acidosis in the animals (*3*). However, since acetazoleamide is a drug with rather diffuse actions, it seemed desirable to determine the effect of carbon dioxide without any injected agent on the susceptibility of rats to audiogenic seizures.

The audiogenic seizure apparatus used has been described elsewhere (doorbell method, *1*). A rubber tubing inlet was provided in the lid of the seizure chamber to allow the introduction of gases. Mixtures of gases prepared consisted of carbon dioxide and air, with enough oxygen added to raise the oxygen concentration to between 20 and 21 percent. The gas delivery rate into the seizure chamber was approximately 8 lit/min.

Rats were placed in the seizure chamber 2 minutes before the bell was turned on, and the various mixtures of CO_2 were run into the chamber during this time. Actual CO_2 levels at the end of 2 minutes in the seizure chamber were determined by the Haldane method (*4*). Stimulation time was 1.5 minutes, with CO_2 administration being continued throughout.

Table 1. Inhibition of audiogenic seizures by various levels of carbon dioxide. There were 24 trials in each series.

CO_2 treatment (%)	Seizures	Percentage of seizures (CO_2 rats/controls)
2.09	14	77.7
Controls	18	
4.79	8	42.1
Controls	19	
8.91	2	11.8
Controls	17	
13.55	0	0
Controls	20	

The rats were males of the Wistar strain weighing 200 to 400 g.

All rats used in this study were originally chosen for their seizure susceptibility. The rats were divided into two groups for testing the effects of the individual levels of CO_2. One group received CO_2 at a given level on the first trial, and the other group served as controls; air was delivered into the chamber for an equal period of time. Five days later, the groups were reversed for the second trial, the former controls receiving CO_2 at the same level and the former CO_2 rats acting as controls.

A seizure was taken as a convulsion, usually clonicotonic. If a convulsion did not occur, a seizure was not counted.

Table 1 shows the gas level-response data that were obtained by administering various concentrations of CO_2 to seizure-susceptible rats and testing for seizures after 2 minutes. The CO_2 levels represent actual concentrations in the seizure chamber as determined by Haldane analysis. The various levels from top to bottom were obtained by running 2.5, 5, 10, and 20 percent CO_2 into the seizure chamber for the specified time interval. For the control rats, it was found that 0.235 percent CO_2 was present in the seizure chamber. It is apparent that CO_2 levels as low as 2.09 percent exhibit a slight effect and that 13.55 percent CO_2 resulted in complete inhibition of seizures. It is interesting to note that 8.91 percent CO_2 resulted in exactly the same values as injected acetazoleamide at 200 mg/kg; the latter values were obtained from a previous study (*1*).

In other studies on the effect of acid-

base balance on the excitability of the central nervous system, convulsions were generally induced by drugs or electric shock. In some cases, anesthetized preparations were used (*5*). The present work and previous studies on audiogenic seizures (*1, 2*) confirm that CO_2 accumulation tends to depress central nervous system excitability and show that this is true in the intact animal.

It has been postulated that the anticonvulsant action of acetazoleamide is due to direct brain carbonic anhydrase inhibition (*6*). There is little doubt that acetazoleamide does inhibit brain carbonic anhydrase, however, in view of increasing evidence suggesting respiratory acidosis resulting from blood carbonic anhydrase inhibition after administration of large doses of acetazoleamide (*3, 7*); it seems premature to ascribe the anticonvulsant effect of acetazoleamide to such a localized action.

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References

1. W. G. Mitchell and E. Ogden, *Am. J. Physiol.* 179, 225 (1954).
2. B. E. Ginsburg, *Proc. Assoc. Research Nervous Mental Disease* 33, 39 (1954).
3. W. G. Mitchell and R. C. Grubbs, unpublished data.
4. W. M. Boothby and I. Sandiford, *Laboratory Manual of the Technic of Basal Metabolic Rate Determinations* (Saunders, Philadelphia, 1920).
5. J. G. Dusser de Barrene and W. S. McCulloch, *J. Neurophysiol.* 2, 314 (1939); J. G. Dusser de Barrene, *Am. J. Physiol.* 124, 631 (1938); G. H. Pollock, *J. Neurophysiol.* 12, 315 (1949); I. H. Wang and R. R. Sonnenschein, *ibid.* 18, 130 (1954).
6. J. G. Millichap, L. D. Thatcher, P. M. Williams, *Federation Proc.* 14, 370 (1955); J. G. Millichap and D. M. Woodbury, *J. Pharmacol. Exptl. Therap.* 113, 39 (1955).
7. J. F. Tomashefski, H. I. Chinn, R. T. Clark, *Am. J. Physiol.* 177, 451 (1954); R. H. Shepard *et al., Federation Proc.* 13, 135 (1954).

