Observations on Luminescence in the Deep sea fish, *Paratrachichthys prosthemius*

Y. HANEDA*

(With 1 plate and 3 Text-figures)

Since BUCHNER (1921, 26) and PIERANTONI (1914, 18) published their so-called Intracellular Luminous Symbiosis Theory, describing the special relationship existing between luminous bacteria and luminous animals, much interest has been aroused in this problem. Bacteriologically, however, it has since been confirmed that this theory is limited only to some species of fish and squid. These special types of luminous fish and squid, although not possessed of luminosity in themselves, possess a duct for the culture of luminous bacteria and have power to produce or extinguish their light. Some are even possessed of reflector, lens to intensity their light. BUCHNER and PIERANTONI described the luminous bacteria as always occurring within the cell and transmitted to offspring by means of the egg, in this manner infecting the second generation. However, after much study, it has been concluded that this is not the case ; rather, it is believed that the luminous bacteria is present externally, passing through external openings into the light organs to settle during the larval stage of the fish. In other words, the bacterial infection is secondary and not transmitted by means of the egg.

Moreover, fish or squid associated with luminous bacteria are found distributed from pure shallow water, through mid-water, to deep sea water, while self-luminous fish and squid are never found in pure shallow water.

Fish associated with luminous bacteria have been studied in rather great numbers in Japan.

In 1916. while in Japan, HARVEY saw the light organs of the knightfish, *Monocentris japonicus*, and predicted that luminous bacteria would be found in the light organs. Later, Yasaki (1928) cultivated luminaus bacteria from the organs and confirmed Dr. HARVEY'S prediction.

The fish of the families Gadidae and Macrouridae possess luminous glands on their ventral regions. KISHITANI (1930) examined the luminous duct of the *Physiculus japonicus* of the Gadidae and discovered it was an open type of gland containing a symbiotic luminous bacteria, *Micrococcus physiculus*.

YASAKI and the author (1936) had reported ten species of the Macrouridae, which

*Director of the Yokosuka City Museum

are closely related to the Gadidae and have luminous organs of the same type, and the author (1938 - 1951) had added one species of the Gadidae and four species of the Macrouridae. From each fish of these two families, he obtained several strains of luminous bacteria, taking the strains from various species of the fish caught at different times and in different localities. All had the same general biological characteristics, varying only in their optimum temperature, and he is led to believe that all these strains of luminous bacteria belong to the same group which includes the *Micrococcus physiculus*.

The fish of the families Acropomatidae and Leiognathidae also possess luminous glands containing symbiotic luminous bacteria. The light glands of these fish lie inside the body, and the light passes through a translucent area of muscles, the author obtained pure cultures of luminous bacteria from two species of *Acropoma* and found their general biological characteristics quite different.

Other luminous fish of the same general type as the *Acropoma* are belong to the Leiognathidae. These are pure shallow water forms and are abundant in the waters of southern Japan and tropical Asia. Externally this fish does not present any unusual features, and it is only by careful observation of the living fish by night that its luminosity becomes apparent. The source of light is a swollen ring of glands which encircles the oesophagus.

From the various species of Leioghathidae, caught at different times in Japanese and tropical waters, he was able to obtain pure cultures of luminous bacteria.

Recently, KUWABARA (1955) discovered a new type of luminous fish, *Paratrachichthys prosthemius* JORDAN et FOWLER, of the family Trachichthyidae. He reported that the luminous organ of this fish is situated just before the anus, forming a black scale-less depression 1.8mm wide in a fish of 70mm total length, and that this apparatus, which belongs to the so-called "open type" luminous organ, consists of such element as a black scale-less depression, luminous gland, and lens. He pointed out that a structure of an unknown nature extends from the scale-less depression back ward along each ventral side of the body, but it could not be determined whether this structure was an element of the luminous organ or not. Furthermore, KUWABARA was not able to observe the light emission from the organ, nor did he has the chance to cultivate any luminous bacteria from it. His observations were purely anatomical and histological.

