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On the Morphology and Physiology of the Branchial Gland in Cephalopoda

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(I) INTRODUCTION

Since 20's of the present century the endocrine phenomenon of some organs found in invertebrates has drawn attention of a number of investigators (HANSTRÖM 1937, von der WENSE 1938, KOLLER 1938, LELU 1938, UMEYA 1953, EULER & HELLER 1963). In cephalopods particularly SERENI (1927-30) published a series of papers on the physiology of the activity of chromatophores in the integument, came to the conclusion (SERENI 1930) that, (1) the activity of chromatophores is regulated by a chemical substance contained in the blood, and (2) the active substance is tyramine which is secreted from the posterior salivary gland. In 1935, I concurred this view of SERENI (TAKI 1935), but further observations have induced me somewhat sceptical about the endocrine nature of the named gland.

In cephalopods, there are several organs which were proved to be endocrine in nature. Of the branchial gland, one of such organs, HUTCHINSON (1928) carried out an extirpation experiment in *Octopus* in Naples, and described briefly that the gland in question might be endocrine. SERENI (1930) also, by similar experiments made at Naples, found that the growth of the operated animals was completely arrested, so he concluded that this organ probably functions for the growth of the body. Later MITOLO (1938) in an excellent paper, has confirmed that the extract of the branchial gland activates the heart. This work has marked a great progress to the solution of this difficult problem.

I took up this problem in the Marine Biological Laboratory of Hiroshima University, Mukaishima near Onomichi, Hiroshima Prefecture in 1934, when I have been resident there as a staff, independently of MITOLO, and a paper containing the preliminary accounts was read in October 1939 at Tokyo (TAKI 1940).

The present paper is to record (1) the anatomy, histology and embryology of the branchial gland in various cephalopods, of which our knowledge remains fragmentary so far, and also (2) some experimental proofs of the endocrine nature of the branchial gland, with special reference to the pathological histology of organs of the operated animal which has been entirely left unnoticed by previous authors.

This work was originally suggested by Prof. T. KOMAI[1], of the Zoological Institute of the Kyoto University, and I should like to express my cordial gratitude to him for his constant advice and encouragement. The experiment was carried out in the Marine Biological Laboratory of the Hiroshima University, Mukaishima near Onomichi[2]. My best thanks are due to the late Prof. Y. ABE and to Prof.

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2) At present: Mukaishima Marine Biological Station, Faculty of Science, Hiroshima University, Mukaishima, Hiroshima-Ken.

3) At present: Emeritus Professor of Hiroshima University.

Y. OZAKI[3], the former directors of the Laboratory, for their kindness in giving me all facilities for the present study. In 1938 a part of the expense was defrayed by a grant from "Tosyogu Tercentenary Memorial Endowment" of the Imperial Academy, and in 1939 and 1940 the study was assisted by a fund from the Education Department. I tender my warmest thanks to the Academy and Department for this help.

Concerning the outline of my results an abstract was published in June 1943 (TAKI 1943), in Japanese.

(II) MATERIAL AND METHODS

The materials employed in my observations and experiments were obtained from the fishermen near the above-mentioned Laboratory. The neighbouring sea of the Laboratory, Bingo Nada, a part of the Inland Sea of Seto, is favoured by the abundance of the cephalopods throughout the year. The octopods are caught with a trawling net or with a fishing-pot without getting any injury, which is highly favourable for such a study.

For fixing organs of all sorts for histological purposes, Nawaschin's fluid was mainly used, as it preserves the branchial gland in good condition. The sections were cut 4–6 μ in thickness for the branchial gland, and for other organs 6–8 μ.

The decapods (*Sepia*, *Loligo* and *Sepioteuthis*) do not live long in the aquarium, so that the experiment was carried out exclusively on octopods. Among the more abundant forms in the neighbourhood of the Laboratory the commonest one, *Octopus vulgaris*, was used throughout the experiment, because it is scarcely possible to keep *O. minor variabilis* or *O. ocellatus* for a long period in the aquarium. For some special purposes, however, for example, the extirpation of the branchial gland, these species were also used. The detailed account of the technique for keeping octopods in the aquarium and also of the operative techniques are given elsewhere (TAKI 1941).

Throughout this paper, unless otherwise stated, the material is *Octopus vulgaris*, and the tissue was fixed with Nawaschin's solution, and stained with Delafield's haematoxylin and eosin. When necessary, the colour was determined according to the colour standard (WADA 1935).

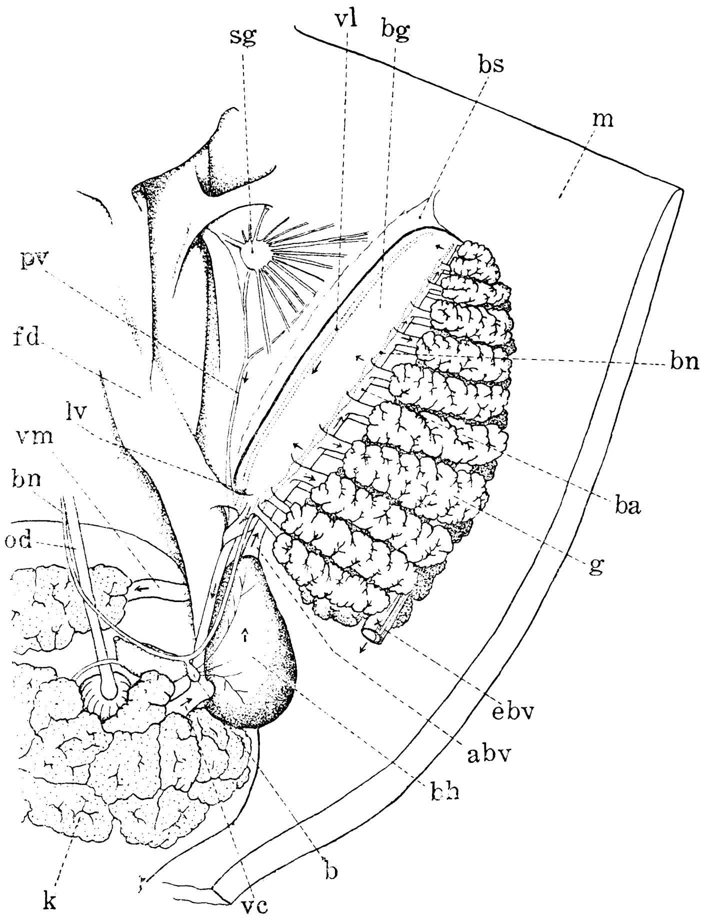
(III) MORPHOLOGY OF BRANCHIAL GLAND

A. Anatomy

The branchial gland was first described by TILESIUS in 1801. Later its anatomy and histology have been recorded by many authors, such as CUVIER (1817), MAYER (1835), GRIFFIN (1900), JOUBIN (1885), CUÉNOT (1891), KOWALEVSKY (1894), SCHAEFER (1904), WILLIAMS (1909), ISGROVE (1909), GRIMPE (1913), TURCHINI (1923, '23a), ROBSON (1926), HUTCHINSON (1928), SERENI (1930), YUNG Ko Ching (1930). Our knowledge of the organ, however, remains unsatisfactory in many points. In the following the anatomy of the gland will be given in detail, mainly in reference to that in *Octopus vulgaris*.

a. Form and Colour

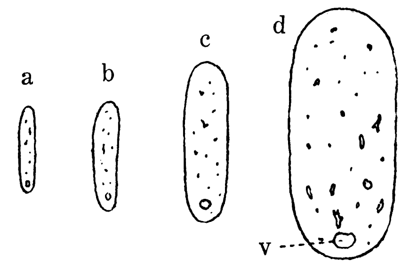
The branchial gland is attached intimately to the dorsal side of the gill on each side along its entire length, and is connected on the dorsal side with the internal wall of the mantle by a branchial suspensorium (Text-fig. 1). In *Nautilus* the gland is not well differentiated, though its existence is obvious (GRIFFIN 1900). In the Decapoda the gland is partly imbedded in the gill, but in the Octopoda it is isolated from the gill. HUTCHINSON (1928) has ascertained the existence of the



Text-fig. 1. Diagram showing the position of the left branchial gland of *Octopus vulgaris*, seen from the ventral side. The efferent branchial vessel is cut at the posterior end. The arrows show the circulatory direction of the blood. abv, afferent branchial vessel ; b, branchial ganglion ; ba, branchial artery ; bg, branchial gland ; bh, branchial heart ; bn, branchial nerve ; bs, branchial suspensorium ; ebv, efferent branchial vessel ; fd, depressor muscle of funnel ; g, gill ; k, kidney ; lv, position of ligature of branchio-lienal vein ; m, inside of mantle ; od, oviduct ; pv, pallial vein ; sg, stellate ganglion ; vc, vena cava ; vl, branchio-lienal vein ; vm, vena mesenterica.

gland in *Spirula* also. Thus, it may be concluded that the gland exists in all members of the Cephalopoda.

Form. In *Nautilus* it has no clear-cut form. In Decapoda, it is a long rod, tapering at both ends, and compressed laterally, so that in cross section of the middle portion it is ellipsoid, oval or tetragonal. In *Octopus* it is nearly oblong and flat, somewhat tapering at both ends, in cross section it is ellipsoid. It is very thin in *O. ocellatus*, but thicker in *O. vulgaris* and quite thick in *O. dofleini*. Thus the gland tends to be thicker in accordance with the increase of the size of the animal (Text-fig. 2). This relation seems to be found in some abyssal species of Octopoda also, as follows:



Text-fig. 2. Cross section of the middle portion of the branchial gland in its fresh state of, a, *Octopus ocellatus* ; b, *O. minor variabilis* ; c, *O. vulgaris* ; d, *O. dofleini*. v, Vena branchio-lienalis. ×2.

(Family Opisthoteuthidae) *Opisthoteuthis japonica*. The gill is extremely shortened, and when seen from the dorsal side, the branchial gland lies between the gill and branchial heart, only loosely connected with the gill, imbedded in a rather thick, gelatinous branchial suspensorium; the gland is somewhat disc-like and thin, coloured light old rose.

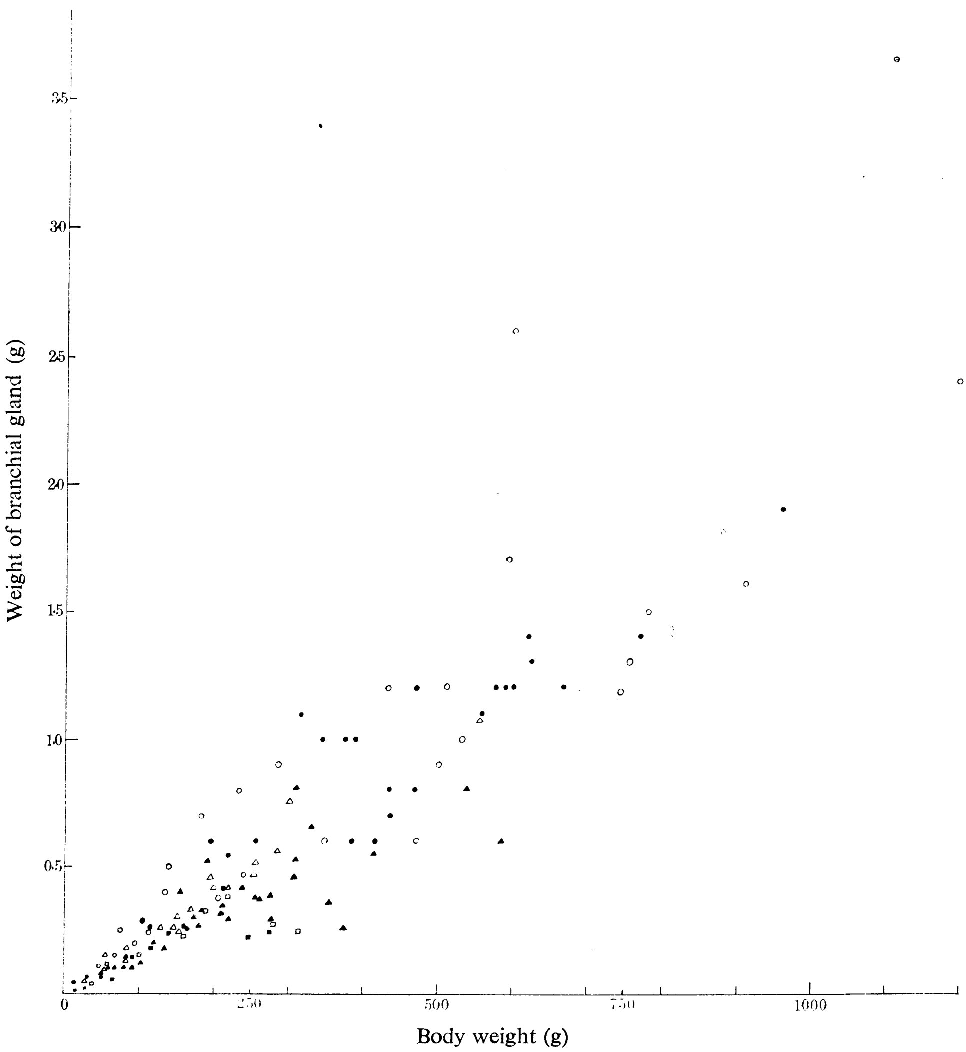
O. californiana. When seen from the dorsal side, the branchial gland is concealed under the gill, but seen from the ventral side, it is rather spoon-shaped, the main part broad, rather thick and anterior part is slender and protruded into the axis of the gill, seashell pink in colour and compact in consistency.
(Idioctopodidae) *Idioctopus gracilipes*. As already shown in the figure (TAKI 1963, text-fig. 11), the gill leaflets are degenerated and gaps are seen between each leaflet, but the branchial gland is very conspicuous, which is opaque, milky white and compact in consistency, but its capsule is very thin, transparent and inconspicuous, and numerous minute (0.05 *mm* in diameter) purplish chromatophores are scattered on the dorsal side of the gland along its entire length. The gill is semitransparent, gelatinous and soft; the gland lies along the entire length of the gill, tapering to both ends, its cross-section is roundish ellipsoid.
(Octopodidae) *Berrya hoylei* (cf. TAKI 1963, text-fig. 18, bg) The gland is thin but broad (2.2 *mm*) in comparison with the size of the gill (6 *mm* long).
Sasakinella eurycephala (TAKI 1964, p. 313) The gland is thin, the general shape resembling that of the juvenile specimen of *Octopus ocellatus*.
(Alloposidae) *Alloposus mollis*. Total length of the animal 185 *mm*. The gland is shorter than the gill, lying along the gill except the proximal one-third; it is very thin (0.5-0.7 *mm*) and broad (4 *mm*).

In the preserved material, the gland tends to become somewhat roundish by the contraction of muscular capsule which envelops the whole gland, and also by the pressure of other neighbouring organs (for example, gill, mantle, etc.). The relation is well observed in the fresh state and in a somewhat less degree in the preserved state.

