precipitate, which at first has a tendency to rise, will settle out on shaking. Centrifuge and decant, testing the supernatant fluid with aurin for complete precipitation of aluminum. Dissolve the precipitate in 1 cc. of 6 N hydrochloric acid and dilute with 10 cc. of water, add 1 cc. or glacial acetic acid and 5 cc. of 6 N sodium hydroxide (prepared from metallic sodium), mix and let stand 30 minutes. Throw down the iron precipitate in a centrifuge and decant into a Nessler tube graduated at 50 cc., add 2 cc. of 6 N hydrochloric acid and 1 cc. of glacial acetic acid. Dilute to about 38 cc. with water and add 5 cc. of a 1 per cent solution of the ammonium salt of aurin tricarboxylic acid. Let stand 10 minutes and add 5 cc. or a slight excess of 10 per cent ammonium carbonate in ammonium hydroxide (1 to 2). Make up to 50 cc. Compare against standards of 0.01, 0.02, 0.03, etc., mg. of aluminum containing 3 cc. of 6 N hydrochloric acid, 5 cc. of 6 N sodium hydroxide and 2 cc. of glacial acetic acid. Dilute the standards and develop the color the same as for the unknown.

Analyses have been made on the blood of 13 miscellaneous hospital cases. Repeated analyses have also been carried out on pig's blood. In all instances the amount of aluminum was less than 0.2 mg. per 100 cc. When larger quantities of blood (10 to 30 cc.) were used the results appeared to be somewhat lower. Excellent recoveries of added aluminum have been obtained.

This is a preliminary report.

[1]Hammett, L. P., and Sottery, C. T., *J. Am. Chem. Soc.*, 1925, xlvii, 142.

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Respiratory Function of the Swimbladder in Lepidosteus.*

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Many investigators have contributed evidence pointing to the probable origin of air-breathing vertebrates from certain physostomous fishes, which apparently have some organ capable of supplementing respiration in times of need. Certain Ganoids and Dipnoians have been credited with this transition of vertebrates from water to land.

* This work was done under the supervision of Dr. Frank A. Stromsten.

From anatomical data alone, some authors have declared the swimbladder to be a respiratory organ in several genera of fish, including Lepidosteus. It has been observed that Lepidosteus and a few others apparently do inhale atmospheric air,[1, 2] but so far as the writer knows, there exists no proof that this inhaled air is carried into the swimbladder, which would be absolutely necessary if this organ is to be considered an organ of respiration. The existing hypothesis that the swimbladder has such a function is based upon the following facts: (a) that it has a cellular, spongy appearance internally,[3] (b) that blood is distributed to a rich capillary network in its walls, then collected and carried directly to the heart,[4] (c) it has a connection with the pharynx by the *ductus pneumaticus* and glottis and (d) that apparently the animal gulps air into the mouth.[1]

In this article it is shown experimentally that Lepidosteus actually inhales atmospheric air into the swimbladder, where it serves in supplementing the respiration, both normally and to a much greater extent when the oxygen content of the water is low.

The method of procedure has been: (1) to carry on experiments with normal animals in deoxygenated water, allowing part of them to come to the surface for air, and confining others below the surface; (2) to inject melted paraffin through the glottis into the swimbladder of live animals, and run them against controls in normal and deoxygenated water; (3) analysis of gas from the swimbladders of the fish under various conditions, immediately following inhalation of air and again in the interval between inhalations, and (4) analysis of air from above deoxygented water (covered with a film of paraffin oil) in an air tight chamber where an animal is living.

Normal animals live 20 days in a small amount of deoxygenated water if allowed to come to the surface and gulp in air, but they die in 5 or 6 hours if confined below the surface. Control animals live for days with no apparent discomfort when confined by a screen, if a current of oxygenated water passes through the container. This seems to show without doubt that there is supplementary respiration. The average gram hour rate of consumption of oxygen by the fish has been worked out, and shows that the volume of the swimbladder and the normal rate of inhalations is enough to supply sufficient air for the needs of the body.

The paraffin injected animals die in 4 to 8 hours when placed in deoxygenated water while normal control animals live for days in the same water and other injected controls are able to live in normal tank water for at least 10 days. The opercular movement of these

animals is about one-third faster than the normal. The injected fish were autopsied and showed a good plug of paraffin in the swimbladder, so that air could not be drawn in. This would indicate that the swimbladder is the organ used for supplementary respiration.

Gas was drawn from the swimbladder with a sampling tube, the canula of which was inserted through the glottis, and analysed for oxygen and carbon dioxide with a Haldane apparatus. Analyses from normal animals in normal tank water show: immediately following inhalation (averages) 7.2 per cent oxygen and 1.59 per cent carbon dioxide; in interval between inhalation, 3.78 per cent oxygen and 2.44 per cent carbon dioxide. Analyses immediately following inhalation in animals which had been disturbed, and were very active, showed 8.03 per cent oxygen and 2.39 carbon dioxide. Activity of the animal seems to cause the carbon dioxide to pile up. Active animals exposed to air for 30 minutes gave the following gas analysis; immediately following inhalation, 10.06 per cent oxygen and 4.1 per cent carbon dioxide, and in the interval, 4.8 per cent oxygen and 4.7 per cent carbon dioxide. Analyses from animals kept in foul water showed: immediately following inhalation, 8.6 per cent oxygen and 3 per cent carbon dioxide, in the interval, 5.02 per cent oxygen and 5.1 per cent carbon dioxide.

The analyses of the air from above the water where an animal is confined in an airtight vessel also gave results which indicate that the fish uses the air in respiration. Some blood analyses are to be tried later in connection with this work.

This is a preliminary report.

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[1] Wilder, B. G., *Proc. Amer. Assoc. Advance. Sci.*, 1875, xxiv, 151.

[2] Day, F., *Proc. Zool. Soc. London*, 1868, 274.

[3] Hoeven, James van der, *Arch. f. Anat.* (Müller), 1841, viii, 221.

[4] Hyrtl, C. J., *Sitzler. Akad. Wiss. Wien.*, 1852, viii, 235.