The author obtained some living specimens of this fish and had an opportunity to observe luminescence in his study of the fish, he was able to determine that it is a luminous fish of the indirect emission type and that its source of light is due to luminous bacteria which live symbioticaly in the duct.

Material.

Paratrachichthys prosthemius, known in Japanese as "Haridashi-Ebisu" belongs to

the family Trachichthyidae found in southern japanes waters. Living at depths of 50-100 fathoms, this fish is aways caught by trawler, as is usual with such mid-water specimens. They are taken most abundantly during the winter, and the total length of the adult fish is about 60-75mm.

Externally this fish does not present any unusual features, and it is only by careful observation of the living fish by night that its luminosity becomes apparent.

In September, 1956 the author obtained some living specimens of this fish from Manazuru harbor, Sagami Bay, and in December of the same year he also obtained a great number of specimens of this fish at the Owase fish market in Mie prefecture.

Luminous organ.

In the authors experimental observations of this fish, he preserved the whole body in a fixative of formaline-prepared sea water. The material was cut at 10μ transversely by celloidin and parraffin, and stains used were haematoxylin-eosin and also alminium morine (500.000 times diluted solution) for observation under fluorescent microscope.

Since the luminous gland is not visible on the surface of the body, this fish, externally and in daylight, have the appearance of ordinary non-luminous fish. The source of the light is a luminous gland situated surounding the papilla in which the rectum terminales lying between and behind the bases of the pectoral fin. According to his observations, the complete luminous organ therefore consists of three, or possidly four components:

(1) luminous gland and its lens and opening; (2) cloudy translucent keel muscle; (3) filiform body which extends backward from the luminous gland and possibly (4) a shutter for the light spots which may be either opened or closed by the chromatophores.

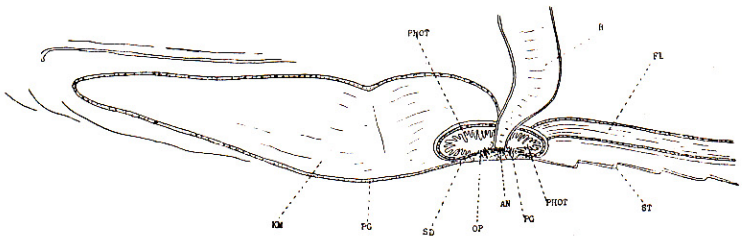

Fig. 1. Longitudinal section of the luminous organ of *Paratrachichthys prosthemius*, showing luminous duct (PHOT), opening of the duct (OP), pigment (PG), inner capsule (IC), keel muscle (KM), scale-less depresion (SD), rectum (R), and filiform body (FL).

Luminous gland and its opening.

The luminous gland (PHOT) is furnished with ducts which pass downwared and outwared, opening on the exterior, where the gland encircles the rectum. In a specimens of 70mm. total length, it measured 2.1mm. long, 1.1mm. in width, 0.7mm. thickness. The skin in this area is transparent and heavily loaded with black pigments.

In the gland, bacteria occur in the film of material which lie on the inner surface of

the epithelial cells. The openings (OP) of the ducts are situated near the anus. The capsule of the luminous gland is composed of two layers; outer and inner. The inner layer (IC) is furnished with chromatophores (PG), and it is perhaps due to their expansion and contraction that the light can be respectively shut off and displayed. The luminous gland of this fish are very similar to that found in the Gadidae and Macrouridae. Beneath the luminous gland lies a transparent substance, through which the light of the luminous gland passes to the outer surface.

Cloudy translucent keel muscle (KM).

The cloudy translucent keel muscle, or thorax, situated before the anus in this species, is very similar to that found in *Acropoma* and Leiognathidae. In the cited 75mm. long specimen, this keel muscle measured 20mm. in length. The light, from its luminous gland source, diffuses through these muscle areas, from whence it is externally visible. These keel muscle serve as a lens.

