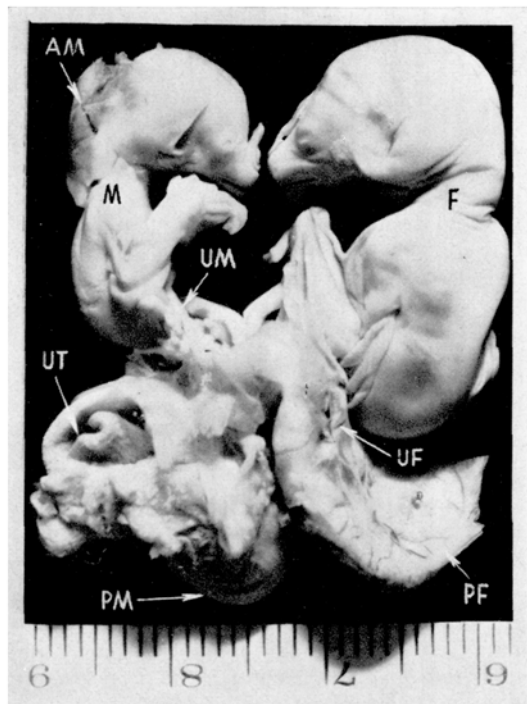


assumes that the corpus luteum in the left ovary disappeared in early pregnancy and that no other structure took over its function enabling thus the right ovary to ovulate; it is known in the bats that the corpus luteum disappears very early⁴. In *lyra*, the ovaries of the two

gravid females collected during March did not show corpora lutea, these having disappeared at least two months prior to parturition, if not earlier.

In view of the absence of data on the above two points, viz., the presence of sperm in the right horn prior to March and the disappearance of the left corpus luteum to enable the right ovary to function, it appears more reasonable to assume that both ovaries ovulated simultaneously and fertilization was probably also simultaneous. The left zygote gained developmental predominance over the right and the right blastocyst marked time, the cause for this delay being not known. Cases of deferred or delayed implantation are not unknown among mammals. Nidation, therefore, should have taken place in this bat having twins at different periods of life of the blastocysts.

It is difficult to say what would have happened to the smaller foetus at the time of parturition of the larger; probably it would have aborted.



Heterosexual twins of *Megaderma*, $\times 2$. AM part of amniotic membrane of male, F female twin, M male twin, PF placenta of female, PM placenta of male, UF umbilicus of female, UM umbilicus of male, UT uterus.

Zusammenfassung. Ungleichgeschlechtliche Zwillinge von verschiedener Grösse von *Megaderma lyra lyra* (Microchiroptera) wurden im April letzten Jahres in einem trächtigen Weibchen gefunden. Um die Grössendifferenz der Zwillinge zu erklären, wird angenommen, dass in diesem Falle zwei Eier befruchtet worden waren, dass die linke Zygote einen Vorsprung über die rechte gewann und dass deshalb die Implantation der rechten Blastocyste verzögert wurde.

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⁴ H. MATHEWS, Proc. zool. Soc. London 111, 289 (1941).

Photosensitivity of the Pineal Organ in the Teleost, *Salmo irideus* (Gibbons)¹

Direct photosensitivity of the pineal organ has been claimed in a number of teleost fishes from which the lateral eyes had been removed. However, the evidence is only indirect, being based either on colour changes¹⁻³ or behavioural tests⁴⁻⁶. In the present experiments, direct evidence is given of light sensitivity of the pineal organ (epiphyseal vesicle) in the rainbow trout, *Salmo irideus* (Gibbons).

