in whom one superior cervical ganglion had been removed, either on the right or on the left side, I have injected at various times various doses of pituitrin—1 c.c., 2 c.c. and 3 c.c.—through the marginal ear vein. *At no time did a dilatation of the pupil on the operated side follow these injections, neither soon nor late. Both pupils, however, and especially that of the eye on the operated side, showed a constriction of short duration immediately after the injection.*

Here we meet, then, with a definite difference between the action of adrenalin and pituitrin which, in some cases, might assist in the identification of the nature of the blood-raising principle found to be present in some fluids.

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Observations on the relation of carbon dioxide and oxygen to the development of certain amphibian embryos.

By **A. M. BANTA.**

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The following observations were made during the past two seasons upon material kept for other purposes.[1]

Eggs of all the species mentioned below were placed when fresh or in early cleavage in artesian water containing 1.32 per cent. of CO_2, the amount normal to large open ponds here being only about 0.04 per cent.

Ambystoma punctatum.—Development was at the normal rate with no mortality of embryos traceable to the CO_2 in the water. The larvæ likewise lived fairly well, though in many cases not so well in the CO_2 water as in pond water.

Spelerpes bilineatus.—Cleavage and later development were probably at the normal rate, but there was a large mortality percentage in standing or running artesian water. The mortality was less in standing water from ponds but thoroughly oxygenated water comparatively free from CO_2 was necessary to get the highest percentage of developing embryos.

[1] Observations on *Spelerpes*, *Rana pipiens* (?), and *Rana sylvatica* were made on material kept in collaboration with Dr. R. A. Gortner, of this station.

Bufo lentiginosus.—Only a small percentage developed at all and none beyond early cleavage.

Hyla versicolor and *Hyla pickeringii.*—A few went through cleavage but none beyond.

Rana pipiens (?).—Mortality was very large during early stages but perhaps 5 per cent. developed until the embryos were considerably differentiated and about 3 mm. in length. None developed further. In two of the ponds observed all the eggs and embryos of this species died much as those placed in CO_2 water in the laboratory. These were ponds practically without aquatic plants and containing great quantities of decaying leaves and other plant debris and therefore doubtless had much CO_2 in the water.

Rana sylvatica.—The development was apparently at the normal rate. In many cases the larvæ hatched, but though active for a day or two and clinging to the jelly mass and sides of the jars in the usual fashion took no food and developed no further. All died within a few days. In other jars where considerable masses of the eggs were placed and where large numbers died before hatching the larvæ in the interior of the masses and thus least exposed to the CO_2 water survived longest.

Often the eggs of the inner portions of the egg masses of *Rana sylvatica* and *Ambystoma tigrinum* (all of which were otherwise developing normally) show a retarded development, progressively the more so the farther the eggs are from the periphery of the mass, due to insufficient oxygen. In one egg mass of *Ambystoma* the exterior embryos of the mass were 10.2 to 11 mm. long and almost ready to hatch, while at the other extreme those deepest within the mass were only 3.6 mm. long and still in the late neural groove stage. In most cases in the ponds where the *Ambystoma* eggs are laid, as well as in jars in the laboratory, a portion of the interior of the masses die before hatching. In one pond near the laboratory protected from wind and containing very quiet and poorly oxygenated water perhaps 90 to 95 per cent. of the eggs die each year. That insufficient oxygen is the cause was indicated by the low percentage mortality in a few masses left almost entirely exposed to the air by the lowering of the level of the pond. These conditions were imitated in the laboratory with the result

that in some cases 60 to 100 per cent. of the eggs of egg masses kept in a depth of 12 to 18 inches of quiet, poorly oxygenated water died, while in similar adjoining vessels eggs less than half submerged developed almost without exception.

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Direct observation of cell division in mammalian tissue.

By **ROBERT A. LAMBERT.**

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Connective tissue cells of the rat cultivated outside the body in rat plasma for as long as seventy-six days formed the material for observation. For maintaining activity for such a period, the pieces of tissue were transferred to fresh plasma at intervals of five to ten days. Measures employed by Carrel for rejuvenating the cells in old cultures were found to be unnecessary.

We have found that the process of karyokinetic division may be followed in the living cells. The earlier phases often escape notice. The later phases, however, may be observed with ease even with the lower powers of the microscope. In actively growing cultures the time required for the entire process is from forty to sixty minutes. The time elapsing between the divergence of the masses of daughter chromosomes to complete division of the cytoplasm averages about ten minutes. In slowly growing cultures the process may be retarded. A half hour to an hour after division is required for the daughter cells to develop the form and staining qualities of the resting cell. These time periods relate to observations made on cells at 35–37°C. At lower temperatures (25–30°) the process is slower.

Cells containing numerous fat droplets have been seen to divide as rapidly as cells free from fat.

Amitotic division has not been observed.

Cells after division have been followed and further division noted. The size reached by these daughter cells was approximately that of the parent cells. This observation affords proof that true growth takes place in cells cultivated in vitro.