Filiform body

KUWABARA stated that a structure of an unknown nature extends from the scale-less depression. By observation of living specimens in the dark, however the author was able to ascertain that this definitely is an element of the luminous organ, and it has been titled as above. This characteristic structurs consists, histologically, of muscle tissue, as KUWABARA mentioned, inaddition however, this muscle tissue is cloudy-translucent, as described, and diffused light will pass through it easily to the end of muscle tissues. Thus, in outer appearance, this body is very similar to the U form and filiform luminous gland of *Acopoma;* but it is to be noted that, histologically, this characteristic structure is nothing more than muscle tissue, and is not a luminous gland itself. It is nevertheless an important element of the luminous organ. In the same 75mm. specimen, this filiform body measured 31mm. in leugth, 1mm in diameter. A great number of chromatophores appeared in the capsule of this filiform body.

Chromatophores.

A great number of branched chromatophores (PG) are schattered throughout the inner capsule of the luminous gland and also in the capsule of the long filiform body and the skin of the keel muscle, or thorax. It is possible that these chromatophores serve as a mechanism for screening the light emitted from the gland.

As previously stated this fish by its unusually large luminous area, utilizing in fact the keel muscles and filiform body of its extremely complicated body structure. The luminous gland is situated surounding the anus, and may be considered to be of an indirect or reflecting type, functioning be reflected light in the same as *Acropoma* and Leiognathidae; but this fish is not only provided with chromatophores for displaying

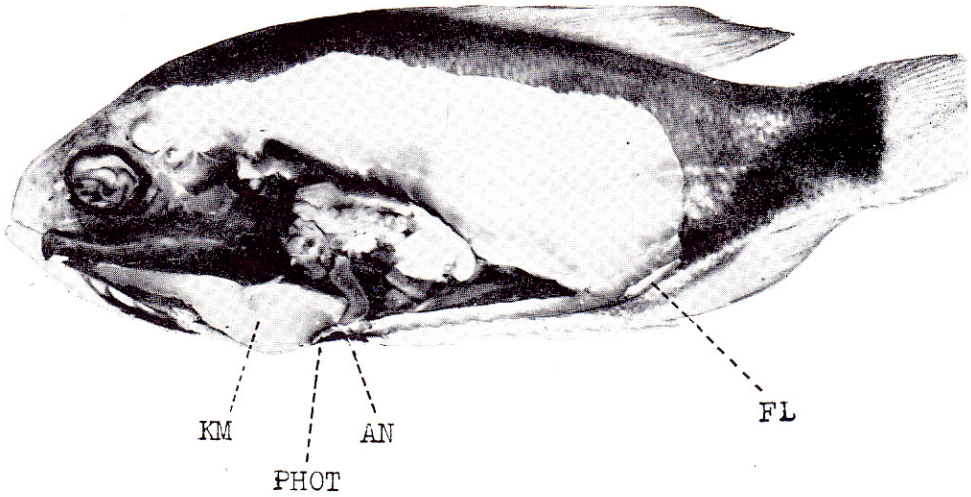

Fig. 2. Section of the luminous organ of *Paratrachichthys prosthemius*, showing luminous duct (PHOT), Keel muscle (KM), filiform body (FL) and anus (AN).

its luminescence, it has a far more complex luminous organ than *Acropoma* or Leiognathidae.

Remarks on the luminescence.

Since the luminous gland is not visible on the surface of the body, this fish, externally and in daylight, have the appearance of ordinary non-luminous fish.

