

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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## 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 m $\mu$  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 m $\mu$  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 m $\mu$ , quinolinic acid at 268 m $\mu$ , urocanic acid at 277 m $\mu$ , and tyrosine at 280 m $\mu$ . 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,

not a function of leaks. Also, stray light is distributed throughout the light beam and is not affected materially by changing slit widths. Decreasing the light path through the sample reduces the proportion of stray light but also causes a corresponding decrease in the magnitude of density read for a given sample. The proportion of stray light can also be reduced by the use of purer monochromatic light, which can be obtained with special light sources, filters, or additional monochromators. For example, when light from a mercury arc (H100-A4) passed through one Beckman monochromator was used as a source for a second Beckman spectrophotometer, accurate measurements could be made on solutions with background optical densities exceeding 5. Similar values were obtained with the Cary spectrophotometer, which has a double monochromator.

It is not practical to attempt to define specific limits of usefulness for any instrument, since the optical density at which serious errors are obtained is a function not only of the light source, monochromator, and phototube but also of the wavelength selected and the absorption spectrum of the samples. Therefore, each instrument must be tested with substances of known extinction, the backgrounds desired, and the required wavelengths in order to determine the conditions that permit accurate measurements.

The limitations of optical instruments discussed here have been understood by spectroscopists for many years. The purpose of this communication is to emphasize a source of error that may be significant in biological studies. I am indebted to Drs. F. Brackett and R. Olson for many helpful discussions.

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## Sex Differences in the Packed Cell Volume of Vertebrate Blood

The existence of sex differences in the packed cell volume (PCV) of human blood seems to be established (1). Sex differences in PCV among the lower, as well as the higher, vertebrates is reported here.

Blood was obtained from the exposed heart in frogs (*Rana pipiens*) stunned by a head blow (2), from the axillary vein in unanesthetized pigeons (*Columba livia*), and by cardiac puncture through a cervical approach in unanesthetized turtles (*Pseu-*

Table 1. Statistical analysis of sex differences in the packed cell volume of vertebrate blood.

Animal and sex		No. of animals	Mean PCV (ml/100 ml blood)	t-values
Frog	M	304	30.1* $\pm$ 7.7	8.0
	F	233	24.8 $\pm$ 7.4	
Turtle	M	101	27.9 $\pm$ 7.0	3.6
	F	101	24.3 $\pm$ 6.5	
Pigeon	M	112	58.5 $\pm$ 5.9	2.63
	F	100	56.4 $\pm$ 5.6	
Guinea pig	M	67	47.1 $\pm$ 4.5	9.5
	F	67	39.8 $\pm$ 4.4	

\* Mean  $\pm$  standard deviation.

*demys* sp.), and through the intercostal spaces in etherized guinea pigs (*Cavia porcellus*). Only mature animals were used.

The Wintrobe method was followed (3), with the blood centrifuged for 1/2 hr at 3000 rev/min. The anticoagulant in frogs was 3 percent sodium citrate, added just to the lowest mark of the hematocrit tube. For the other animals, 0.8 g of potassium oxalate and 1.2 g of ammonium oxalate were dissolved in 100 ml of distilled water, 0.5-ml samples were withdrawn and dried, and to each sample 1 ml of blood was added. It is assumed that hematocrit corrections for anticoagulant were unnecessary and irrelevant to the present study.

Table 1 shows a statistically significant sex difference in the PCV in representatives of each vertebrate class studied. The Student t-test showed all differences to be significant at the 1-percent level. Among mammals, sex differences in PCV are controversial in dogs, rabbits, and rats (4).

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*A paradox is never terrifying to the scientist. Faraday wrote to Tyndall, "The more we can enlarge the number of anomalous facts and consequences, the better it will be for the subject, for they can only remain anomalies to us while we continue in error."*—GILBERT N. LEWIS, *The Anatomy of Science*.