30°C. The phage action is observed the following morning. The phage reactions on the agar slide are similar to those noted on the agar plate, varying degrees of activity being observed.

This method was used in comparison with that of Williams and Rippon for some 500 cultures. Since then an additional 500 cultures have been run with the slide method alone. The agar-slide method appears to have these advantages over the agar-plate method.

1) It is a less exacting technique. The plate method requires the use of very fine capillary pipettes, which are difficult to make and handle without breaking. Great care must be exercised in placing extremely small drops, approximately 0.01 ml, on the agar plate to avoid transferring culture from one plate to another and to avoid spreading of the drop over a larger area of the agar than is designated for it by markings on the bottom of the plate.

2) It takes less time to carry out the test with slides. Agar placed on a slide dries almost immediately, whereas when agar plates are poured they must first harden and then be dried for 60 to 90 min.

3) It uses only a fraction of the volume of culture medium required by the plate method.

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19 August 1954.

Original Adaptive Significance of the Tetrapod Limb

The origin of the tetrapod limb marked one of the major advances in the evolution of the vertebrates. The ancestral crossopterygian fishes had probably supplemented gill respiration with air breathing for a long time, but the reorganization of the paired fins into supporting legs and feet brought a major shift in genetic and functional emphasis, and opened the way for the extremely rich and complex evolution of the land vertebrates. The geologic record of tetrapod origin is still imperfectly known, and there has been much speculation concerning possible events in this transition. The tetrapod limb is usually interpreted as an adaptive modification that was directly useful for land locomotion in the relatively dry Upper Devonian, perhaps enabling the first amphibians to escape overland from receding swamps and pools to areas that might retain more water (*1*). Possible stages in evolution, from ancestral swimming fin through hypothetical intermediates to the walking leg, have been figured by several authors (*2–4*). This note considers an alternate selective advantage that this modification may have given to its first possessors: that, originally, the tetrapod limb may have served chiefly as a more efficient digging adaptation that facilitated estivation in the dry season.

Recent amphibians seek moisture and avoid dryness. Typically, they disperse from increasingly adverse, but still damp, sites only if the surrounding areas are sufficiently moist to attract, rather than repel, invasion. If the adjacent region is dry, they huddle into the damp debris and mud that remain from the evaporating pond. In semiarid climates the predominant amphibians are burrowing forms that can go underground to avoid desiccation. Newly transformed young amphibians normally linger in the vicinity of the pond margins until there is sufficient rain to permit dispersal, and the young of burrowing species commonly "dig in" soon after metamorphosis.

The extant groups of amphibians are, of course, greatly different from the original stock. The earliest known amphibians in the Upper Devonian were considerably larger than most of the modern forms, and in the adult stage they probably bore scales and were more effectively protected against desiccation. But their delicate young stages would certainly be vulnerable to environmental hazards, and a breeding population could not be maintained in an area for a geologically significant length of time unless the successive year-classes in the colony had reasonably continuous access to sufficient moisture to meet their basic needs.

The behavior of many extant vertebrates indicates that both land surface locomotion and burrowing can be carried on successfully without the tetrapod leg and foot. Certain fishes can make temporary or even regular land excursions. For example, the mudskippers (*Periophthalmus* sp.) and the climbing perch (*Anabas* sp.) are well known for their ability to move about on damp land surfaces (*5*). Many kinds of fishes (certain eels, flatfishes, and so forth) habitually secrete themselves in the loose sandy or muddy bottom of their aquatic habitat. Some of the major groups of burrowing land vertebrates are legless (caecilians, snakes, and certain families of lizards); but these legless tetrapods are derived groups, and subterranean habits are their normal way of life rather than a seasonal or occasional adjustment in behavior.