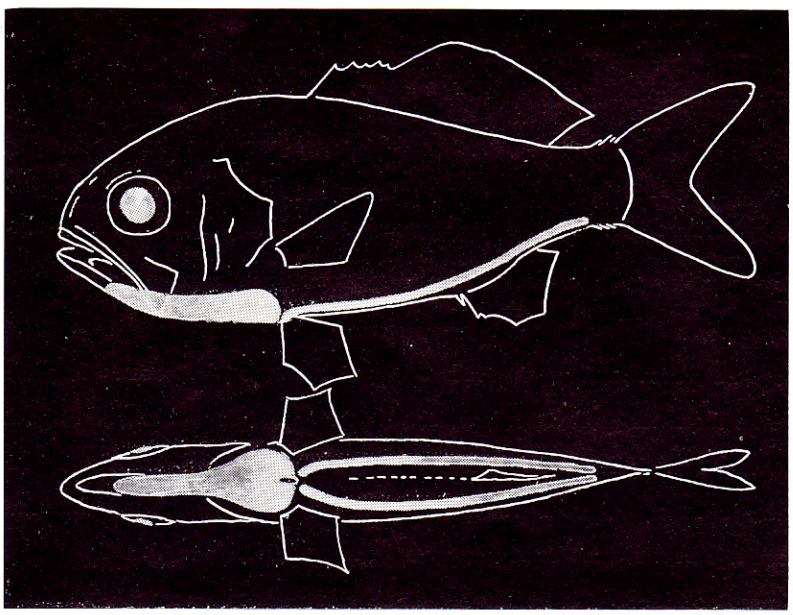

Fig. 3. Diagram of luminous areas of *Paratrachichthys trosthemius.*

A specimen of this fish were put into an aquarium of sea water in a dark room and their luminescence was studied. No luminescence could be seen when the fish were viewed laterally a diffuse bluish-white luminosity of keel muscle could be seen. It did not shine brilliantly, but was feeble and diffused. If the fish received a strong stimules or was removed from the water the keel muscle before anus (the lower part of the thoracic region) lighted up brilliantly.

The long filiform body extends backwards from the luminous gland along each ventral side of the body also become luminous. The author observed a mild bluish luminescence extending along part of the filiform body, from the luminous gland to a point situated at about half of its total length, and some times a mild bluish luminescence appeared along the whole length of the filiform body when a strong stimules is given the fish, or when the fish is taken from the water, the intensity of luminescence increases greatly, owing to the contraction of the chromatophores scattered in the capsule of the luminous gland and in the skin of the ventral area. After death, the light cannot be seen from the expansion of the chromatophores in the transparent capsule covering the luminous body.

Contents of the Luminous Gland

Emulsion of the contents of the ducts were made in sterile sea water and were examined in the dark. The whole of the emulsion will be luminescent, but if it is allowed to stand, only the upper layer exposed to the atmosphere will glow while the deeper lower layer becomes non-luminous. When shaken up, it was again uniformly distributed in the tube. If it was centrifuged, the luminosity was concentrated in the sediment at the bottom of the tube and the fluid above was clear and not luminous. If this precipitate was well mixed with sterile sea water the whole mixture become uniformly luminous, but if it was mixed with distilled water, it failed to show any luminosity. Luminescence is greatest at a temperature of 15-24°C. When the temperature is raised, it increase and finally disappears 45°C; above this point it finally ceased and could not be reactivated when cooled again to 15-24°C.

Microscopically this emulsion is made up of disintegrated particles of gland cells will innumerable bacteria, and it appears from the results of these experiments that the contents of the luminous gland consist of luminous bacteria. In order to test this, isolation experiments were carried out by the usual methods of cuture in artificial media.

Culture of the Luminous Bacteria

The luminous gland was removed from the fish body and washed in water. For the purpose of removing germs from its surface, it was brought in alcohol and washed again in water. The gland was open with a sterised knife, and the luminous matter in the gland was taken for the culture, after 10-15 hours a small, round, transparent and luminous colony appeared. From this start a pure culture which was free from any contaminating bacteria was obtained. It would be made clear, that the same species of fish always possesses the same species of bacteria. 30 different strains of the luminous bacteria were obtained by the usual bacteriological techniques from 30 of this fish. The characters of these bacteria are as follow: Morphology-Coccoid, 1.2μ in diameter; Non-

capsulated; Gram-negative. Agar-plate-20 houres at 20°C: round, convex, amorphous, transparent colonies up to 1mm in diameter; smooth glistening surface. Agar-slope-20 hours at 20°C: Abundant filiform. Gelatine stab-7 days at 20°C: Abundant. filiform growth. No-liquefaction· Potato-10 days at 20°C: Abundant, raised opaque yellowish growth. Potato changed yellowish. Metabolism-Aerobic. Opt. temp. 15-24°C. Limit of pH. 6.8-9.5 in pure culture. Luminescence. Biochemical-Indol −. Gas. product +.