Colour. In *Octopus* as well as in *Sepia* the gland is of about the same colour as the gill or pallial muscles, or somewhat deeper than these, namely a *sulphur yellow*. This makes its existence rather inconspicuous.

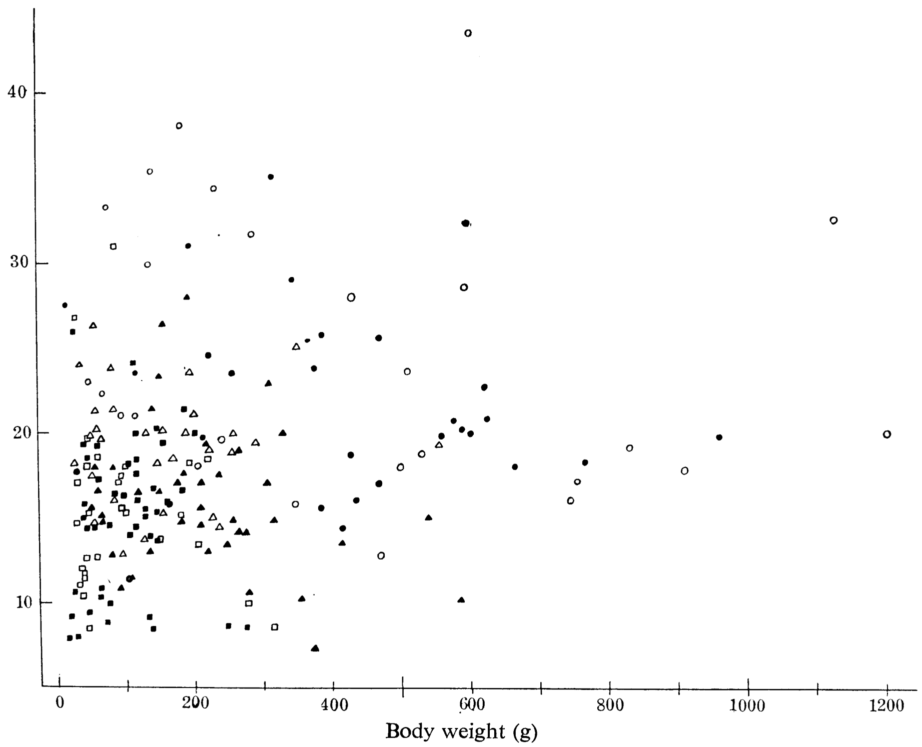
b. Relative Weight

The weight of the branchial gland (a pair) was measured after the muscular investment was removed from a preserved material (Text-fig. 3), and its ratio to



Text-fig. 3. Showing the relation between the weight of body and that of branchial gland in three species of *Octopus*.

● ♂ ○ ♀ *O. vulgaris* ▲ ♂ △ ♀ *O. minor variabilis* ■ ♂ □ ♀ *O. ocellatus* the body weight was calculated (Text-fig. 4). As shown in these figures, the weight of the gland in relation to the body weight is rather highly variable, being mostly 0.0015 - 0.0025 in both Octopoda and Decapoda. However, in the three species of *Octopus* of which I have seen numerous specimens, there seems to exist tendencies that (Table 1),



Text-fig. 4. Showing the ratio of weight of branchial gland to that of body in three species of *Octopus*. Ordinate : $\frac{\text{Weight of branchial gland}}{\text{weight of body}} \times 10{,}000$. Marks as in Text-fig. 3.

i) the weight of the gland in different species is proportional to the weight of the animal, namely it is heavier in *O. vulgaris* than in *O. ocellatus*, and intermediate in the medium-sized *O. minor variabilis*;

ii) it is heavier in female than in male;

iii) its proportional weight decreases with the growth of the body.

In *O. vulgaris* and *minor variabilis* the female has a larger proportional weight ($^3/_{10,000}$) than male, but in *O. ocellatus* the sexual difference is very small. It should be noted that in this species the ovary is loaded with a great amount of yolk in the breeding season, so that if the weight of the gonad is disregarded, the sexual difference becomes manifest as in other two species (Table 2). It may be added that the weight of the testis is so small, 0.5–1.5 g, as can be disregarded.

In the giant Octopus, *Paroctopus dofleini*, the weight of the branchial gland may attain more than 10 g; in one male weighing 3833 g, a rather small individual for this species, the gland weighed 5.6 g. In the large Eledonid species of the Antarctic Sea, *Megaleledone senoi* (TAKI 1961, p. 304, text-fig. 5, br: a matured female specimen), I showed that the branchial gland is obviously heavy in weight, namely 4 g in the right side, and its ratio to body weight is 28. This value is a little larger than that of female *O. vulgaris* (24.69), and an expectation that the larger species has a larger value of ratio of weight of branchial gland to body weight is positively affirmed.

Among the Decapoda measurements were taken of many individuals of *Sepia esculenta*, *S. kobiensis*, *Sepiella japonica*, *Sepioteuthis lessoniana*, *Loligo japonica*, *L. kobiensis*, *Sepiadarium nipponianum*, *Euprymna morsei*; the ratio ranged between 8.3 - 26.0, no sexual difference being observed.

Table 1. The ratio (× 10,000) of weight of branchial gland to that of body in three species of *Octopus*.

Species	*O. vulgaris*		*O. minor variabilis*		*O. ocellatus*	
Sex	♂	♀	♂	♀	♂	♀
No. specimen	29	26	41	33	52	30
Mean	21.28 ± 0.65	24.69 ± 0.86	16.12 ± 0.43	19.15 ± 0.38	15.02† ± 0.39	15.57 ± 0.58
Standard deviation	5.21 ± 0.47	7.53 ± 0.49	4.10 ± 0.31	3.22 ± 0.27	4.22 ± 0.28	4.72 ± 0.41
Difference: ♀ minus ♂	3.41		3.03		0.55	

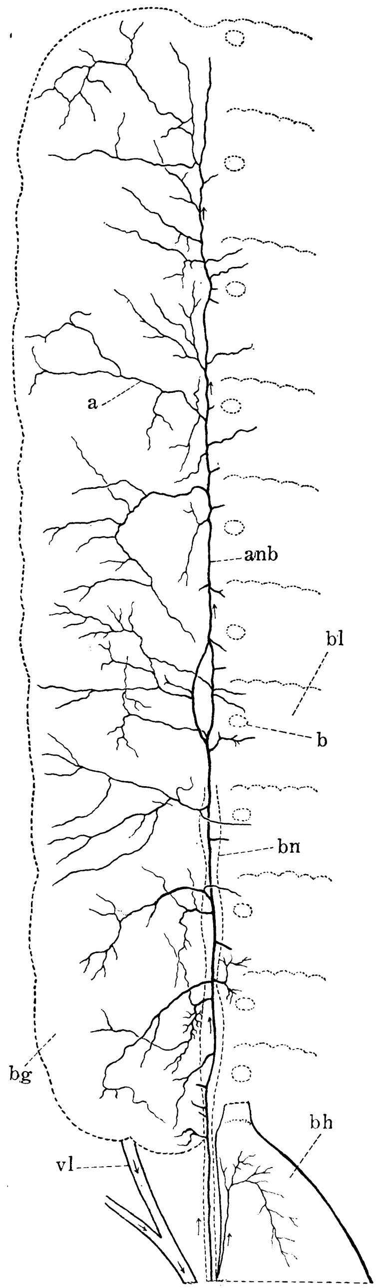
Table 2. The ratio of weight of the branchial gland to that of body in the female of *O. ocellatus*, with or without weight of gonad ; only 5 examples were chosen. (*18.1−†15.0=3.1)

No. specimen	(I) Weight of body g	(II) Weight of ovary g	(I)∼(II) g	(III) Weight of branchial gland g	(III)/(I) × 10,000	(III)/((I)∼(II)) × 10,000
a	277	47.9	229.1	0.27	9.7	11.8
b	191	32.1	158.9	0.33	17.3	20.7
c	100	14.9	85.1	0.18	18.0	21.2
d	49	6.3	42.7	0.10	20.4	25.4
e	45	10.3	34.7	0.04	8.9	11.5
				Mean	14.9	18.1*

C. Vascularisation

i. Artery

Octopoda. A small artery, A. cordis branchialis, starts on each side from



Text-fig. 5. Artery of the branchial gland in *O. vulgaris*. a, artery of the branchial gland ; anb, Arteria nervi branchialis ; b, cross section of the axis of branchial leaflet ; bl, branchial leaflet ; bh, branchial heart ; bn, branchial nerve ; vl, branchio-lienal vein.

the base of the anterior ventral side of the systematic heart, just at the point where the large arteries, A. pallialis medianus and A. aqueductus Krohni start. This artery reaches the branchial heart and one of its branches, A. nervi branchialis, goes to enter the gill with the branchial nerve. The artery goes through the entire length of the axis of the gill, partly imbedded within the branchial nerve, sending branchlets to the gill leaflets and the branchial gland (Text-fig. 5, a) and its capsule. JOUBIN (1885) described the existence of this artery only in *Octopus*, but not in *Eledone*; though ISGROVE (1909) shows its presence in the latter species too. SCHAEFER (1904) is entirely ignorant of this artery.

ISGROVE (1909) names it simply a branchial artery, but this term is ambiguous and should be avoided. GRIMPE (loc. cit.) recognizes that this artery attains the capsule of the branchial gland, but he is not aware of the distribution of this artery into the branchial gland, saying:" Dass die Blutdrüse [branchial gland] selbst davon Zweige erhält, scheint mir zweifelhaft zu sein" (p. 592).

The artery is so small as easily to escape notice, but its existence may be shown by injecting a small quantity of coloured fluid into the systematic heart, after the aortae, vena cava and branchial veins have been carefully ligated.

Decapoda. WILLIAMS (1909) has described of a small branch artery of the anterior aorta found at about the posterior third of the length of the liver on each side, "one branch (3′) passes through the supporting membrane of the gill to the branchial gland and the gill" (p. 56 ; pl. 1, fig. 6).

ii. Afferent Vein [1]

The branchial gland is also abundantly supplied with afferent veins (Text-fig. 6, av). In *Octopus vulgaris*, this afferent branchial vessel sends out about ten branch veins lying perpendicular to its course. The veins have thin but strong walls ; they ramify into several branchlets at about the distal third or at about the middle of the width of the branchial gland. The vessel finally ramifies into capillaries.

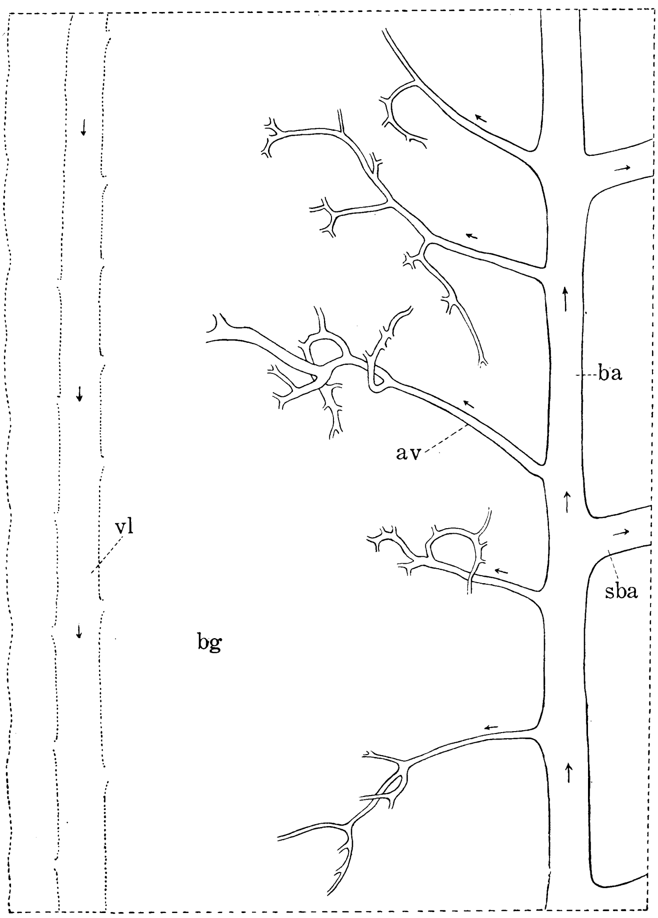
iii. Efferent Vein

The capillaries which ramify in radial directions in the matrix of the gland, gather together and finally enter a thick straight vessel, which runs along the entire length of the gland on the dorsal side (Text-figs. 1 and 6, vl). This vessel, named vena branchio-lienalis[2] by GRIMPE (1913, p. 582) according to the German terminology "Kiemenmilz" for the branchial gland, forms the efferent vessel of the branchial gland. It leaves the gland at its postero-dorsal corner and unites with the pallial vein, to pour finally into the branchial heart on its dorsal side.

The vena branchio-lienalis is the largest vessel in the gland and located at

1) Though this is a branch of the branchial artery, I think it appropriate to call this "a vein," to distinguish it from the true artery, as in the case of the hepatic vein of the liver in higher vertebrates.
2) Though the branchial gland can hardly be homologized with the spleen of vertebrates, this term may be retained for convenience' sake.

nearly the same position in all cephalopods (Text-fig. 2.v). SCHAEFER (1904, p. 17) took this vessel for an artery ("Vas afferens der Blutdrüse"), while SERENI (1930) considered that outgoing blood enters the renal vein and branchial heart. In *Nautilus*, as mentioned above, which has no histologically differentiated bran-



Text-fig. 6. Showing the afferent vein of the branchial gland (av) in *O. vulgaris*; the vein is traced up to the end of a hard endothelium. ba, branchial artery; sba, secondary branchial artery. Other letters as in Text-fig. 1.

chial gland, yet an efferent vessel homologous with the vena branchio-lienalis of Dibranchiate Cephalopods is found. This fact is noticed by SCHAEFER (1904, p. 15, pl. 1, fig. 1, v), who denied the presence of the branchial gland in this animal.

It is a remarkable fact that in the branchial gland the afferent vein is quite large and the artery is relatively small, and both the arterial and venous bloods are mixed there. Dissection shows that endothelium is only present in the afferent vein (Text-fig. 6).

d. Innervation

The branchial nerve, the terminal part of the visceral nerve, runs through the axis of the gill. In *Octopus* it has about 7 ganglia (ganglia branchiale) in the gill and a number of fine nerves issuing from its entire length on both sides. The ventral branches enter the branchial leaflets (ramus branchialis), while the dorsal branches supply the capsule of the branchial gland. There is no branch for the branchial gland itself. I have ascertained this by examining the giant octopus (*Paroctopus dofleini*). I have also looked in vain for a nerve from the stellate ganglion which might go to the branchial gland through the branchial suspensorium.