One-year-old specimens, about 10 cm in length, were anaesthetized with tricaine (MS-222), immobilized by intramuscular injection of a small dose of tubocurarine chloride, and perfused with a stream of oxygenized tap water through the gills. In some experiments the lateral eyes were removed. In order to get access to the pineal complex, the integument on top of the head and the frontal bones were taken off, the epiphyseal vesicle being clearly seen dorsal to the cerebrum as a reddish, nearly globular evagination in the posterior part of the forebrain where meningeal melanophores are absent. After removing the cartilaginous plate covering the pineal vesicle with the aid of ophthalmic lancets, responses were led off

by means of microelectrodes (steel needles electrolytically sharpened and insulated except at the tip⁷) guided by means of a micromanipulator.

Some of the results are illustrated in the Figure. Illumination by white light of the pineal vesicle produces, after a latency of about 30 msec, inhibition of the spontaneous discharge. With short stimuli (0.1 sec) activity gradually reappears after cessation of the stimulus (Figure, A') while with longer stimuli (0.65 sec) the inhibitory effect is only transient (Figure, A''). Thus, illumination of the pineal vesicle in fishes is followed by both inhibitory and excitatory changes of nervous activity. Photosensitivity of the pineal organ is not evenly distributed over the organ, activity being recorded mostly from the surface

¹ K. VON FRISCH, Pflügers Arch. ges. Physiol. 138, 319 (1911).

² W. HOAR, J. Fish. Res. Bd. Canada 12, 178 (1955).

³ O. SCHÄFER, Pflügers Arch. ges. Physiol. 278, 62 (1963).

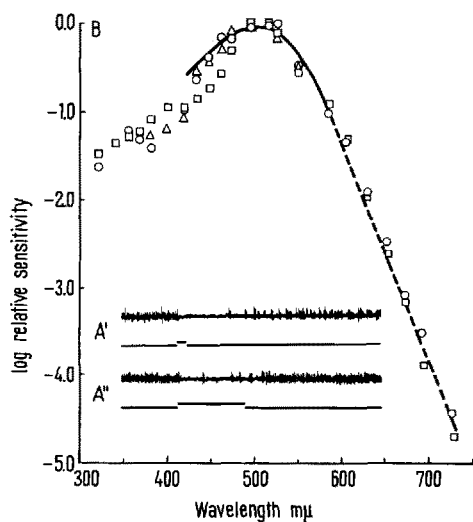
⁴ E. SCHARFF, Z. vgl. Physiol. 7, 1 (1928).

⁵ C. M. BREDER and P. RASQUIN, Bull. Amer. Mus. Nat. Hist. 89, 325 (1947).

⁶ J. DE LA MOTTE, Naturwissenschaften 50, 363 (1963).

⁷ J. D. GREEN, Nature 182, 962 (1958).

at some places near the midline. Following exposure to strong light, the threshold luminance which evokes a response is greatly increased as compared to the dark adapted threshold, 0.75 lm/m^2 . After cessation of light adaptation the threshold falls exponentially by 2 log units after about 20 sec of dark adaptation, and 5 log units



Rainbow trout (*Salmo irideus*). A: Microelectrode recording from the exposed pineal organ (epiphyseal vesicle) showing the activity of several sensory units. Exposure to light indicated by upward deflection of the lower beam. Stimulus duration 0.1 sec (A'), 0.65 sec (A''). B: Relative spectral sensitivity, after dark adaptation, of the photic response of the pineal organ. Measurements made at wavelengths between 321 and 727 $m\mu$ by determining the energy causing the smallest perceptible decrease of impulse frequency. Equal quantum intensity spectrum. Three different animals indicated by different symbols. For comparison the absorption curve of visual pigment 505 $m\mu$ ⁸ is shown (continuous line).

within 30 min. Full dark adaptation of the pineal vesicle in the rainbow trout requires about 1 h or even more.

Spectral sensitivity of the inhibitory response of the pineal organ in *Salmo irideus* is highest in the blue-green and declines towards either side of the spectrum (Figure, B). A comparison of the sensitivity function with the absorption spectrum of known photopigments shows that visual pigment 505 $m\mu$ ⁸ fits the sensitivity data fairly well. Recently, it was found that visual pigment solutions prepared from the lateral eyes of the rainbow trout, *Salmo irideus*, contained a mixture of two photosensitive pigments, one with λ_{max} at 533 $m\mu$, the other with λ_{max} at 507 $m\mu$ ⁹. From the present measurements it is tempting to conclude that only visual pigment 507 $m\mu$ may be responsible for the action of light on the pineal organ in the rainbow trout¹⁰.