The details of the bacteriological work will be presented elsewhere.

Acknowlegment:

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To Professor K. HIDAKA, chairman of the Committee on the Research of Marine Resources sponsored by the Japanese National Commission for UNESCO, for having given him the opportunity to carry out this present work; to Mr. T. IWAI of the Fisheries Department of Kyoto University who gave him the valuable specimens of this fish in 1952.

The author would also like to express his warmest appreciation to the Committee on the Reseach of Marine Resources for financial aid.

Explanation of plate IV.

Fig. 4. Abdominal view of the papilla in which the rectum terminales, showing the position of the luminous gland by the white dotted line.

Fig. 5. Longitudinal section of the luminous organ of *Paratrachichthys prosthemius*, showing luminous gland (PHOT), pigments (PG), keel muscle (KM), and filiform body (FL).

Fig. 6. Transverse section of the filiform body (FL), and its pigments (PG).

Fig. 7. Luminous bacteria, cultivated from the luminous duct of *Paratrachichthys prosthemius*.

References

BUCHNER, P. 1921, 1930. Symbiosen bei Leuchtenden Tiersen. Tier und Pflanze in Symbiose. Berlin, 2nd ed : 664-736; Ist ed. 1921. : 340-400.

〃 1926. Tierisches Leuchten und Symbiose. Berlin. : 58.

HANEDA, Y. 1938. Ueber den Leuchtfish, *Malacocephalus laevis* (LOWÉ). J. Med. Sci. Ⅱ; Biophysics, 5 : 355-66. Also Japan. J. physiol., 3 : 318-26, in Japanese.

〃 1939. New type of luminous organ of the fish. Reiko (Cold Light) Ser. 1. : 45-55. In Japanese.

〃 1940. On the luminescence of the fishes belonging to the family Leiognathidae of the tropical pacific. Palao Tropical Biol. Station Studies. 2 : 29-39.

〃 1950. Luminous organs of fish which emit light indirectly. Pacific Sci., 4 (3) : 214-27.

〃 1951. The luminescence of some deep-sea fishes of the families Gadidae and Macrouridae. Pacific Sci., 5 (4) : 372-78.

KISHITANI, T. 1930. Studien über die Leuchtsymbiose in *Physiculus Japonicus* HILGENDORF, mit der Beilage der zwei neuen Arten der Leuchtbakterien. Sci. Rept. Tohoku Univ. 5 : 801-23.

KUWABARA, S. 1955. Some Observations on the Luminous Organ of the Fish, *Paratrachichthys prosthemius* JORDAN et FOWLER. J. Shimonoseki College of Fisheries. 4 (2) : 81-85.

PIERANTONI, U. 1914. La Luce degli insetti luminosi e la simbiosi ereditaria. R. C. Accad. Napoli 20 : 15-21.

〃 1918. Gli organi simbiolici e la luminescenza batterica dei Cefalopodi. Pubbl. Sta. Zool. Nopoli 2 : 105-146.

YASAKI, Y. 1928. On the nature of the luminescence of the knight fish, *Monocentris Japonicus* (HOUTTUYN). J. exptl. zool., 50 : 495-505.

YASAKI, Y. and Y. HANEDA. 1935. Bioluminescence of the deep-sea fish, Macrouridae, J. Appl. Zool. Tokyo, 7 : 165-76 In Japanese.

YASAKI, Y. and Y. HANEDA 1935. Über einen neuen Typus von Leuchtorgan in Fische (*Acropoma Japonicum*). Proc. Imp. Acad. Japan. 12 (2) : 55-57.