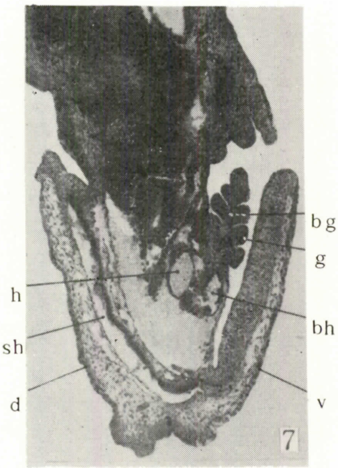
Bauer (1909, p. 261) also states: "Ueber die Innervierung der Kiemendrüse ist nichts bekannt." Pfefferkorn (1915) describes the branchial nerve of *Eledone* and *Octopus* in detail, but he shows none of the branchial gland. In short, the glandular part of the branchial gland has no nerve supply and this fact has an important bearing on the function of the gland.

B. Embryology

I have examined some embryos of *Sepia esculenta*, *Sepiella* and *Octopus vulgaris*. As is known, the gill develops first as an evagination from the ventral surface of the visceral mass, on each side, which consists of a superficial ectodermal layer and a mesodermal matrix (Kölliker 1844, Joubin 1885, Faussek 1901, Ranzi 1931).

In the early embryo of *Sepia esculenta* (mantle length 0.8 *mm*, Text-fig.7), the gill rudiment is a simple-pinnate process, with 5 leaflets on each side. The covering epithelium is compact, while the mesoderm which forms the axis of the gill as well as the interior of the leaflets, is rather loose, homogeneous, and consists of 1–3 cell layers. In this stage the branchial gland (Text-fig. 8, bgr) forms the whole central core of the gill rudiment, other organs have not appeared yet. In an older embryo with 1.0–2.4 *mm* mantle length the branchial gland is not yet differentiated. In still older embryos (mantle length 2.8 *mm*, Text-fig. 9; mantle length 3.2 *mm*, Text-fig. 10) the branchial leaflets (9–10 on each side) are elongated and covered by a thin film, having affinity with haematoxylin. The interior is filled with loose tissue containing small cells. The branchial gland is made up of cells larger in size and compactly gathered together. In still later embryos (mantle length 4 *mm*, branchial leaflets 16 on each side) the leaflet has begun to ramify, and the histological characteristics of the branchial gland are more manifest, the component cells being larger and showing strong basophily which is unique among the various tissues of this stage.

In the sagittal section the gland appears as a broad band, attached to the dorsal side of the gill ; the blood vessels run from the ventral to dorsal side and the branchio-lienal vein is already formed. When the embryo hatches out (mantle length 4.8 *mm*, Text-fig. 11), the tissue of the gland is clearly differentiated, and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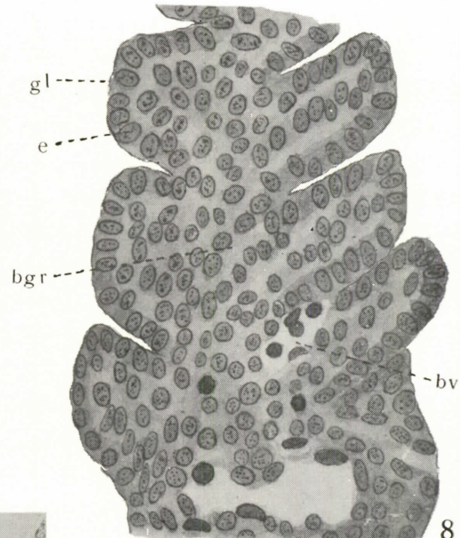
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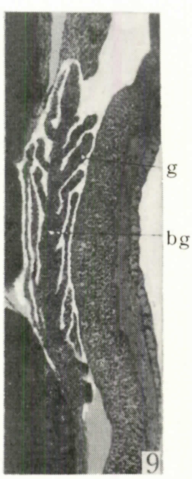
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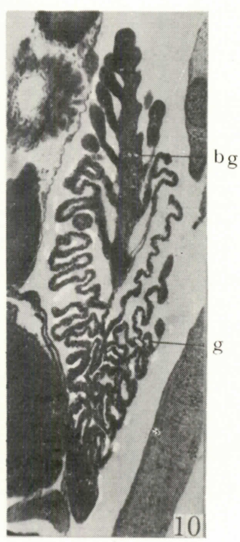
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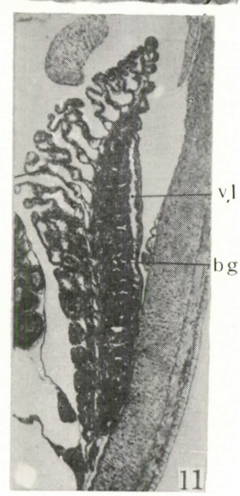
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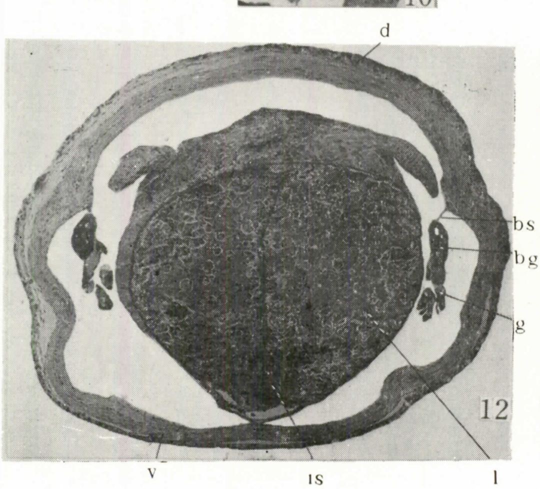
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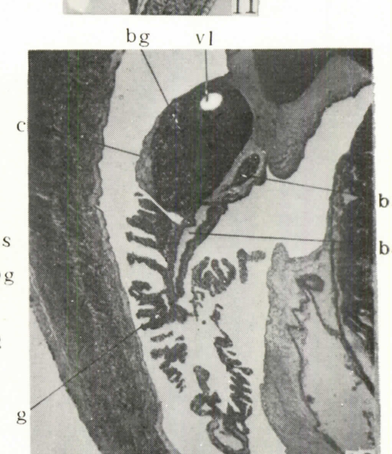
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the capsule may be discriminated as a single-layered epithelium.

In the newly hatched larva of *Octopus vulgaris* (mantle length 1.4 *mm*, Text-fig. 12), the branchial gland is marked by its staining characteristic, though it is quite small as compared with that of *Sepia*. In the free-living young (mantle length 5.5 *mm*, Text-fig. 13), the tissue is nearly completely differentiated as in an adult animal.

Thus it is clear that the branchial gland is derived from the mesoderm, and its development is due to (a) the increase of the glandular cells, (b) the formation of blood vessels in the intercellular spaces, which gradually develops into a complicated vascular system, and (c) the differentiation of the capsule, which consists of an ectodermal and a mesodermal connective tissue.

It may be noted that (1) the basophilic nature of the cytoplasm appears quite early, (2) but the secretory globules are not formed at the time of hatching. Probably the secretion begins some time after the larva has entered the free swimming life. Incidentally it may be added that the posterior salivary gland contains abundant secretion granules in the newly hatched larva of *Octopus* (stage shown in Text-fig. 12).

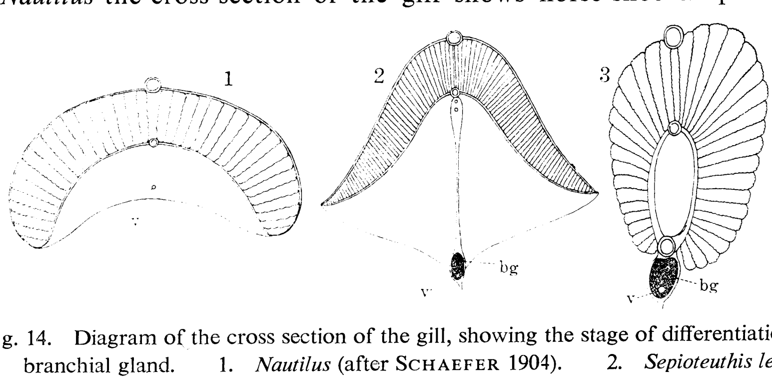
Text-figs. 7-11. Development of the branchial gland in embryos of, Text-figs. 7-10, *Sepia esculenta* (× 16) and Text-fig. 11, *Sepiella japonica* (× 10).
7. Sagittal section; mantle length 0.8 *mm* (NAEF's stage XII).
8. Part of the gill of the same, magnified (× 390).
9. Coronal section; mantle length 2.8 *mm* (NAEF's stage XIV).
10. Coronal section; mantle length 3.2 *mm* (NAEF's stage XVI).
11. Sagittal section, mantle length 4.8 mm (NAEF's stage XX).

Text-figs. 12-13. Cross section of the branchial gland in the larvae of *Octopus vulgaris*. × 10.
12. Larva just hatched out.
13. Young octopus, mantle length 5.5 *mm*.

ba, branchial artery ; bg. branchial gland ; bgr, rudimentary tissue of the branchial gland ; bh, branchial heart ; bn, branchial nerve ; bs, branchial suspensorium ; bv, rudiment of blood vessel, containing amoebocytes ; c, capsule of the branchial gland ; d, dorsal side of mantle ; e, epithelial layer covering the gill ; g, gill ; h, systematic heart ; is, ink sac ; gl, gill leaflet ; l, liver ; sh, shell rudiment ; v, ventral side of mantle ; vl, vena branchio-lienalis.

C. Comparative Anatomy (Text-fig. 14)

In *Nautilus* the cross section of the gill shows horse-shoe shaped branchial



Text-fig. 14. Diagram of the cross section of the gill, showing the stage of differentiation of the branchial gland. 1. *Nautilus* (after SCHAEFER 1904). 2. *Sepioteuthis lessoniana*. 3. *Paroctopus dofleini*. bg, branchial gland ; v, vena branchio-lienalis.

lamellae, the central region of the axis of the gill represents the branchial gland of Dibranchiate Cephalopods (GRIFFIN 1900, ROBSON 1932). In the Decapoda the gill is compressed laterally, and the branchial gland is clearly differentiated, and situated on the dorsal side of the gill, partly imbedded in the axis of the gill. In the Octopoda, the branchial lamella is so strongly compressed laterally that the branchial gland is situated between the gill and the mantle. This series seems to indicate the stage of gradual differentiation of the gland in different groups of Cephalopods ; attaining the maximum in the Octopodidae. ROBSON (1926, p. 1344; 1932, p. 128) says that the gland tends "to be smaller in the Cirrate and other abyssal Octopoda," which can be assumed as a rudimentary or degenerative feature.

The branchial gland is called hypobranchial gland by some authors, but it is not homologous with the hypobranchial gland of Prosobranchs (TARAO 1935), Opisthobranchs (GUIART 1901), nor of the Protobranchiate bivalves (Nuculidae and Solenomyidae), because they are different in the location in the body, development and also in function. The branchial gland is found only in the Cephalopoda and in no other class of Mollusca.

Mainly on account of our ignorance of its real function, the organ has been called variously by previous authors[2]. To me "branchial gland" seems to be the most appropriate name of the organ.

D. Histology

a. General Structure

In structure the branchial gland is essentialy identical in both Octopoda and Decapoda. It is composed of (1) the capsule, (2) the gland cells and (3) the blood vessels. The capsule consists of an epithelial layer on the surface and a connective tissue lying beneath. The epithelium is composed of columnar cells and covered by a thin cuticula, and scattered sparsely with albumen glands and mucous glands. The connective tissue contains longitudinal and oblique muscles, as well as fibrilles and blood vessels which are especially abundant in the middle layer of its thickness.

The blood vessels, which are the branches of an artery and two veins, appear abundantly in sections. The branches of the efferent vein (vena branchio-lienalis) are especially well developed in all parts of the gland. They have comparatively large calibre and very thin wall. This feature apparently serves for retarding the blood stream, and for the easy penetration of the secreted matter.

The gland consists of numerous acini encircling the capillaries. The gland

1) Glande hypobranchiale (TURCHINI 1923, YUNG KO Ching 1930).

2) Glande de la branchie, glande branchiale (JOUBIN 1885) ; corpo o ghiandole o milze branchiale o peribranchiale (SERENI 1930, MITOLO 1938) ; Corpus branchiale, Kiemenbanddrüse (HANSTRÖM 1937, 1939) ; Branchialkörper (BOETTGER 1933) ; Branchialdrüse (KOLLER 1938); Milz, Blutdrüse od. Kiemenmilz (MEYER 1906, 1913 ; GRIMPE 1913 ; SCHAEFER 1904 , NAEF 1923) : Kiemendrüse (BAUER 1909).

cells are uniform in structure; they are polygonal in section, and each of which is enveloped with a cementing membrane, forming a stroma. The gland is enveloped by a thick membrane. The endothelium of arteries is rather thick, and shows depressed nuclei, but that of smaller veins and capillaries is very thin, and insensibly changes to the cementing membrane. This feature is well shown in materials fixed with Flemming's or Champy's solutions, in which gland cells show conspicuous shrinkage and become separated (Text-fig. 17). JOUBIN (1885, p. 114) noticed the absence of capillaries in the gland, but this view is incorrect. Under high magnification we find that the so-called blood lacunae are lined with endothelium, and this fact has been pointed out by SCHAEFER (1904).

b. Course of Circulation of Secretory Globules

The secretion globules are poured into the capillaries, which are very well developed in the central core of the gland. They are next transported by means of branchio-lienal vein into the branchial heart, and then into the gill through the branchial artery, which sends blood to the branchial lamella as well as the branchial gland. It is probable that a certain amount of blood circulates in the route between the branchial gland and the branchial heart, without being aerated in the gill, so that a part of the secretion can not be distributed in the body. But the majority of the secretion globules seem to be transported by way of the branchial lamella, branchial vein, systematic heart, aortae and arteries.

c. Gland Cell

i. Gland Cell and Secretion

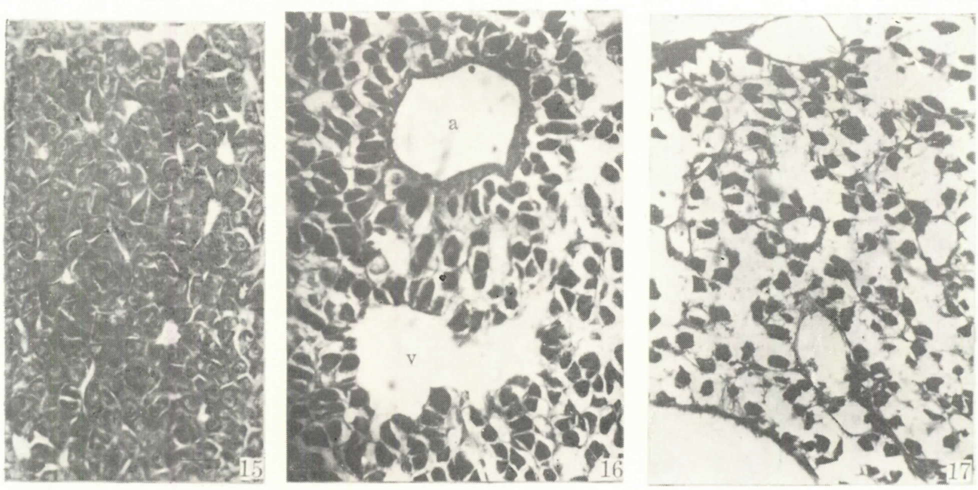
The nucleus of the gland cell is relatively large, spherical, and contains rather conspicuous chromatin network and relatively large and vesicular nucleolus weakly stainable with basic dyes. The nucleus is situated generally near the base of the cell; when the secretion globule is enlarged, it becomes depressed.