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Use of Glycine in Bovine Semen Extenders Stored at 5° and – 78°C

The survival time of bovine spermatozoa has been increased with the use of a 3-percent glycine extender beyond the time, obtained with use of a 3.2 percent sodium citrate extender when stored at 4°C (*1*). This advantage of glycine over sodium citrate was believed to be due to the low electrolyte content and to the maintenance of the functional integrity of the sperm cells. Evidence has been presented to indicate that amino acids play an important role in the physiology of the sex organs (*2*). Further evidence has been presented to demonstrate that certain amino acids increase the life span of sea urchin spermatozoa, but that they are not used metabolically. It has been found that glycine extends the viability of fowl spermatozoa (*3*).

Gassner and Hopwood (*4*) have reported that the amino acids found in bovine semen, in order of apparent concentration, are glutamic acid, alanine, glycine, serine, and aspartic acid. The concentration of any of the five amino acids is about the same in both plasma and whole semen.

Tyler and Rothschild (*2*) observed that when whole semen was incubated for 3 hours to permit metabolism of fructose by spermatozoa, none of the amino acids were utilized. The purpose of amino acids in semen appears to be other than for metabolic functions. Since they are amphoteric in reaction, they may be a part of the natural buffer system. Glycine was selected as the amino acid to be used in this study because of its natural occurrence in semen and because of the indication of its possible use that was given by other workers (*1*).

Three-percent glycine in double-distilled water is not isotonic to bovine semen. Freezing-point determinations in this laboratory indicate that 2.05-percent glycine in double-distilled water with added antibiotics is isotonic to semen. It was decided to test the 2.05-percent and the 3-percent levels of glycine against 2.94-percent sodium citrate (dihydride), which would serve as a control, since it has been in common use in bovine semen extenders for many years.

The semen was collected from dairy bulls in an artificial vagina. It was transferred immediately to a test tube that was stoppered to prevent contamination. The semen was then divided into three equal portions and extended with the following: (i) an extender made up of 50 percent egg yolk and 50 percent by volume of 2.94-percent sodium citrate (dihydride) solution; (ii) an extender made up of 50 percent egg yolk and 50 percent by volume of 3-percent glycine solution; and (iii) an extender made up of 50 percent egg yolk and 50 percent by volume of 2.05-percent glycine solution. The rate of extension was 1 part bovine semen to 20 parts of extender.

Table 1. Influence of glycine-yolk and citrate-yolk extenders on the motility of bovine semen stored for 7 days at –78°C.

Extender	Ejaculates (No.)	Avg. motility (%)
3% glycine	10	50.8 ± 5.36
2.05% glycine	10	49.7 ± 5.66
2.9% citrate	10	50.7 ± 6.16

The antibiotic levels used were 0.2 g streptomycin and 0.06 g penicillin per 100 ml extender. The extended semen was then cooled in a refrigerator at 5°C for 5 hours. A small sample was then taken for storage at 5°C. The remaining portions were extended by adding at a rate of 1 to 1 a solution consisting of (i) 16 percent glycerol by volume in 2.9-percent sodium citrate (dihydride) solution; (ii) 16 percent glycerol in 3-percent glycine solution; and (iii) 16 percent glycerol in 2.05-percent glycine solution, respectively. The final concentrations of the components of the three extenders were as follows: (i) 67 ml of 2.9-percent sodium citrate (dihydride) solution, 25 ml of egg yolk, and 8 ml of glycerol; (ii) 67 ml of 3-percent glycine solution, 25 ml of egg yolk, and 8 ml of glycerol; (iii) 67 ml of 2.05-percent glycine solution, 25 ml of egg yolk, and 8 ml of glycerol.

The extended semen was then sealed in glass vials and equilibrated for 18 hours at 5°C. It was then frozen in a bath containing acetone and Dry Ice. The rate of freezing was 1°/min from 5° to – 15°C and 3 to 4°/min from – 15° to – 72°C. The samples were transferred to a storage cabinet containing Dry Ice in methanol and stored at – 78°C. The samples were thawed on the seventh day and the percentage of motile spermatozoa was observed. The results of this trial are presented in Table 1. An analysis of variance indicated that the differences observed were not significant. The motility observations on the extended semen stored at 5°C are presented in Table 2. After 4 days of storage, there appeared to be little difference between extenders. After 6 days of storage, the glycine-yolk extenders appeared to give better results than the citrate-yolk extenders. The *p*H observations indicate that glycine does not buffer the extended semen as well as sodium citrate.

Table 2. Influence of glycine-yolk and citrate-yolk extenders on the motility and *p*H of bovine semen stored at 5°C.

Storage (days)	Ejaculates (No.)	Avg. motility (%)			Storage (days)	*p*H of extended semen		
		2.05% glycine	3% glycine	2.9% citrate		2.05% glycine	3% glycine	2.9% citrate
2	10	64.4	65.4	63.4	1	6.45	6.40	6.78
4	10	58.4	59.4	58.7	5	6.32	6.32	6.73
6	10	48.4	45.0	40.0				