抄　　録

深海魚ハリダシエビス *Paratrachichthys prosthemius* の発光に就て

羽根田弥太

ハリダシエビス ***Paratrachichthys prosthemius*** は100尋内外の中層性の深海魚で、大きさは 70—75 mm、発光器が外部に現はれていないので、他の深海発光魚に見られるような特徴のある形をしていない。桑原誠之氏（1955）は解剖学的、組織学的見地より肛門に接して、直腸をとりまく腺構造の器官があり、肛門の近くに開孔を持つ開孔式発光器であると考えた。然し、発光腺より体側の両側に長く走つている組織は外観がホタルジヤコ ***Acropoma hanedai*** の発光腺に似ているが、その構造は筋肉束であつて、その作用は不明であると述べている。但し、同氏はこの魚の発光を実際に観察していないし、又、開孔式発光器であるから発光腺内容は発光バリテリアであると考えたが培養試験を行つていない。

著者は、この魚の生時の発光状態を観察した結果、桑原氏が単に直腸をとりまく発光腺、肛門の前方の鱗を欠く凹所を発光器と考えたのに反して、著者はこの魚の発光器は以上の器管のみでなく、光を強めるレンズの役割をなすと考えられ肛門前方の腹部竜骨筋、桑原氏が不明の器官と考えた腹側両側を走つている筋肉組織も発光器の一部と考えている。

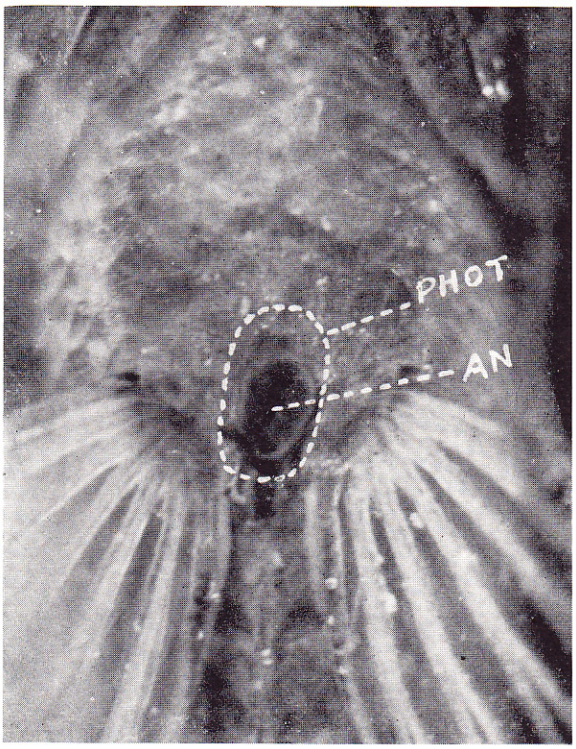
発光腺は体長 70 mm の魚では長さ 2.1 mm、巾 1.2 mm、厚さ 0.7 mm の楕円形の器官でやや後方の中心部を直腸が通つている。この器官は内外二層の透明なカプセルに包まれ、内層中には多数の樹枝状の黒色色素斑がある。構造は腺で、腺細胞の間に発光バクテリアが培養されている。多数の毛細孔が肛門の周囲に開孔している。

肛門より前方の胸部竜骨筋は乳白色半透明で光をよく通すと共に光を拡散する性質があり、発光腺に接しているのでこの筋肉はレンズの役割をし、この筋肉そのものが発光する如くである。この状態はホタルジヤコ ***Acropoma***, ヒイラギ Leiognathidae の腹部の竜骨筋と全く同じである。

桑原氏が不明な器官と考えた腹部両側を走つている筋肉束はやはりレンズの役割をし、この筋肉束の一端は発光腺に接しており、発光腺の光は、細長い筋肉束の先端まで光が達し、従つて、魚の両側の細長い器官が弱い青白い光を放つている。

魚をつかむとか、強く刺戟をすると、胸部竜骨筋（KM）と腹側の両側を走つている器官（FL）が青白く光り、明滅する。これは発光腺及びこれ等レンズ組織の表面の皮膚に散在する黒色色素斑の伸縮によるものである。

発光腺を魚体より切りとり、表面を消毒して、発光腺内容を 3% 食塩加培養基に植えると 20 時間（20° C）で強く光るコロニーが見られる。従て、本魚も亦、ホタルジヤコ、ヒイラギ類と同様に間接照明装置で開孔式発光器を持つた共棲発光魚である。