The cytoplasm is strongly basophilic, which distinguishes the gland sharply from other tissues. As already stated, this feature appears first at the embryonic stage, and is maintained throughout the life. It is stained *violet red* to *lilac* with Delafield's haematoxylin and eosin, and nearly black to grey with Heidenhain's haematoxylin, while the chromatin and nuclear membrane are stained *cotunga purple* with the former dye.

Generally speaking, the cytoplasm of the animal tissue is acidophilic, and if it shows basophily it means some peculiarity of its nature. The scarcity of arterial supply to the branchial gland is apparently nothing to do with the staining peculiarity of the gland cell of the branchial gland. And there must be some other cause.

All of the fixing reagents tried have brought about shrinkage of gland cells which is another characteristics of the cell. In order to minimize such artefact, the following methods were employed : a piece of the middle portion of the branchial gland of a medium-sized *O. vulgaris*, was fixed with the various kinds of fixatives which brought about shrinkage of the cytoplasm in the order : Lewitsky <Nawaschin, Zenker, Flemming weak <Flemming strong, Bouin, 10 % formalin <Benda <Champy.

Among these fixatives, Lewitsky's solution causes no shrinkage of cytoplasm at the periphery of the section, but brings about shrinkage of the internal cells. However, by Nawaschin's (Text-figs. 15–16; Pl. 1, figs. 11–12) and Zenker's solutions the shrinkage is slight, though the boundary between the secretion globules and cytoplasm is somewhat obscure. But for general use these solutions are satisfactory. So Nawaschin's fluid was used for fixing all kinks of tissues. While,

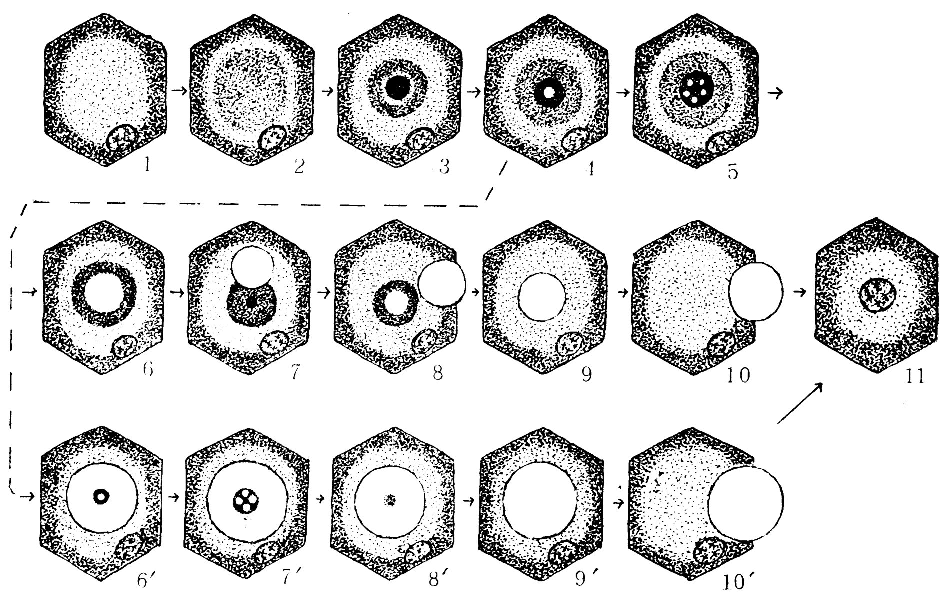


Text-figs. 15-17. Sections of the branchial gland of *Octopus vulgaris*. ×134.
15. Material fixed with Nawaschin's fluid ; a preparation made by the author in which the shrinkage of cytoplasm of the gland cell was in the least degree. 16. Material fixed with Nawaschin's fluid, showing a thick-walled artery (a) and a thin-walled vein (v). 17. Material fixed with Champy's fluid, showing the shrunken component cells and the delicate cementing substance, together with numerous capillaries.

hot sea water and hot Zenker's solution result no shrinkage, but produce vacuoles in the cytoplasm, the most undesirable artefact. Thus it has been hardly possible to fix the gland cell without shrinkage; perhaps this is due to a peculiar physicochemical property of its protoplasm.

The secretory cycle of the gland in *O. vulgaris* may be divided as follows:
(1) In the stage of the height of the secretory activity the cytoplasm is stained deeply with basic dyes. The nucleus is pushed to one side, and the first rudiment of secretion globule is formed in the central portion (Pl. 1, figs. 1–2). The globule is minutely granular and basophilic, and encircled by a layer of cytoplasm of thin consistency. It grows more basophilic and loses its granular appearance and acquires a sharply defined outline. At the centre of the globule there appears a small spherule which is extremely basophilic of variable size and form (Pl. 1, figs. 3, 5; Text-figs. 3, 7) The globule is generally only one, but may be 2–3, in each cell.

(2) The globule becomes stained with acidic dyes (Pl. 1, figs. 4–6: Text-figs. 4–8, 6′–8′), namely *crimson* with eosin, but the central spherule retains its basophilic nature, until the time just before the liberation of the globule. There seem to be two types of secretion, namely (i) the change from basophilic substance to eosinophilic one occurs from within (Text-fig. 18, nos. 1–9), and the secretory globule is poured out twice (nos. 8–10), so that each globule is somewhat small ; (ii) the change occurs mainly from outside, and secretory globule is poured out once, and the globule is very large (nos. 6′–10′). It is not clear what this difference means, but perhaps this may be a simple and not fundamental difference, because there is no variation in the ultimate production.



Text-fig. 18. Diagram showing the secretory cycle of the branchial gland cell of *Octopus vulgaris*. Nos. 1-11, first type, nos. 1-4, 6′-10′, 11, second type of secretion. Stippled part basophilic, white part eosinophilic.

(3) The homogeneous, strongly acidophilic refringent globule (Pl. 1, fig. 7 ; Text-figs. 8, 10, 10′) is now sent into the capillary, through the thinnest part of cytoplasmic envelope, so that a large vacant part is left in the centre of the cytoplasm, which is weakly basophilic (Text-figs. 10, 10′).

(4) The central part is gradually condensed, the nucleus returns to the middle, and all the original features of the cell are recovered (Pl. 1, fig. 8).

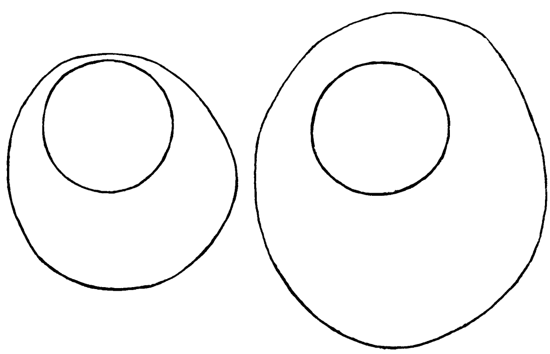
The secretion globule usually occupies the whole bulk of the cell. The secretory substance is apparently subdivided as it is transported from a capillary to large vessels (Pl. 1, fig. 11), at the same time its acidophilic character is gradually lost.

As far as my observation on the organ in *O. ocellatus* and *Sepia subaculeata* goes, the secretory process is much similar to the above, but in *O. minor variabilis* it is somewhat different. In this species aggregates of 20–50 secretory cells may be found in a section (Pl. 1, fig.12). Each cell shows a depressed nucleus and stains entirely *crimson red* with eosin, although the cytoplasm is scarcely visible. In the other species the secretory cells are scattered here and there, never forming aggregates.

The activity of secretion is apparently variable, though the secretion seems to be continued throughout the year in all species of the Cephalopods examined.

ii. Vital Staining

When the component cells of the gland are isolated they become spherical (Text-fig. 19), 15–20 μ in diameter and containing a nucleus 8 μ in diameter. The cytoplasm is extremely soft, and disintegrates by slight injury and flows out in a colloid film, retaining only the nucleus in its original state.



Text-fig. 19. Outlines of two isolated cells of the branchial gland, with their nucleus, in a living state. ×2000.

The nuclei[1], the immature secretory globule[2] and the central spherule of secretory globule[3] are stained in various degrees with methylen blue, thionin, brilliant cresyl blue, toluidin blue, trypan blue and Janus green, but the large, highly refringent secretory globule[4], which is ready to be secreted, does not take any stain. When neutral red or Nile blue sulphate is applied, there appear a number of deeply stained globules of various sizes scattered all over in the cytoplasm, of which the peripheral ones are larger (Pl. 1, fig. 10). The large secretory globules stain weakly with Nile blue sulphate. In Sudan III the small granules are stained but not the larger secretory ones. These facts suggest that the globules are of fatty or lipid nature. YUNG KO CHING (1930), applying neutral red to the branchial gland of *Sepia*, concluded these globules as to be vacuoles, and denies the presence of fatty or other inclusions (p. 350), but without any cogent reason given for the view. I could find a cross band in the secretory globule, neither in the fresh nor in the fixed material, under the polarised micro-

1), 2), 3), Basophilic in the fixed preparation.
4) Eosinophilic in the fixed preparation.

scope. Nevertheless, it may be surmised from the above findings that there is some lipid substance or its compound in the cytoplasm as well as in the secretory globule.

iii. Some Staining Reactions

(A) Feulgen's nucleal staining. The cytoplasm is stained *eosin pink*, the nucleus *rosolanc purple* and *eugenia red*, thus the differentiation between the nucleus and cytoplasm is very clear (Pl. 1, fig. 9).
(B) Van Gieson's staining. The nucleus and cytoplasm are stained brownish purple, secretory globule yellow, cementing substance and capsule red.
(C) Mallory's triple staining. The nucleus and cementing substance are stained deep *Lyons blue*, cytoplasm *orange yellow*, secretory globule *spectrum red*, capsule *violet red*, ground substance of connective tissue *blue*.
(D) Oxidase reaction. By the Nadi-reaction, no sign of oxidase in the branchial gland was found.
(E) Kon's silver reaction. This reaction was negative in *Octopus*. It is known that the majority of the endocrine organs in mammals show this reaction (Kon 1933).

iv. Physiological Renewal

Necrotic cells are found fairly frequently in the tissue of the branchial gland, even in a healthy animal. Such cells are usually gathered in small groups. The cytoplasm is weakly basophilic or acidophilic and looks homogeneous; the nucleus is very small, condensed, homogeneously basophilic, and shows a pycnotic appearance. This shows that a part of the gland is gradually degenerated and replaced by a new tissue produced by the remaining parts. Thus the tissue is constantly renewed. This process is physiological and not pathological. It is likely that Mayer (1835) and Joubin (1885) have taken the branchial gland for an amoebocytogenic organ, based on the observations of these cells containing pycnotic nuclei.

v. Post-Mortem Change

If a piece cut from the branchial gland is left for some time (30 minutes to 8 hours) in the air, the component cells soon undergo disintegration, which can not be prevented even by keeping the piece at low temperatures (−10° to 0° C). Possibly this disintegration is due to the activity of a powerful autolytic enzyme contained in the gland cell. The secretory globule, however, retains its typical form and staining reaction all through the process, showing that it is not decomposed even under such condition.

vi. Remarks on Histology

Joubin (1885), Schaefer (1904), Isgrove (1909), Sereni (1930) and Mitolo (1938) all simply give that the cell of the branchial gland is polygonal and contains a large vesicular nucleus. TURCHINI (1923, 1923a) for the first time has given a brief description on the secretory activity of the gland cell, which he divides into three phases, and has also confirmed that the secretion actually enters the blood. This process was also observed by HUTCHINSON (1928). YUNG Ko Ching (1930) has given detailed descriptions of the mitochondria and Golgi bodies in the cell of the branchial gland, in fresh or fixed materials of young Decapods, but without showing the large secretory globule at all. He did not find the pycnotic nucleus noticed by TURCHINI, possibly because of that his material was too young or the secretion was rather inactive.

CAZAL & BOGORAZE's description (1949) agrees generally with my result; for example, these authors give the diameter of the gland cell to be 15–25 μ in *Octopus*, but the species name of the material employed is not shown. Concerning the statement, "Les région centrale juxta-nucléaire est plus claire, un peu acidophile, parfois d'aspect vacuolaire. Certaines cellules contiennent deux ou trois noyaux." (p. 226) I think that this shows a transitional stage of the formation of the secretory globule, not the definite structure of the gland cell itself. I did not observe, however, a case of a multinuclear gland cell ; this may be plausible, as new gland cells are added by cell division in the consecutive series of physiological renewal, and then multinuclear cell may be formed accidentally. While, "absence de conduit excréteur," one of histological features of the branchial gland which they give, is erroneous ; I have already shown that vena branchio-lienalis transports the secretion to the blood stream of the body.

It is a curious fact that nearly all previous workers did not notice the shrinkage of the cytoplasm in the glandular cell of the branchial gland; perhaps they took it for the failure of their microtechnique. Only JOUBIN (1885) clearly described this change in an early embryo of *Sepia*, in which the shrunken cell is connected one another with "prolongements" (p. 94; pl. 4, fig. 23),though he did not show such shrinkage in an adult *Ommastrephes* (pl. 5, fig. 10).

(IV) PHYSIOLOGY OF BRANCHIAL GLAND

There are two methods for studying the endocrine function of the branchial gland : (1) the observation of the symptoms which appear after the suppression of its activity, by ligating blood vessels, extirpating the gland, or indirectly, by placing the animal in abnormal physiological conditions, such as castration, inanition or anaemia, and (2) the examination of the physiological effect of the extract of the branchial gland on other individuals with intact or extirpated branchial gland.

A. Suppression of Function of Branchial Gland

a. Ligature of Branchial Vein

The branchial vein is separated from other organs at the part where it enters the visceral mass, so that it is quite easy to ligate this vessel (Text-fig. 1, bv).

When ligature is done successfully, the aerated blood can not return the systematic heart. Then the blood becomes stagnant in the gill, and the function of the branchial heart is depressed. Furthermore circulation in the afferent vein of the branchial gland is stopped, and causes the decrease of the function of the branchial gland. This technique, however, gives hinderance to the function of the gill, so that only the unilateral operation is feasible. Several octopods were subjected to this experiment, of which one may be described in the following (Table 3; Pl. 2, fig. 1). By 103 days after the experiment of the animal, the branchial vein had lost its connection with the systematic heart at the ligated position,the branchial heart had been reduced in size and probably had stopped pulsation. The gill was in anaemic condition, and the tissue showed clear sign of degeneration. The capsule of the branchial gland and especially the branchial suspensorium edematised. Histological examination showed that the branchial gland of the ligated side had no secretion, and was undergoing degeneration, while that of the unligated side apparently functioned normally.[1] In this case the animal kept its activity in nearly normal degree by the hypertrophy and compensatory function of the branchial gland of the unligated side.