Inferences concerning the possible behavior and ecology of ancient fishes on the basis of habits of highly differentiated modern forms that are only remotely related to them must be made with caution. In some cases, however, there is geologic evidence to support such inferences. For example, it is well known that the extant species of lungfishes of South America and Africa are able to withstand seasonal drouth by burrowing into the mud and estivating, and recently Romer and Olson (*6*) reported the remarkable discovery of Permian lungfishes found in upright cylindrical burrows under conditions that indicate that the individuals had been fossilized in estivating sites. It is apparent that seasonal escape underground from a drying swamp is a very ancient behavior pattern in certain evolutionary lines of dipnoans. The possibility that some of the early crossopterygians were also capable of effective burrowing can only be speculated, on present knowledge. But the skeletal structure of the paired fins of the few crossopterygians in which it is well known, for example, *Eusthenopteron* (*2*), compared with the general trends of foot specialization in tetrapods, suggests that the foot (the part of the limb that has always been the most difficult to account for) can be understood most easily if it is interpreted as, originally, a digging specialization. In *Eusthenopteron* the proximal elements in both the pectoral and the pelvic fins resembled fairly closely the corresponding tetrapod elements, but the distal units were radial bones that had no very clear tetrapod resemblances, and the fin terminated in a thin flap. Westoll (*3*) suggested, as had a number of earlier authors, that the digits and their adjacent supporting bones are new structures rather than remodeled fin elements, and he proposed the terms *archepodium* for the proximal elements that were derived from the ancestral fin and *neopodium* for the new distal structures. He postulated (*7*) that the tetrapod limb originated only once, and that its establishment in the affected population was relatively rapid.

A dry era, such as the Upper Devonian, would be a particularly inauspicious time for the emergence of aquatic animals onto the land. The prospective new environment would then be at its worst for such animals, and it would be much more likely to select adaptations that would permit more effective direct use of available water supplies. The evolution of the fin into a strong footlike structure with good muscular control over its terminal segments would seem to confer greatest immediate functional and ecologic advantage as a more efficient digging mechanism that would enable the proamphibian to remain in contact with the retreating moisture by following it downward seasonally (perhaps digging into more resistant sediments), and thus remaining in the vicinity of established seasonal water holes rather than wandering off into a hostile environment. It is possible that the amphibians long remained a small and obscure group, gradually gaining in relative importance as they outlasted the contemporary swamp fishes that were less well equipped to survive in restricted habitats in a difficult climate. The real acceleration in amphibian evolution probably began later, when climatic changes began to provide better all-year moisture conditions and to open up a greater variety of near-shore ecologic niches. With pedal structure, air breathing, and associated modifications already established, the amphibians would be preadapted to extend their activities onto humid land areas.

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20 September 1954.

Potential Errors in Spectrophotometry with Optically Dense Solutions

The recent availability of photomultiplier detectors for ultraviolet spectrophotometers has introduced a possibility for obtaining false optical density measurements when solutions of high optical density are studied. In this, and other laboratories, it has become common practice to determine increments of optical density by compensating for the background density with similar solutions as reference samples. In particular, reactions have been followed below 290 mμ in the presence of protein and nucleotides, which absorb strongly in this region. It has been noticed that the addition of a given amount of absorbing material to solutions of high optical density yields a density increment less than that given by an identical addition to a solution of low density.

One theoretical explanation for this observation is the failure of Beer's law because of association of molecules. This explanation does not apply to the dilute solutions of various compounds tested. Another explanation is found in the limitations of the optical instruments used. As is well known by students of optics, every monochromator gives, in addition to the selected wavelengths, a certain amount of light of random wavelength, the so-called "stray light." The purpose of this communication is merely to indicate how stray light can be a source of significant error when the photomultiplier is used.

The failure of the spectrophotometer to detect densities above a high background was noticed in this laboratory during studies on the enzymatic formation of nicotinic acid. In these the absorption spectrum of pyridine compounds in the region 260–270 mμ could not be detected in incubation mixtures, although it was easily determined after deproteinization. The ability of the spectrophotometer to detect densities above high backgrounds was subsequently tested with the Beckman DU spectrophotometer using dilute solutions of adenosine at 260 mμ, quinolinic acid at 268 mμ, urocanic acid at 277 mμ, and tyrosine at 280 mμ. In each case solutions were made to give theoretical densities of 1, 2, 3, 4, and 5 by diluting proportional amounts of a stock solution to a given volume with buffer. Theoretically, each cuvette read against the previous one (the first one against buffer) should give a density of 1. The observed values in a typical experiment with adenosine were 1.0, 0.96, 0.88, 0.575, and 0.15. Similar values were obtained with each of the other compounds tested.

It is possible to obtain valid measurements of high optical densities if the light used is sufficiently monochromatic. It is obvious that when the response of the phototube to unselected light approaches or exceeds that to selected light penetrating the background, further changes in the selected light will have little influence on the phototube output. Therefore, the solution to the problem lies in reducing the ratio of stray to selected light. It should be emphasized that stray light is an intrinsic property of the monochromator,