"Evolution"—"Development"—Anatomical and Cerebral Features and the Pathological Consequences

WE recently published papers in the oceanographic field which contained in their titles the words "development"[1], "evolution"[2], "triple junctions"[2] and "fingers"[3]. These key words were dispatched by computers to thousands of child psychiatrists, biologists, neurologists and medical practitioners. Hundreds have requested reprints. We are curious to know how many are going to request a reprint of the present communication on the same basis.

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[1] Davies, D., *Sci. J.*, **5A**, 2, 39 (1969).
[2] McKenzie, D. P., and Morgan, W. J., *Nature*, **224**, 125 (1969).
[3] Turner, J. S., *Deep Sea Res.*, **14**, 599 (1967).

BIOLOGICAL SCIENCES

Prostaglandins—an Experiment

I AM writing not about prostaglandins but about requests for reprints of a *Nature* article[1] about them. In the first two months after this was published, I received 615 reprint requests of which 53 per cent came from the USA or Canada. Thirty-eight per cent of these, and 16 per cent of those from other countries, bore the name of the sender only in typewritten or rubber-stamped form. Of the handwritten names, internal evidence suggested that many were not personal signatures.

This kind of experience is doubtless very common. How can one assess the probability that a rubber-stamped card (probably sent by a technician who has scanned *Current Contents* for key-words) indicates anything more than a general interest in a subject and a disinclination to go to the library? The answer may partially be provided by the response to the present letter. Anyone who reads this is asked not to request a reprint.

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[1] Pickles, V. R., *Nature*, **224**, 221 (1969).

Absorptivity and Quantum Yield of Bleaching in Bovine Visual Pigment$_{500}$

VISUAL pigments are conjugated proteins containing either retinal or 3-dehydroretinal as the chromophoric prosthetic group. The molar absorptivity (ε) of the pigments at their absorption maximum in the visible spectrum has been determined either by measuring the retinal or the apoprotein content in a given amount of visual pigment. Knowledge of the molar absorptivity of visual pigments is of practical importance because concentrations are most easily determined by spectrophotometric methods. In addition, this parameter has a theoretical interest in that it enables one to calculate the quantum yield (φ) of bleaching.

Wald and Brown[1] determined the amount of all-*trans* retinal oxime formed from a given amount of illuminated visual pigment after reaction with hydroxylamine and found an $\varepsilon_{500\ nm}$ of 40,600, assuming one chromophore per molecule. A similar value was found by Shichi *et al.*[2] using a chemical determination of the retinal liberated from illuminated visual pigment. The determination[3] of the molar absorptivity ($\varepsilon_{500\ nm}$), however, performed by measuring the amount of protein in purified samples of visual pigment, gave a value of 23,100. A new attempt was therefore made to determine the molar absorptivity of visual pigment by at least two different methods to find the cause of the disagreement.

Table 1. MOLAR ABSORPTIVITY OF BOVINE VISUAL PIGMENT$_{500}$ DETERMINED BY AMINO-ACID ANALYSIS

	$A_{500\ nm}$	μmole protein	$\varepsilon_{500\ nm}$
Sample I	0·417	0·01914	21,786
		0·01924	21,673
Sample II	0·350	0·01638	21,367
		0·01646	21,263
Average			21,524

Native visual pigment$_{500}$ was purified as the cetyltrimethylammonium bromide complex[3]. Duplicate samples of known absorptions obtained from two separately purified visual pigment$_{500}$ preparations were hydrolysed with 6 M HCl for 24 h at 110° in sealed, evacuated tubes and were then analysed on the amino-acid analyser. Assuming a molecular weight of 26,400 for the visual pigment apoprotein, the stable amino-acid residues aspartic acid, glutamic acid, glycine, alanine, leucine and phenylalanine were used to calculate the amount of protein present on the basis of the previously known composition[4]. Independent measurements in several laboratories have led to the conclusion that the molecular weight of visual pigment is indeed around 28,000 (refs. 2–4). The molar absorptivity of the sample could then be calculated in a direct way and the results are shown in Table 1.

Attempts were then made to determine the molar absorptivity as described by Shichi *et al.*[2], who determined the retinal content of visual pigment using the method of Futterman and Saslaw[5]. This method is based on the colorimetric reaction of the aldehyde group of retinal with thiobarbituric acid. Although excellent reproducibility and accuracy were obtained with standard all-*trans* retinal, only very erratic and nonreproducible results were obtained with visual pigment. The $\varepsilon_{500\ nm}$ values determined by this method, using the same pigment samples as were used for amino-acid analysis, ranged from 35,000 to more than 50,000. The variability and low yield of the reaction probably arise because other amino groups of the protein compete for the potential aldehyde group of retinal, as was shown by Ball *et al.*[6] for a series of interactions between amino groups and retinal, and possibly as a result of other effects such as oxidation or reduction of the aldehyde group. All the competing reactions would tend to reduce the colour yield with thiobarbituric acid and thus produce an apparent increase in $\varepsilon_{500\ nm}$. Similar considerations may apply to the oxime method[1] of determining the retinal content of visual pigment.

The photosensitivity of visual pigments is defined as the product of the molar absorptivity and the quantum yield of change ($\varepsilon\varphi$). Dartnall *et al.*[7,8] found the photosensitivity of frog visual pigment to be 23,000 and Dartnall[9] later reported that the photosensitivity of a series of visual pigments ranges from 26,300 to 28,500. With the $\varepsilon_{500\ nm}$ value obtained above and with the knowledge that the $\varepsilon_{500\ nm}$ of bovine and frog visual pigment are practically identical[10], it is possible to calculate the quantum yield of bleaching as 1·07 to 1·32 depending on the value of photosensitivity chosen (φ = photosensitivity/ε). Because it seems improbable that the quantum yield is larger than 1·0 in this system, either the molar absorptivity reported here is somewhat too low or the calculated photosensitivity is somewhat too high, or both. Nevertheless, the con-