Table 3. Growth relation of two animals in which the left branchial vein is ligated. Duration, 103 days. Body weight in g.

Date (1937—38)		No. experiment 1 ♂	2 ♂
4.	X.	450 g	410 g
26.	X.	598	580
5.	XI.	695	595
24.	XI.	835	744
14.	XII.	834	819
29.	XII.	935	900
15.	I.	1016 (killed and fixed)	897 (killed and fixed)
Increase in body weight		566 g	487 g
The same,	%	125 %	119 %

b. Ligature of Branchio-lienal Vein

Since the secretion of the branchial gland is gathered and transported by the branchio-lienal vein, the ligature of this vein will check the function of the branchial gland. After the mantle septum is excised and the mantle is turned over, the branchio-lienal vein is ligated with a silk thread at the posterior end of the branchial gland (Text-fig. 1, vl). This operation can be done on both sides at

1) A similar abnormality (loss of connection between the branchial vein and auricle, on one side) was observed in three cases found in nature. At autopsy no serious lesion was found except a moderate atrophy of the branchial heart and kidney of the affected side. The origin is not clear altogether, but such cases may be happened to be caused by the fighting among these carnivorous animals.

the same time. The operated animal does not show any bad symptom except that the colour change in the integument is more or less suppressed and the animal remains brown all the time, without presenting dark or pale phase. In about two days, however, the animal becomes as vigorous as before. When the experiment is ceased, a dilute methylen blue solution is injected into the branchial gland. It is distributed in the interior of the mantle through the blood vessel of the capsule of the branchial gland, since the branchio-lienal vein is completely interrupted by the ligature. It is likely then that, by the compensatory dilatation of the capillaries in the capsule of the branchial gland, which takes place a few days after the operation, enables the transport of the secretion. As is well known, the delicate capillary anastomoses are well developed in *Octopus*.

This experiment shows that, for the life of the animal the branchial gland should be connected with blood vessels and also that the secretion can be transported by other vessels than the branchio-lienal vein. Thus it is shown that the function of the branchial gland can not be suppressed by this means.

c. Extirpation of Branchial Gland

Through the above, the decisive result may be obtained by the extirpation. But, if the branchial gland of a healthy animal is cut off with scissors even partially, it dies in a few hours. Such result is plausible, as the gland is abundantly vascularised and the haemorrhage does not stop at the wound in marine Mollusca in general. The operation is done successfully as follows: the branchial gland is cauterised by applying a red-hot platinum wire on the capsule. This operation should be done gradually; after the capsule is partially destroyed and the tissue of the gland becomes very soft and milky white in colour, the gland is taken off carefully with forceps. In the control animal a part of the integument or the gill is cauterised in about the same degree as the experimented animal.

As will be shown below, I came to the conclusion that the branchial gland is obviously an endocrine organ in Cephalopods, so that I propose to use such terms as branchialectomy, branchial insufficiency etc. in analogy of the terminology current in vertebrate endocrinology.

i. Incomplete Branchialectomy

The branchial gland was unilaterally extirpated. The operated animals appeared quite healthy and took as much food as controls, though they were inferior in growth to the controls. The remaining branchial gland showed a conspicuous hypertrophy; autopsy showed it to be nearly twice as heavy as normal. The existence of one branchial gland thus gives no serious effect on the life of the octopod.

ii. Symptoms of Branchialectomy

The symptoms which can be observed after branchialectomy may be summarized as follows:

A. The first period: The effect of the branchial insufficiency is seen first in the behaviour of the animal which becomes more sluggish than usual. It also takes little food. The colour change of the integument occurs only weakly; the tegumental injury due to banding[1] appears as a dark ring around the base of the arms.

B. The second period: The animal feeds no more and becomes very sluggish, the colour chang is largely suppressed and the animal sits usually in the corner of the aquarium or near the water surface, with all its arms gathered on the body. The injured area of the base of arms is enlarged, and its middle part becomes desquamated, exposing the hypodermic connective tissue.

C. The third period: The animal is in moribund condition (Pl. 3, fig. 1); the integument is completely pale and the colour change is no more seen; the desquamated area is much enlarged, the mucilage secretion in the integument is completely suppressed, the frequency of respiration greatly decreased, the animal usually attaches on the wall of the aquarium, and the arms have lost the contracting power and hang down in clusters, with each arm more or less coiled[2]. The body is edematised, especially in the arm and the mantle (Pl. 3, fig. 4A). The animal eventually dies.

In the following the symptoms of abranchialism is described in detail according to the prominent characters:

Table 4. Number of bivalves devoured by branchialectomised and control animals. Duration, 16 days. BG, branchial gland.

Date (1938)	Operation	No. experiment CU 3 ♂	No. control C+U 1 ♀
3. XI.	Left BG cauterised	—	—
5. XI.	Left BG extirpated, right BG cauterised	52	40
7. XI.	Right BG 3/4 extirpated	—	—
8. XI.	———	10	8
9. XI.	Completely branchialectomised	—	—
10. XI.	———	3	0
12. XI.	———	6	30
13. XI.	———	1	21
14. XI.	———	0	28
18. XI.	———	0	51
19. XI.	———	0 (died)	29
Total number of bivalves devoured		72	208
Body weight { At beginning		412 g	282 g
Body weight { At end		464 g	381 g
Increase in body weight		52 g	99 g
The same, %		13 %	35 %

1) At the operation the animal is enclosed in a cloth bag and all the arms are banded to immobilise the animal. (cf. TAKI 1941).

2) There is a tendency that the arm of *Octopus* is spirally coiled in moribund condition ; this is well shown if a fresh *Octopus* is put into formalin solution when its arms still have the contracting capacity.

(A) Pathological Ecology and Anatomy

1. Appetite

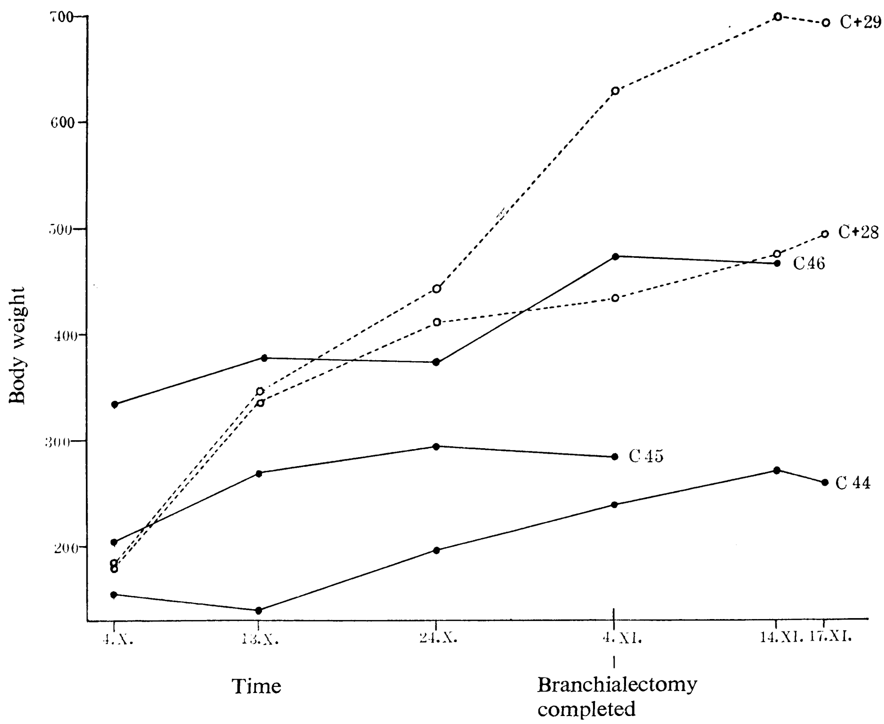
The appetite of the animal can be measured by the amount of food it takes, when it is kept isolated. The common bivalve, *Tapes semidecussata*, is given as food. The octopus opens the shell with its arms and eats the flesh. The cauterisation gives obvious effect to the appetite of the animal, branchialectomy still more. During the operation the animal feeds nearly as much as the control, but after the complete branchialectomy it sooner or later gives up feeding, and enters a hunger condition. The difference in the amount of food in both groups becomes much more pronounced as time goes on. The control animal usually sits on the floor of the aquarium, while the branchialectomised one is often attached to some substratum near the water surface, in a state of anorexia (Table 4). Autopsy shows the alimentary canal to be nearly empty; the urine production seems to be remarkably suppressed.

2. Growth

The anorexia is practically identical with the decline in growth rate, but here it will be dealt with separately. The imcompletely branchialectomised animals keep on growing but completely operated ones do not. The latter animal shows a hunger symptom, though the body weight does not show any marked decrease. This is due to the edematisation of the connective tissue in the body, especially in the third period of abranchialism (Table 5; Pl. 3, figs.2–3).

Table 5. Growth of *O. vulgaris* in branchialectomised and control sets of animals. Body weight in g ; duration, 44 days.

Date (1937)	Operation	No. experiment			No. control	
		C 44 ♂	C 45 ♀	C 46 ♀	C+28 ♀	C+29 ♂
4. X.	Both BG cauterised	156 g	204 g	335 g	282 g	285 g
13. X.	————	149	271	375	338	345
24. X.	————	198	295	374	413	444
4. XI.	Completely branchialectomised	240	284 (died)	475	435	631
14. XI.	————	272	—	468(died)	475	700
17. XI.	————	258(died)	—	—	495	695



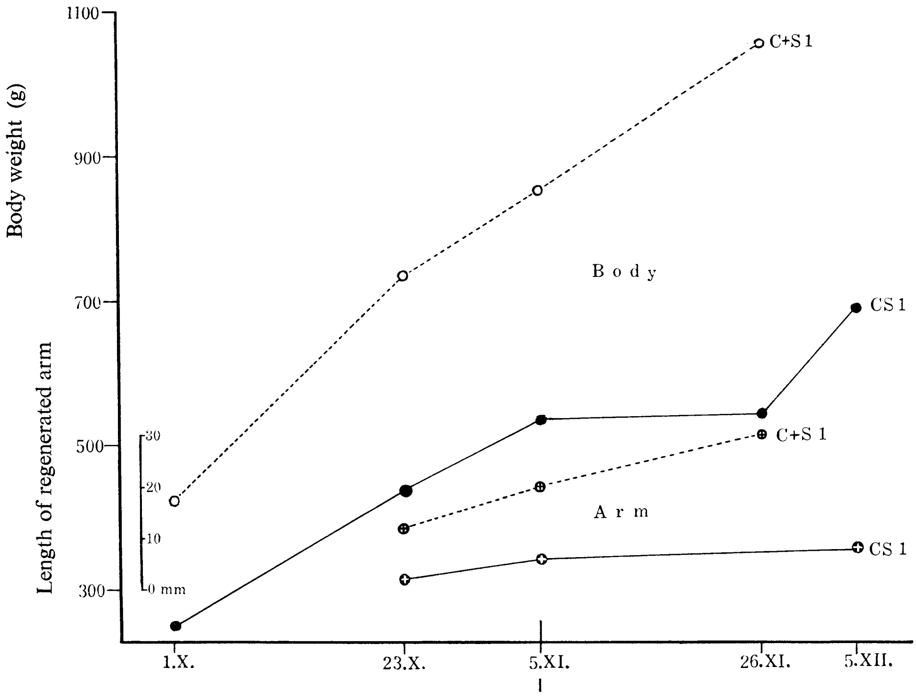
Text-fig. 19. Growth of *Octopus vulgaris*, in branchialectomised and control sets of animals. Data given in Table 5. See Pl. 3, figs. 2-3.

3. Regenerating Power

The tip of an arm and a small area of the integument in the dorsal mantle were amputated and their regeneration was observed. The branchial deficiency clearly reduces the regenerating power. The existence of even a small piece of the gland is sufficient for normal regeneration. This is obviously shown by Table 6, Text-fig. 20 and Pl. 4, fig. 2.

Table 6. *O. vulgaris*. Regeneration of the arm tip, together with the growth of the body, in branchialectomised and control animals. Duration, 65 days.

Date (1938)	Operation	No. experiment: CS 1 ♀ — Body weight	No. experiment: CS 1 ♀ — Length of regenerated arm	No. control: C + S 1 ♀ — Body weight	No. control: C + S 1 ♀ — Length of regenerated arm
1. X.	Branchial-	249 g	0 *mm*	428 g	0 *mm*
23. X.	ectomy	437 g	2 *mm*	735 g	12 *mm*
5. XI.	completed	538 g	6 *mm*	856 g	20 *mm*
26. XI.	———	544 g	——	1055 g (killed)	30 *mm*
5. XII.	———	692 g	8 *mm*	——	——



Text-fig. 20. *O. vulgaris*. Regeneration of the arm tip, together with the growth of body, in branchialectomised and control animals. Data given in Table 6, See Pl. 4, fig. 2.

4. Frequency of Respiration and Muscular Inertia

The frequency of respiration does not change under incomplete branchialectomy, but it does under complete branchialectomy, the change becoming more marked to symptoms of the deficiency. When it decreases to less than half the normal number, the animal dies sooner or later. In *O. vulgaris* the frequency in

Table 7. Frequency of respiration per minute in branchialectomised and control animals. Duration, 21 days.

Date (1938)	Operation	No. experiment		No. control	
		C U 2 ♀	C U 3 ♂	C+U1 ♀	C+U2 ♀
3. XI. } 9. XI. }	Branchialectomy completed	—	—	—	—
17. XI.	————————	27	27	28	27
18. XI.	————————	28	9 (moribund; killed)	33	34
24. XI.	————————	19 (moribund; killed)	—	—	—
Body weight	At beginning	413 g	412 g	282 g	427 g
	At end	740 g	464 g	381 g	575 g

medium-sized individuals is about 30 per minute (POLIMANTI 1913, PELSENEER 1935, p. 35) (Table 7). It is known that the frequency of respiration agrees with that of the pulsation of systematic heart (PELSENEER, loc. cit.), so that this frequency may be made an indicator of the vitality of the animal.

It is obvious that branchialectomy causes muscular inertia of the animal, which leads to the failure of the pulsation of the heart and respiration. The sucking strength of the arm decreases with the progress of branchial deficiency so much that the animal is no longer capable of opening the shell given as food. Just before death the animal often emits ink, which is poured out without ejaculation. This is due to the relaxation of the sphinctor muscle of the ink duct.

5. Symptoms in the Integument

A. Decrease of contracting power of dermal muscles: In the last state of abranchialism no wrinkles are formed by stimulation; the elasticity of the skin decreases so that it is easily torn.