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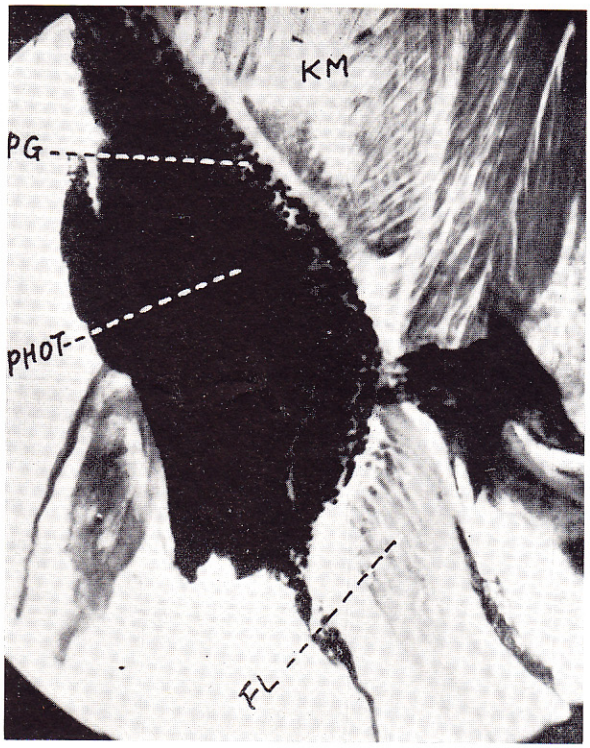

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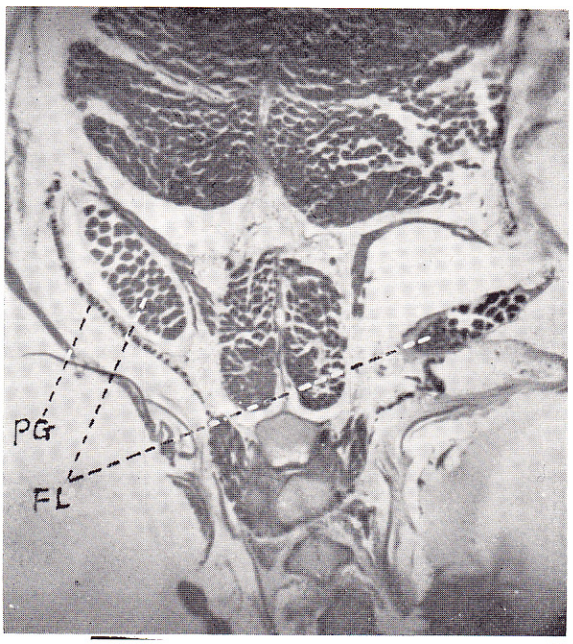

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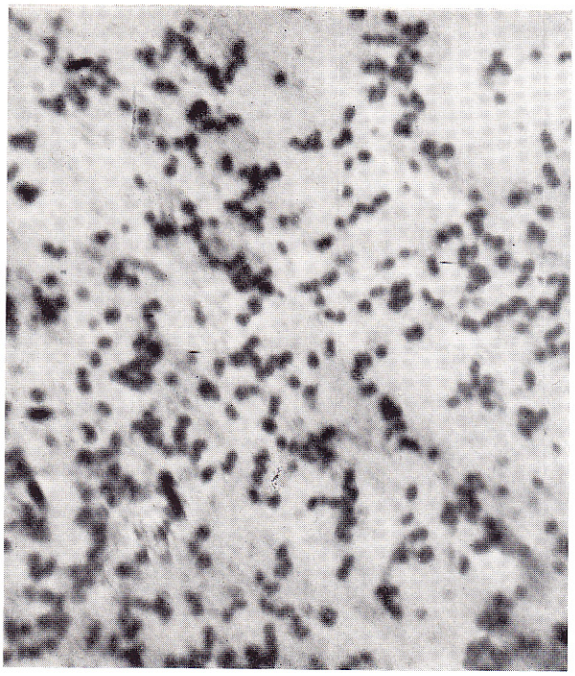

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