

The strips were then exposed to osmium tetroxide vapours. The staining reaction was prompt and intense for melatonin<sup>11</sup> and slow for serotonin<sup>12</sup> (Figure 6). The strips were decolorized by treatment with periodic acid 10% solution.

**Discussion.** These results show that osmiophilic granules are present in the rat pineal gland and that their number increases with age. The high content of the pineal body in serotonin and melatonin, and the staining of these two substances after exposure to osmium tetroxide vapours, supports the view that serotonin and melatonin are components of the osmiophilic granules. The granules seem to contain also lipids, because they are soluble in xylene. The positive PASM staining of the granules observed in the phase-contrast microscope might suggest

that the PASM material visible in the light microscope is the same material which is stained by osmium tetroxide.

Finally, the perivascular localization of the granules induces speculation that they enter the blood stream as a product of secretory activity.

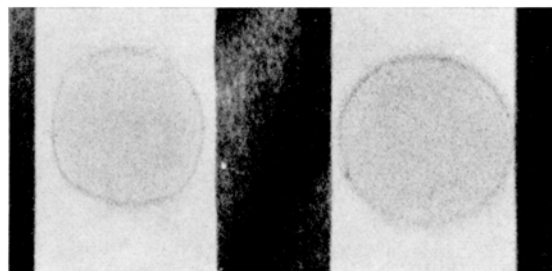


Fig. 6. Serotonin and melatonin staining after 1 h exposure to osmium tetroxide vapours.

**Riassunto.** Sezioni di pineale di ratto impubere e adulto sono state esaminate al microscopio a contrasto di fase ed al microscopio ottico dopo colorazione secondo la tecnica di JONES. Gli autori confermano l'esistenza di una attività secretoria che aumenta con l'età e prospettano la possibilità che nel secreto siano contenute serotonina e melatonina.

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<sup>11</sup> N-acetyl-5-methoxytryptamine.

<sup>12</sup> 5-Hydroxytryptamine.



Fig. 5. Argyrophilic material is localized around and inside the perivascular space and around the capillary wall. Bouin fixation. Paraffin and beeswax embedding. PASM, Schiff and hematoxyline stainings. Light microscopy. Magnification  $\times 1700$ .

### Differential Implantation of Twin Blastocysts in *Megaderma* (Microchiroptera)

While examining the reproductive physiology of the bats of Rajasthan desert, we came across a gravid female *Megaderma lyra lyra* Geoffroy during the month of April last year having two different sized foetuses.

Normally *Megaderma* does not carry two conceptuses at a time but there have been occasional instances of twinning in *spasma*<sup>1</sup>. So far as we know, there has been no record of twinning in *lyra*.

This note is not only intended to record the occurrence of occasional double foetuses in *lyra* but also to report, for the first time, the more important fact of the difference in size between the two. The larger female foetus (Figure) was present in the left horn of the uterus while the smaller male occupied the right. The placentae are separate, and each embryo has its own umbilical cord and blood vessels.

Neither of the ovaries showed a corpus luteum in the above gravid female. All the same, we are led to infer that both ovaries are active in *lyra*, and in both horns of the uterus progestational changes are brought about. In order to explain the difference in size of the twins, it may be argued that the left ovary ovulated first, the fertilized

egg implanted in the left horn and a placenta formed; the right ovary ovulated after the left and the sperm sojourning in the right tube were activated, one of them fertilizing the egg. It is rather doubtful if after the formation of a corpus luteum in the left ovary, the right would ovulate; the sperm may be assumed to be viable as has been described in some wintering bats<sup>2</sup>; HAMLETT<sup>3</sup> thinks that retention of sperm in the tract is likely in Megadermidae. However, examination of the non-pregnant horn of the uterus of two gravid females during the middle of March this year disclosed the absence of sperm in the tubes; in these the pregnant left horn measured  $16 \times 19$  mm and  $21 \times 18$  mm respectively. These probably represent mid-term (?) uteri as parturition in these bats is usually noticed in the middle of May. At any rate, this does not preclude sperm having entered the right horn and having been digested prior to the date of capture of the two pregnant *lyra* by us. The argument advanced above also

<sup>1</sup> A. BROSSET, J. Bombay Nat. Hist. Soc. 59, 1 (1962).

<sup>2</sup> W. A. WIMSATT, Anat. Rec. 83, 299 (1942).

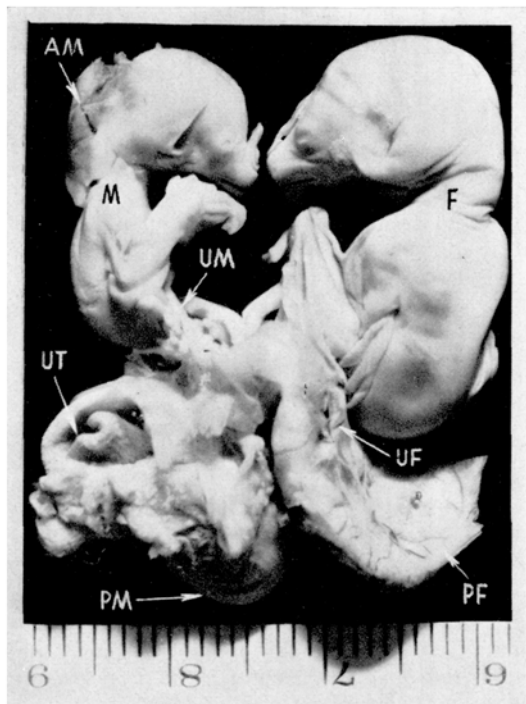
<sup>3</sup> G. W. D. HAMLETT, J. Mammal. 16, 135 (1935).

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 early<sup>4</sup>. 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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Department of Zoology, University of Rajasthan, Jaipur (India), June 24, 1963.

<sup>4</sup> H. MATHEWS, Proc. zool. Soc. London 111, 289 (1941).

### Photosensitivity of the Pineal Organ in the Teleost, *Salmo irideus* (Gibbons)<sup>1</sup>

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 changes<sup>1-3</sup> or behavioural tests<sup>4-6</sup>. 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 tip<sup>7</sup>) 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

<sup>1</sup> K. VON FRISCH, Pflügers Arch. ges. Physiol. 138, 319 (1911).

<sup>2</sup> W. HOAR, J. Fish. Res. Bd. Canada 12, 178 (1955).

<sup>3</sup> O. SCHÄFER, Pflügers Arch. ges. Physiol. 278, 62 (1963).

<sup>4</sup> E. SCHARFF, Z. vgl. Physiol. 7, 1 (1928).

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

<sup>6</sup> J. DE LA MOTTE, Naturwissenschaften 50, 363 (1963).

<sup>7</sup> J. D. GREEN, Nature 182, 962 (1958).