B. Failure in the colour change: The ability of colour change gradually decreases and completely lost in the moribund state, becoming thoroughly pale.

C. Decrease of slime production: This is seen with the progress of branchial deficiency, so that the surface of the skin becomes rough.

D. Desquamation: As already stated, the banded area of the integument appears at first blackish, which is gradually enlarged and desquamated as the branchial deficiency proceeds. Thus there appear irregular whitish spots or bands around the base of the arm (Pl. 1, fig. 16), though the tip of the arm remains intact. In the control animal no tegumental lesion appears. The desquamation does not appear in healthy animals. It extends along a thin groove of tegumental wrinkles, so that the outline is irregular and does not always agree with the banded area.

6. Gill

By the extirpation of the branchial gland, the gill becomes shortened (Pl. 3, fig. 4A). The axis of the gill is supported by a rod-like process consisting of a connective tissue of hard consistency, somewhat resembling a cartilage (Pl. 4, fig. 1). But this fact shows that the power of maintaining the length of the gill axis of the gill supporter is weak. KOWALEVSKY (1894) once stated that the branchial gland merely serves for a support to the gill as a kind of axial skeleton (cf. BAUER 1909, p. 261), but this view can not be entirely ignored. It is true that, the axial contraction of the gill is chiefly due to the failure of fibrilles contained in the axis of the gill, but the branchial gland serves to support the gill merely by the fact that it is naturally located beside the gill. But this is, of course, of a secondary meaning.

7. Anaemia and Blood Pressure

One of the most important symptoms of abranchialism is anaemia. Autopsy of branchialectomised animal shows that the gill leaflet is contracted, yellowish brown in colour and the blood vessels are also contracted. The blood pressure, though not measured, is evidently low. In the control animal the gill as well as blood vessels are well swollen and coloured bluish. MITOLO (1938) also confirmed the decrease of blood pressure by branchialectomy.

8. Gnoad

Branchialectomy apparently results no atrophy to the testes, vas deferens, Needham's sac and penis. In one case, however, I have observed degeneration of ovary into a small brownish clump, while its capsule and oviduct with oviducal ball was normal (Pl. 3, fig. 6). This state somewhat resembles that seen in ovaries of starved animal, because the branchialectomised animal does not feed. However, it is supposed that there may be an intimate relationship between the existence of the branchial gland and the maintenance and development of the ovary, as in this case only the ovary was particularly degenerated.

9. Edema

Another prominent symptoms of branchial deficiency is the edema, which occurs some time after the branchialectomy has been completed gradually. It does not appear when death ensues immediately after branchialectomy, or in the state of incomplete branchialectomy. The edema attacks the connective tissue of all parts of the body, but especially the hypodermis. The mantle and arm become swollen considerably (Pl. 3, fig. 4A), though the change appears more in one of the organs than the other. In one case the eye became nearly completely concealed under edematised coating (Pl. 3, fig. 5). The inside of the mantle is not usually edematised. Next, the edema is prominent in the visceral sac, and also in the eye-ball, crop, stomach and intestine, gill, branchial heart, systematic heart and gonad. The edematised tissue is transparent, soft and jelly-like. It is clear that the marked increase in the body weight seen at the last stage of branchial deficiency is due to this edematisation of the body.

10. Vitality

It is clear from the above observations that the existence of the branchial gland is indispensable for the normal vitality of the animal, and its complete deficiency invariably causes death. The experiments show that the loss of vitality after branchialectomy is conditioned by the following factors:

A. The velocity of operation: Rapid extirpation results early death, the gradual one allow the animal to live much longer.

B. The age of the animal: Younger animals die earlier than older ones.

C. The season of the year: The vitality is high in autumn and winter, and low in summer.

These facts suggest that the branchial gland has an important bearing on the metabolism of the body. Also, as will be described later, the animal gets very weak and sluggisth when its branchial gland is in a pathological condition. Thus, it is clear that the branchial gland is quite important for normal life of the octopus.

(B) Pathological Histology

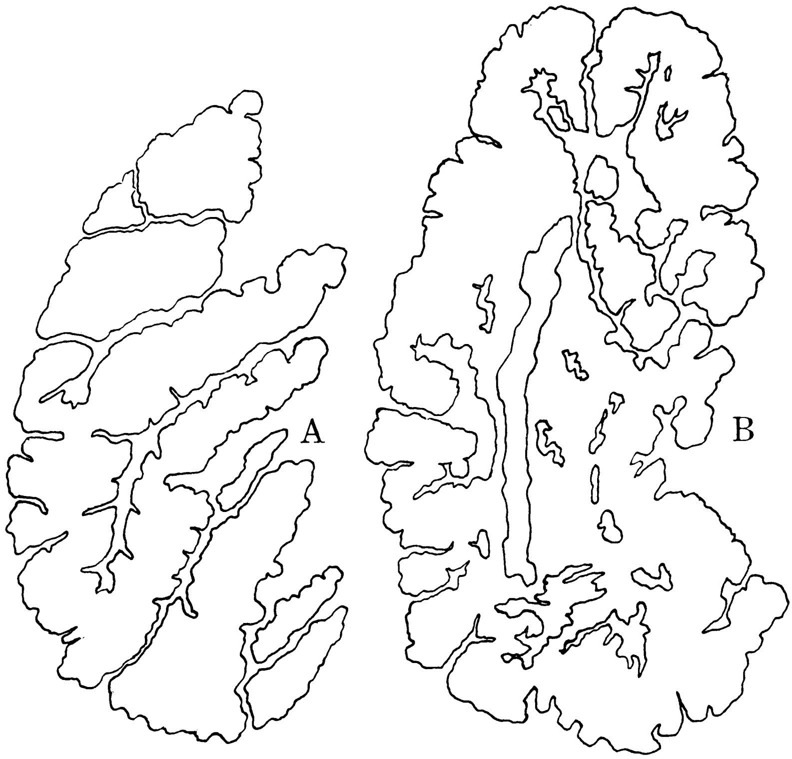
The common feature of all sorts of tissues in the branchialectomised animal is anaemia and atrophy, which is manifested in their poor staining capacity in histological preparation. The edema is induced by an enormous increase of the ground substance of the connective tissue which has small staining capacity, besides stellate cells and isolated fibrilles scattered therein.

1. Integument

Item	Branchialectomised octopus (Pl. 4, fig. 4)	Control (Pl. 4, fig. 5)
A. General feature	thick and swollen	thin and contracted
B. Surface	nearly smooth	well wrinkled
C. Epithelial layer	partly desquamated or disintegrated, with a regenerated tissue here and there	not desquamated
D. Mucous gland	atrophied	well developed
E. Chromatophore layer of banded area	partly desquamated	not desquamated
F. Muscular layer beneath chromatophore	partly disintegrated	not disintegrated
G. Hypodermic innermost connective tissue	thickened	not thickened
H. Edema	present	absent

2. Gill

Item	Branchialectomised octopus (Text-fig. 21,A)	Control (Text- fig. 21,B)
A. Outline of leaflet	small	large
B. Ramification of leaflet	simple	complicated
C. Blood in vessel	nearly none	well congested
D. Connective tissue	well developed, tending to fulfill the interior	not developed
E. Axis of gill and wall of blood vessel	edematous	not edematous



Text-fig. 21. Outline of the longitudinal section of a branchial leaflet of a branchialectomised (A) octopus and its control (B). ×8.

3. Branchial Gland

In the branchial gland of the incompletely branchialectomised animal, the tissue of a cauterised part is completely dissociated, leaving a homogeneous, weakly eosinophilic colloid mass. In the marginal part the glandular cells are in various stages of necrosis, and the cytoplasm and nucleus show eosinophily instead of normal basophily. The change may be graded as follows:

Grade	Cytoplasm	Nucleus
A	normally granular, deeply stainable	of a normal size, nuclear membrane distinguished, chromatin condensed
B	more or less granular, weakly stainable	smaller than a normal size, pycnotic
C	melted	quite small, pycnotic
D	melted	disappeared

At distant places from the operated area there is a healthy tissue containing normal secretion.

4. Branchial Heart

Item	Branchialectomised octopus (Pl. 4, fig. 3)	Control
A. Cell membrane	not prominent, atrophied	prominent
B. Nucleus	smaller, shrunken irregularly	larger, rounded
C. Xanthin concrement at periphery of branchial heart	uniformly smaller; yellowish brown	generally larger; dark purplish brown
at centre of branchial heart	smaller: brown	larger; blackish

The xanthin concrement contained in the cell of the branchial heart is known to be waste product absorbed from the venous blood ; its larger size and deeper staining ability show that the high activity of excretory function of the branchial heart.

5. Pericardial Gland

A. Cell size	smaller	larger
B. Staining ability	weakly stained	deeply stained
C. Nucleus	smaller, shrunken	larger, rounded
D. Cytoplasm	scarce, condensed	voluminous, hyaline

6. Kidney

Item	(Pl. 4, fig. 6)	(Pl. 4, fig. 7)
A. Blood vessel	nearly vacant	congested in capillaries
B. Thickness of epithelium	partly quite thin	generally thick
C. Fold of epithelium	loosely gathered, not in lobules	compactly gathered, in several lobules
D. Excretory canal	smaller	larger
E. Excretion	scarce	abundant
F. Cell boundary	less sharply defined	sharply defined
G. Consistency of cytoplasm	uniformly, moderately thin	perinuclear part thin, peripheral part thick
H. Nucleus	irregularly shrunken	not shrunken
I. Chromatin	not sharply defined	sharply defined, with prominent network

7. Systematic Heart

A. Edematous coating	present	absent
B. Muscular wall	thin	thick
C. Eosinophilic character	very weak	strong

8. Liver

A. Thickness of epithelium	thin	thick
B. Eosinophilic granule	scarce	abundant
C. Conglomerate	scarce	abundant ; in various stages of secretion
D. Rod margin (Stäbchensaum) at upper end	absent	present
E. Staining ability of nucleus	weak	strong
F. Shrunken nucleus	present	almost absent

9. Pancreas

	(Pl. 4, fig. 8)	(Pl. 4, fig. 9)
A. Thickness of epithelium	thin	thick
B. Upper end of cell	smooth	not smooth ; vacuolated
C. Cytoplasm	nearly homogeneous	not homogeneous ; fibrillar structure present
D. Nucleus	not sharply defined	sharply defined

10. Posterior Salivary Gland

A. Intertubular connective tissue	scarce ; generally widely separated from tubules in histological preparations, due to the loss of elasticity	scarcely separated
B. Contents of tubule	scarce; each element partly separated	abundant; each element gathered compactly

11. White Body

A. Germinative centre	scarcely present	present ; quite prominent
B. Blood lacuna	nearly vacant	filled with fresh amoebocytes
C. Lipoid globule	absent	present
D. Production of amoebocyte	nearly scarcely done	abundantly done

12. Amoebocyte

A. Granule	scarce	numerous
B. Vital staining with Dahlia purple		
cytoplasm	neutral grey	grayish lavender
nucleus	Indian lake	Corinthian pink

The staining reaction of the amoebocyte suggests that the branchialectomised octopus suffers acidosis. In short, the degree of atrophy is variable in different organs ; the secretory part of the posterior salivary gland, oviducal gland and testis show little change.

B. Effect of Inanition (Pl. 2. figs. 3, 5 ; 6–7 ; Table 8)

The octopod was kept in hunger state in order to bring forth atrophy of organs, and thus to suppress the action of the branchial gland. Each individual was kept in a separate aquarium. The duration of enduring inanition is variable according to the season and age of the animal. The experiment was stopped before the death of the animal and histological preparation was made of it. The emaciated animal has lost more than 40 % of the initial weight, and also much of its muscular strength. The colour change was suppressed ; it was coloured dark gray, the reddish brown phase being almost disappeared. The branchial gland showed conspicuous atrophy (Pl. 2, fig. 6); the cell as a whole is quite small, especially the cytoplasm is scarce and hardly stained, though basophilic, the nucleus is deformed, the secretory globule is entirely atrophied. The animal in such a state eventually dies.

The inanitiated animal shows much the same atrophic change as branchialectomised ones, although no edema is seen in the former. Possibly the branchial gland, though atrophied, retained the capacity of secretion up to the last stage, as indicated by the existence of atrophied secretory globule in the gland. In this case death is due to inanition and not by the mere atrophy of the branchial gland.

1) Records of inanition experiment are scarce in Cephalopods; JULLIEN (1928) reports that *Sepia officinalis* survived 26 days in inanition.

Table 8. Decrease or increase of body weight (g.) in inanitiated and well-fed octopods. Duration, 130 days. See Pl. 2, figs. 3, 5.

Date (1939)	No. experiment H 1	No. control H + 1
9. II.	525 g	452 g
25. III.	480	526
1. V.	429	623
4. VI.	340	649
19. VI.	290	678
Decrease or increase in body weight	− 229 g	+ 226 g
The same, %	− 44%	+ 50%

C. Effect of Gonadectomy

The gonadectomy was carried out for both sexes. In the ovariotomised female, the branchial gland shows atrophy. In the castrated male, histological disintegration in the gonoduct system (vas deferens, spermatophoric gland, accessory spermatophoric gland, Needham's sac; but the penis is intact) ensues, but the branchial gland shows no great change.

SERENI (1929) and CALLAN (1940) reported on the castration in *Octopus* ; the latter author found that the castrated male has the power of regenerating the amputated tip of a hectocotylised arm, in spite of the fact that the operation gives serious disturbance to the physiology of the animal. So far as my observations go, (TAKI 1941, 1944) (1) ovariotomy gives some bad effect on the function of the branchial gland, (2) the development of the ovary is suppressed by branchialectomy, and (3) the mutual dependence of the testis and the branchial gland is probably very small, (4) ovariotomy retards the growth of the animal.

D. Effect of Anaemia

The secretion of the branchial gland is transported by the vascular system, so that the decrease in volume of blood probably gives some pathological effect on the animal. The mantle was turned over and the blood was sucked out gradually with a syringe. In one case 6.5 cc blood was sucked out in 39 days, from the branchial heart of an octopus weighing about 300 g in the beginning. The animal became weaker and weaker, feeding very little, but retained the power of colour change until directly before death. There was a severe desquamation in the banded area of the arm, like that found in the later period of abranchialism. The volume of blood in a Cephalopod is estimated as about 1/20 of the body weight (PELSENEER 1935, p. 145), so that this specimen must have had about 15 cc the blood; the blood sucked out probably amounted to 40–50 % of the total, provided that no new formation of blood did occur during the time. The loss of such large amount of blood may be fatal to the animal, but presumably the secretion of the branchial gland was continued in such an anaemic condition, which checked the appearance of edema.

E. Administration of Branchial Gland Extract

To examine the effect of the extract of the branchial gland on other animals, extract of the glandular tissue was prepared and injected into octopods and also to mice, tadpole etc.

a. To Octopus

1. To Branchialectomised Octopus

Injection of a condensed extract to an octopus gave harmful effect, so that a dilute solution was prepared: 0. 1 g of the branchial gland was cut from a healthy octopus, ground with 10 cc of sea water, filtered with a cloth, from which 1 cc was injected intraperitoneally daily. To the control animals 1 cc sea water was injected.