Zusammenfassung. Mikroelektrodenableitung der freigelegten Epiphyse der Regenbogenforelle, *Salmo irideus* (Gibbons), zeigt eine spontane Impulsaktivität, die bei Belichtung des Organs gehemmt wird und auch nach Entfernung der lateralen Augen bestehen bleibt. Nach Helladaptation des Pinealorgans an ein starkes Licht ist während des nachfolgenden Dunkelaufenthalts eine Empfindlichkeitszunahme um das 10^5 -fache festzustellen. Die Spektralsensitivität der Hemmung ähnelt der Absorptionskurve eines Photopigments mit Maximum bei 505 $m\mu$.

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⁸ H. J. A. DARTNALL, Brit. Med. Bull. 9, 24 (1953).

⁹ C. D. B. BRIDGES, J. Physiol. 134, 620 (1956).

¹⁰ The author is indebted to the Deutsche Forschungsgemeinschaft for support in this work.

The Effect of Incubation in Phosphate Buffer of Different pH on the Transplantability of the Mouse Ascites Tumor of Ehrlich

As part of a study concerned with the effect of specific environmental conditions on the viability of mouse ascites tumor cells, samples of these cells were incubated in phosphate buffer solutions of different pH before being injected into recipient mice.

Ascites fluid was withdrawn from tumor-bearing white Swiss mice 7-8 days after inoculation with Ehrlich ascites tumor cells, centrifuged for 10 min at 4000 rpm, and then washed three times with triple volumes of 0.85% saline. 1 cm^3 of these packed cell preparations contained about 34×10^7 tumor cells (counted in a hemocytometer after dilution with 0.1% citric acid). The packed cells were then suspended in an equal volume of 0.85% saline and 0.48 M phosphate buffer was added to make each preparation 0.16 M. Buffers of pH 6.1, 6.4, 6.8, 7.1 and 7.4 were used. After the addition of 1000 units of penicillin, each mixture was incubated in Erlenmeyer flasks at 37° while being constantly shaken. Mixtures were incubated for periods from $\frac{1}{2}$ to 7 h. Following incubation, each preparation was brought to pH 6.1 by the addition of 0.1 N HCl, and diluted with 0.85% saline to different cell concentrations.

1 cm^3 of this suspension was then injected intraperitoneally into 10 white Swiss mice (weighing 20-22 g) and their survival followed for 40 days.

The effect of pH on the viability of the incubated tumor cells is illustrated in the Figure. On incubation for 2 h survival of the tumor cells decreased with increasing pH, until at pH 7.4 no mice died of tumor within 40 days when an inoculum of 1.7×10^6 cells was used.

The Table shows a comparison for 20 representative experiments between the viability of non-incubated cells and cells incubated at pH 6.1 and 7.4 respectively for various periods of time. Whereas 1.7×10^6 cells produced between 50 and 100% mortality, even after incubation for 5 h at pH 6.1, the inoculation of as many as 51×10^6 cells, incubated at pH 7.4 for 2 h, produced no tumor in 40 days. In 8 of the 20 experiments with cells incubated at pH 6.1 the 'break' in viability occurred between 3 and 5 h, while in the remaining 12 the 'break' was between 5 and 7 h.

It should be stressed that there was no appreciable autolysis of these cells during incubation at pH 7.4 for 2 h or at pH 6.1 for 7 h. The cellular DNA content, analyzed according to SCHMIDT and THANNHAUSER¹, remained

¹ G. SCHMIDT and S. J. THANNHAUSER, J. biol. Chem. 161, 83 (1945).