A case of such experiment is given in Table 9. In more than a fortnight from the commencement of daily injection, the experiment animal came to show dark reddish-brown pattern when stimulated (Pl. 1, fig. 15). This reaction is usually seen in a vigorous octopus and not observed in the control animal (Pl. 1, fig. 16). In other points, however, including all behaviours, no particular difference was noticed between the experiment and control animals. The former grew weakened several days earlier than the control. Then the two control animals were subjected to experiments, namely the extract was injected in one of them and other was left as its control. But this was continued only for 10 days. In another experiment the colour pattern also appeared in the experiment animal.

In both the experiments the experiment animal could not be kept in a healthy condition nor was it possible to let it live longer. It did not, however, show such severe edema as in the control; the thick edematous coating of the latter was probably due to the injection of the sea water, which lowered the excretory function.

The histological examination of the above three animals showed that the tissue of the body of the experiment animal is practically normal, the control (J + H2) the most abnormal, and the experiment animal in the series II (J + H1), which may be called as the second control, showed the intermediate condition.

In the control animal the capsules of the systematic heart and the branchial heart exhibited severe edema, also the capsule of the pericardial gland was much thickened and vacuolated. The interior of the pericardial gland also suffered histolysis. The kidney has atrophied, and abundance of minute refractive granules are contained in the nephridial cells, recalling much of granular degeneration in a pathological tissue. The liver and pancreas also presented greater abnormality than in the experiment animal. The white body of the

Table 9. Injection of the branchial gland extract into branchialectomised octopods, with its controls. Duration, from the beginning of injection to the end of experiment, in series I, 49 days; in series, II, 10 days. See Pl. 1. figs. 15-16.

Date (1938-39)		No. experiment (I)	No. control (I)	
		J H 2 ♀	J + H 1 ♀	J + H 2 ♂ (Pl. 2. figs. 2, 4)
		Remarks and Body weight	Remarks and Body weight	
I	3. XII.	453 g	512 g	356 g
		Both branchial glands cauterised		
	4. XII.	Completely branchialectomised		
	8. XII.	Daily injection begun (extract 1 cc)	Daily injection begun (sea water 1 cc)	
	26. XII.	Dark reddish brown pattern appeared	No pattern appeared	
	17. I.	394 g	596 g	357 g
	26. I.	Weakened 456 g (killed)	——	——
II			Experiment (II)	Control (II)
	26. I.		Daily injection begun (extract 3 cc)	(sea water 3 cc)
	2. II.		———	Weakened 595 g (killed)
	5. II.		Weakened 695 g (killed)	———

experiment animal has atrophied, namely the nuclei becoming irregular in size and shape, with chromatin condensed in a small mass, and cytoplasm showing disintegration here and there. These findings show that the animal was in an anaemic condition, inasmuch as the white body is known to be amoebocytogenic organ (NOEL & JULLIEN 1933). The earlier death of the experiment animal may be assigned to this lesion.

The injection of the extract of the branchial gland gives stimulating effect on the function of the alimentary, excretory and circulatory systems, except that of the white body. The atrophy of the white body here observed may be due to the characteristic intoxication caused by the injection.

The recovery of the colour change in the experiment animal may be explained as due to a chemical stimulus by the extract of the branchial gland. SERENI (1925-28) has reported that the chromatophore may be activated pharmacologically.

Next, a piece of the branchial gland was boiled with sea water and extracted, and opaque solution was obtained. Besides, absolute alcohol extract was prepared ; this was a slightly viscous clear solution. The alcohol extract was diluted in 1 % with sea water, and 3 cc of the solution was injected in octopods 400–500 g in weight. A mixture of alcohol and sea water of the same strength was applied to the control animal. Their results obtained were variable; it was found above all that to branchialectomised octopods, injection of such dilute alcohol was harmful.

ii. To Intact Octopus

The extract of the branchial gland prepared as above was injected in intact octopods, while in the control animal a mixture of equal parts of sea water and octopus blood was injected. Three experiment animals died or weakened in 13, 21 or 56 days respectively, and thrombosis in the branchial heart was perceived macroscopically in all of them. In one of the experiment animals a number of concrements were found attached to the ventral surface of the kidney. These findings show that the injected animals have suffered from intoxication of some chemical substance. Also darkening of the colour pattern was seen in a few animals which can be explained as due to a similar intoxication.

Pathological histology of an injected octopus. Experiment number J E 19; body weight 930 g at end, duration of injection, 56 days.

The branchial gland was normal, with abundant secretions. In the branchial heart the xanthin concrement staining with haematoxylin has a vacuolated appearance. Thrombi are abundant in the blood lacunae (Pl. 5, fig. 1). The process of its formation is as follows: a) a compact mass of amoebocytes, b) formation of a vesicle by the fusion of amoebocytes ; the interior of the vesicle is filled with a colloidal substance containing amoebocytes in a reticulum, c) melting of such a reticular structure, especially due to karyolysis of amoebocytes. Such a necrotic tissue appears as an opaque spot at autopsy. Thrombus is also found in the interior of the pericardial gland (Pl. 5, fig. 2); where a thick columnar epithelium is found around the thrombus. In the liver each component cell is quite slender, its cytoplasm is vacuolated, the concrement is also vacuolated. In the kidney the epithelium is quite thick, and the cell contains minute concrements, the cytoplasm is vacuolated.

Next, alcohol extract was injected in the branchial heart of intact octopods. In about 4 hours after injection, increase of respiratory frequency was noticed. After a week, the frequency per minute became 40 in the two experiment animals while it was 24 and 29 respectively in the control. Still 3–5 days later both experiment animals died, the control animals showed nothing particular. It is thus clear that the alcohol extract of the branchial gland gives an acceleration effect to the pulsation of the heart, but administration in excess is fatal to the animal.

b. On Chromatophore

When the branchial gland extract is injected subcutaneously in the octopus, that part of integument contracts and forms a swollen, papillate mass, and the chromatophores of that part expand. As this expansion of chromatophore is due to the contraction of radiating muscles, both of the above two changes may be assigned to the contraction of muscles. This effect can be seen in the integument of various species of *Octopus*, for example, the extract of *O. vulgaris* can give effect to the skin of *O. minor variabilis*, or *ocellatus*, and vice versa. The boiled extract gives a much similar effect.

The branchial gland extracts have no effect on the chromatophores of shrimps (*Penaeopsis*, etc.) or flatfish (*Pleuronichthys cornutus*). The chromatophore-activating substance may be also obtained from the posterior salivary gland and pancreas of *Octopus*; even the extract of the muscle of the mantle or arm and white body can often expand the chromatophores. In short this effect is neither species-specific, nor organ-specific.

c. To Some Vertebrates

i. Mouse

The extract of the branchial gland of *O. minor variabilis* or *O. vulgaris* was injected intraperitoneally, once in four days, into mice (*Mus wagneri albula* KISHIDA), and their growth rate was observed during two months. In the control animal the same quantity of physiological salt soluiton was injected. In four sets of the experiments there was no difference between the experiment and control animals, so that the branchial gland seems to have no effect on the growth of higher vertebrates.

ii. Frog Tadpole

The tadpole of *Rana nigromaculata*, *R. limnocharis* and *Bufo vulgaris japonicus* were reared in finger-bowls, and the branchial gland extract was added into the medium. In ten sets of experiments the administered tadpoles were more or less vigorous, fed more speedily, and metamorphosed earlier than the control (Text-fig. 22). Therefore the branchial gland seems to give a growth-promoting effect to Amphibian tadpoles.

F. Transplantation

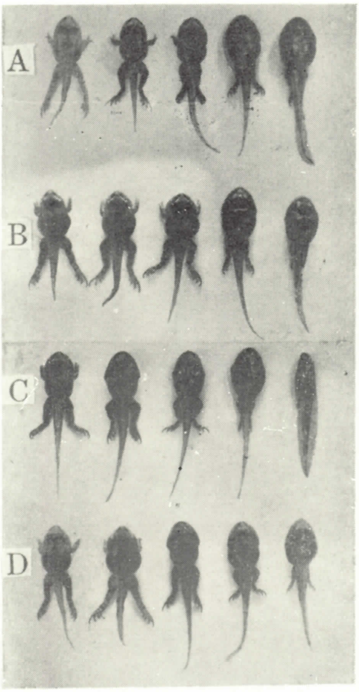
A piece of the branchial gland was inserted into the white body in the eye-capsule. After 52 days, the white body was cut in sections, but no tissue of the branchial gland was found. Also attempts to transplant a piece of the branchial gland into testis or hypodermis were unsuccessful. Perhaps the transplantation of so highly differentiated tissue as the branchial gland is impossible.

G. Pathology of Branchial Gland

Since 1935, I have treated hundreds of octopods for the experiments. Thus, I have been able to confirm that the branchial gland is not infrequently affected by pathological lesion occurring in nature, and this is usually accompanied by low vitality of the animal. These natural abnormalities of the branchial gland may be divided into three categories: the abnormality in size and its histological disorders.

a. Insufficient Development

As stated above, the size of the branchial gland is variable, but when it is extremely small, it is thought to be teratological. In my experiments on branchialectomy, one of the control animal did not feed at all. Autopsy showed that the branchial gland was quite small (Text-fig. 23); as compared with that of a normal octopus of nearly the same weight:



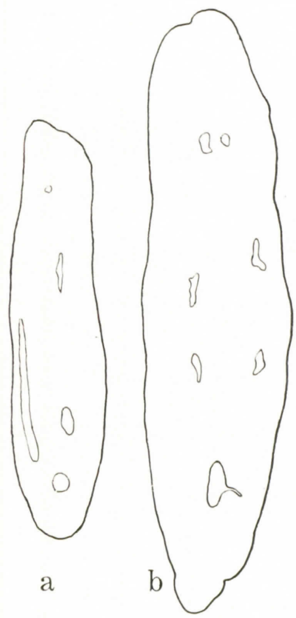
Text-fig. 22. Tadpoles of *Rana limnocharis* reared from 29. IV. to 4. VI. 1939 (36 days). A. tadpoles administered with boiled extract ; B, the same with ordinary extract; C, D, control groups. Notice that in A and B three out of five tadpoles (or young frogs) have fore-legs, while in C and D one or two have fore-legs. Also notice the earlier atrophy of the tail of the experiment animal figured on the left.

Specimen	Body weight	Size of branchial gland : cross-section	Text-fig. 23
abnormal	350 g	3.8 × 0.8 mm	a
normal	340 g	5.3 × 1.4 mm	b

Probably this abnormal gland weighed only about 1/5 that of the normal gland. The gland contained no secretion, and histolysis was seen in the peripheral part. It is quite clear that such small size and histological disorder were correlated with the low vitality of the animal. A similar case was found also in *O. ocellatus*.

b. Anaemic Infarct

The branchial gland of *Octopus vulgaris* often has white dot- or ring-markings which are undoubtedly the sign of a local anaemic infarct, because ; a) the affected part is usually greyish white and opaque, b) and takes no stain when



Text-fig. 23. Cross-section of the branchial gland in (a) abnormal and (b) normal octopods. × 12.

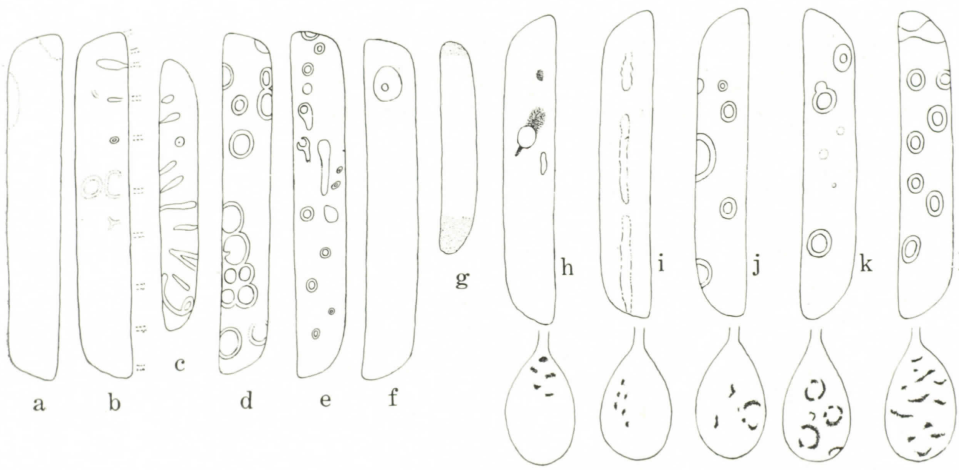
a coloured fluid is injected in the intact part, c) the tissue is necrotic and shows histolysis, and without vascular connections ; in the living state a kind of fluid is often found in the affected part, which can flow within it when pressed.

i. Occurrence

In *O. vulgaris* such markings are not rare in medium-sized or larger individuals, but I have not yet seen them in very young ones (10-50 g in body weight). They are also found in *O. ocellatus* and *Paroctopus dofleini*, but never in *O. minor variabilis* or Decapods, of which I have seen numerous specimens. There seems to be no sexual difference in its occurrence.

ii. Form

The anaemic infarcts are extremely variable in form and localization; they may be dot, circle, ring, semicircle, arch, rod, plate or their combinations, or of diffused forms ; the localization may be superficial or deeper, peripheral (dorsal or ventral) or axial, transverse or longitudinal (in a rod-like form), isolated or aggregated (Text-fig. 24). The infarct becomes quite indistinct in a preserved specimen, as the tissue as a whole turns opaque.



Text-fig. 24. a–g, Diagrams showing variation in the form and location of infarcts in the branchial gland; h–l, the simultaneous occurrence of infarcts in both the branchial gland and the branchial heart. Dotted line shows the inconspicuous outline, existing generally below surface. In fig. b, branches of the afferent branchial vessel are shown on the right side; in fig. g, the majority, except both ends, is occupied by infarcts.

iii. Anaemic Infarct and Vitality

On May lst, 1939, eighteen octopods which suffered from anaemic infarcts came in my hand, and they were kept in a mass culture. All of them except four

Table 10. Growth relation of *O. vulgaris* whose branchial gland suffered from anaemic infarcts. Body weight in g.

Date (1939) \ No. animal	a	b	c	d
1. V.	255 g	328 g	315 g	361 g
2. VI.	—	345	379	350
19. VI.	212	—	—	—
14. VII.	—	—	—	440
No. days lived	49	32	32	74
Increase or decrease of body weight	− 43 g	+ 17 g	+ 64 g	+ 84 g
The same, %	− 18%	+ 5%	+ 20%	+ 23%

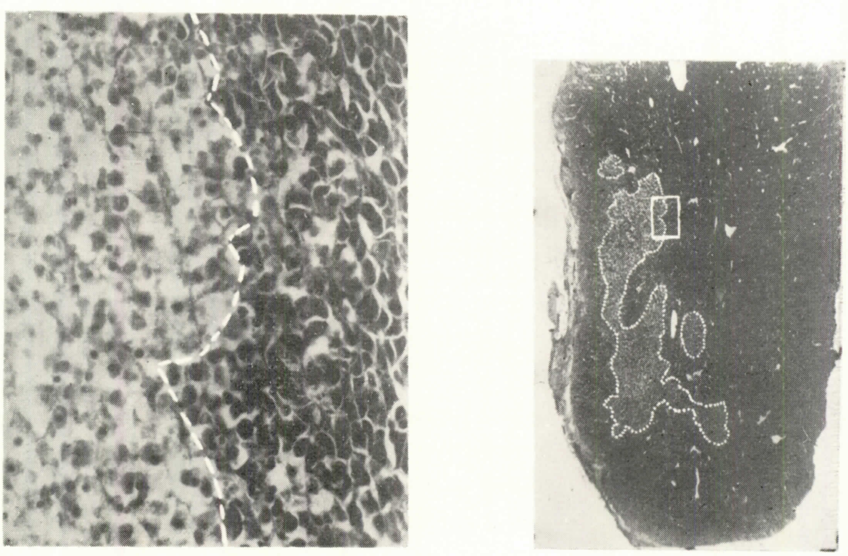
died in a few days. The two of the survived ones showed infection by sporozoans in their intestine (Table 10, a, d). It is noted that (1) if the branchial gland suffers from anaemic infarcts, the vitality of the animal declines, (2) even if the animal can survive and feed apparently normally, its growth rate is quite low (Table 10, b, c).

iv. Pathological Histology

The diseased tissue invariably shows various degrees of necrosis and histolysis. It should be noted that, a similar histological lesion, though slight, is rather frequently met with in an apparently normal branchial gland (cf. "Physiological Renewal" p. 363).

(A) Morbid Area

The morbid area in the cross section has a form as variable as its external form. It may be an irregular circle, oblong or triangle, or ramified (Text-fig. 25). There are cases in which the tip of the area is connected with the blood vessel. In the extreme case nearly the entire matrix of the gland is affected, leaving only a thin marginal zone intact.



Text-fig. 25. Cross section of the branchial gland in *O. vulgaris* (682 g; male), showing an irregular ramified anaemic infarct; the area of which is outlined with white dots. ×65.

(B) Cytolysis and Histolysis

The process of cytolysis may be gradated as follows:

a) The cell diminishes in size according to the degree of shrinkage of the peripheral cytoplasm, and takes a spherical form (Pl. 5, figs. 3–4).

b) The cytoplasm loses gradually its basophilic character and turns acidophilic

(Pl. 5, fig. 5).

c) The nucleus suffers from pycnosis and turns acidophilic, but the shrinkage and basophily of nucleus occur later than the disintegration of the cytoplasm, so that in a certain stage, only the nuclei are conspicuous in the tissue (Pl. 5, fig. 6).

d) The cells also disintegrate into a homogenous colloidal fluid (Pl. 5, fig. 5).

The histolysis apparently ensues histochemical change ; a case was found where minute secretory granules were attached to the endothelium of the branchio-lienal vein and stained bluish cobalt with haematoxylin. The granules, as stated above, are eosinophilic in the ordinary case. In some cases the necrotic tissue is invested with a thin membrane.

v. Infarct in Branchial Heart

When the branchial gland has the infarct, the branchial heart also suffers it in nearly the same degree. Even though both of them are affected on the same side of body, the same organs on the other side may remain intact, showing no bilateral correlation. The first sign of the branchial heart appears as an opaque ring on the skin, showing a local anaemic infarct (Text-fig. 24), which is soon liquefied and becomes transparent, so that it looks dark to the surface view. When a coloured fluid is injected in the branchial heart, the infarct is coloured earlier than the rest. The tissue is disintegrated here and there, the cytoplasm becoming irregularly granular. The xanthin concrements are generally large, and scattered irregularly; their surface is coloured yellowish or stained dark with haematoxylin, but the central core is pale. In the blood lacunae aggregates of amoebocytes may be found. In their central part these cells are more elongated and contain slender nucleus, but in the marginal part they are entirely amoeboid. The general status of the tissue recalls one of the thrombus due to the injection of branchial gland extract into an intact octopus, though in detail there are some slight differences.

In the lumen of the pericardial gland are found a number of pycnotic nuclei, and exceptionally xanthin concrements of the branchial heart as well. Undoubtedly the latter have been brought here by blood stream from the branchial heart.

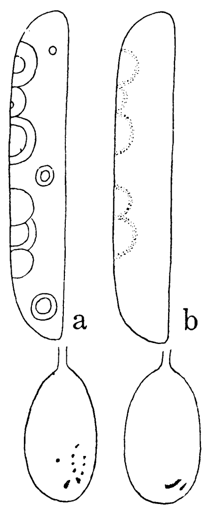
Kidney: The epithelial cell is intact ; in the lumen are found a few spherical, compact masses, of a number of small cells, of which the cytoplasm is nearly gone, but the nucleus retains the chromatin network. It is probable that they have been brought back from the necrotic tissue of the branchial gland and the branchial heart.

These pathological changes reveal that the animal is suffering from a grave disease, as the lesion is not limited to the branchial gland alone.

vi. Recovery from Anaemic Infarct

In the animal survived from the anaemic infarct, the latter is recovered to some extent. Sometimes a complete recovery is observed, the scar becomes invisible outside, or the traces of the original infarct remains as a small scar, pale greyish brown in colour (Text-fig. 26). But no case of advanced infarct was observed, probably because this means the death of the animal.

The healing of the infarct is due to the amoebocytes which enter the morbid tissue ; they first clean the lesion by devouring the residue tissue, and aggregate together to develop a new tissue. The outer part of the healed tissue is crowded by many fibroblasts (cf. JULLIEN 1928) containing elongate nuclei (Pl. 5, figs. 8–9, f), while the inner part is formed by a loose parenchymatous tissue consisting of spherical, undifferentiated cells (Pl. 5, figs. 8–9,a). In a vigorous animal, the healing proceeds in due course and the secretory activity is resumed.



Text-fig. 26. a, Infarcts in the branchial gland and the branchial heart on May lst, and b, the healed condition of the same on June 2nd, 1939. Cf. Table 10, no. b.

vii. Aetiology of Anaemic Infarct

As to the origin of the infarct in the branchial gland and the branchial heart, the following three cases seem possible:

1) In the branchial gland some of the aged cells fall down and are absorbed. Thus the cells will die in mass; and if this loss is not compensated by the multiplication of the cells in the surrounding tissue, a local anaemic infarct will result. But such a lesion will not cover a large area.

2) By the characteristic of the afferent vessel of the branchial gland, an anaemic infarct may occur in a fairly large area. The anaemic infarct usually occurs in the organs which receive the so-called end arteries. This assumpion, however, is not supported by actual observations. The branchial artery sends blood into the branchial gland as well as the branchial leaflets by a strong propulsive power. So that if the capillaries can not admit the afferent blood thoroughly, congestion may ensue which will develop into a stagnant infarct, and its dorsal part falls naturally in an anaemic condition. Cases conforming to this assumption have

been actually observed (Text-fig. 24, b).

3) The majority of the infarct appear in a ring or circular form, which suggests that the colloidal fluid is diffused from a centre.

Concerning the true explanation on the origin of the infarcts in both organs, at present I can only propose a conjecture as follows: At some time a histolytic substance may be produced by unknown particular mechanism in the tissue of the branchial gland, which is rather viscid and heavy in specific gravity, and this substance causes histolysis of the gland, and moreover a part is transported to the branchial heart via vena branchio-lienalis, and similarly dissolve the tissue.

In the branchial heart infarcts are seen on the ventral side and never on the dorsal side; this seems to be due to its heavy nature of histolytic substance. Also the origin of this infarct seems to lie in the branchial gland itself and infarct in the branchial heart is secondariiy provoked; infarcts are more frequently observed in the former and rarer in the latter, and there is practically no case in which branchial heart alone is affected.

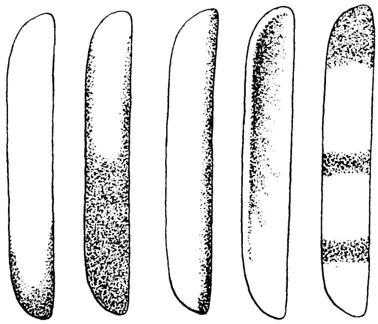
The genesis of infarcts may be temporary, but such lesion necessarily causes the decline of the function of these organs and healing ensues for long. At the same time a further conjecture may be added : the branchial heart may serve to check the bad effect by itself and prevent the propagation of this histolytic substance into other parts of the body. As generally known, the branchial heart is comparatively large in size and deeply coloured in purplish brown in adult octopods, but in juvenile ones and Decapods it is smaller in size and generally pale in colour. The location, size and colouration of the branchial heart may have an intimate relation to the particular function of the branchial gland which is located near by.

The infarct tends to appear more frequently in spring season. This is probably due to the tendency of body fluid to become stagnant in winter, which is favourable to the development of an infarct.

c. Discolouration

In some cases, especially in an octopus of very low vitality, the branchial gland is discoloured so much as to appear thin yellowish brown. The gland in a dead individual is often coloured in various tones of brown. This colour appears in various parts of the gland, for example, (a) the posterior part (Text-fig. 27, a, b), (b) the dorsal or ventral margin (Text-fig. 27 c, d), (c) the transverse band ((Text-fig. 27, e), and (d) the whole branchial gland (Pl. 1, fig. 13).

As for cause of this colouration, it is probable that it is due to a chemical change in the tissue. But if an octopus is killed and left it for several hours in water or in air, no such colouration occurs. It also occurs oftener in the ventral margin, where the gland faces to the gill, than in the dorsal margin ; and when the whole of the gland is coloured, the marginal part is coloured darker than the middle part, no reversal case being seen. In one case the coloured area followed the configuration of the vascular system of the dorsal side; this fact suggests that the discolouration occurs mainly through the blood. At any rate, such a post-mortem change is seen only in the branchial gland, which suggests that it is related to the special chemical nature of its secretion.



Text-fig. 27. Diagram showing variation in the discoloured area of the branchial gland in *O. vulgaris* and *O. ocellatus*. a, b, posterior part ; c, ventral margin ; d, dorsal margin ; e, transverse band.

When the branchial gland is dried by heating in an open air, it turns gradually brown by the oxidation of the tissue. The alcoholic extract of the branchial gland, which contains abundant lipid substances, if evaporated by heating, is discoloured in the like manner.

From these data it is surmised that the discolouration of the branchial gland is due to the oxidation of the chemical component of the tissue after death. The following facts apparently favour this view:

a) In life the gland seems to be devoid of oxidase (see § Staining Reactions, p. 363), so that in a healthy octopus no such discolouration occurs;

b) The gland is mainly supplied with venous blood (branches of the afferent branchial vessel), and arterial blood enters only in a small quantity;

c) The discolouration occurs in the part of the gland where the blood circulation gets stagnant, or the periphery of the gland in which oxidation occurs easilier than in the middle part.

Thus, when the branchial gland is discoloured in a living octopus, a similar decomposition is expected. In life the secretion of the branchial gland is carried out without oxidation, but after death, oxidase is formed probably by the bacterial decomposition, and causes discolouration.

In short, we can measure the vitality of an octopus by the colour of the branchial gland, since both the existence of an infarct and the deterioration of the tissue can be found from the exterior.

(V) DISCUSSION

A. Historical Sketch

Until the end of 20's of the present century, the function of the branchial gland in Cephalopods was not known exactly, and various conjectures have been proposed. TILESIUS (1801), who first recorded the gland, said nothing about its function. Next, CUVIER (1817) took it for a muscular band or ligament. MAYER (1835) was of the opinion that it corresponds to the spleen of vertebrates, giving the name "Kiemenmilz." This name has been widely accepted since. JOUBIN (1885, p. 118), who carried out detailed embryological and anatomical studies on the gill of Cephalopods, extended MAYER's view and stated that it might be an amoebocytogenic organ. This view was followed by many subsequent authors such as WILLIAMS (1902), SCHAEFER (1904), MEYER (1906), ISGROVE (1909), etc. Soon after, however, it was refuted by CUÉNOT (1891) for the reason that, the component cells are larger than the blood corpuscle and show no amoeboid movement, and especially that the nuclei have a different appearance from those in the amoebocytes. While, KOWALEVSKY (1894) regarded as a sort of support to the gill like a cartilage. SCHAEFER (1904), in his work on the anatomy of the Cephalopod gills, proposed ; "Es könnte sich aber um eine Drüse mit innerer Sekretion handeln, welche wie die Thyreoidea dem Organismus Stoffe zuführt, die für sein gedeihliches Fortkommen nötig sind" (p. 14), though he admits MAYER's view at the same time. GRIMPE (1913,p.582), in his anatomical work on the vascular system of Octopoda, says merely that it is apparently an endocrine organ. TURCHINI (1923, 1923a), in his brief notes on the cytology of the branchial gland, expressed that "les éléments de la glande rappellent ceux du lobe antérieur d'une hypophyse de Mammifère." YUNG Ko Ching (1930) concurred him on the endocrine nature of the branchial gland. CAZAL & BOGORAZE (1949), in their paper on endocrine glands in Cephalopods, described briefly the histology of the gland.

The result of the physiological experiment on the branchial gland was first reported by HUTCHINSON (1928), who stated, in a short note, that the gland in question might be of endocrine nature, saying : "The gland can be extirpated in *Octopus vulgaris* unilaterally, through a slit in the mantle. In one such individual which survived 62 days, very marked hypertrophy of the remaining gland was noticed. The haemocyanin content of the blood of this specimen, determined by the refractometric method of Quagliariello, was identical with that of its control" (p. 675). The last sentence is not striking, since the unilateral extirpation does not give any apparent ill effect on the animal.

SERENI (1930) succeeded in extirpating the branchial gland completely, and arrived at the conclusion that the gland secretes an important substance which is necessary for the normal growth. MITOLO (1938) published a detailed account on the physiology of the branchial gland, and showed that the gland secretes a substance which activates the circulatory system. I have been studying the branchial gland independently of MITOLO, and reached a similar conclusion, and an abstract of my paper was read at the General Meeting of the Zoological Society of Japan in 1939 (TAKI 1940) and a short note was published in 1943.

The views of different authors on the function of the gland may be tabulated as follows:

a) On anatomical basis
 i) Organ other than endocrine organ
  1) Muscular band .... CUVIER (1817)
  2) Supporter of gill ...... KOWALEVSKY (1894)
  3) "Spleen" or amoebocytogenic organ...MAYER (1835), JOUBIN (1885)
 ii) Endocrine organ
  4) "Thyroid gland" .... SCHAEFER (1904)
  5) "Anterior pituitary gland" ...... TURCHINI (1923)
b) On experimental basis
  6) Growth-promoting endocrine organ ...... SERENI (1930)
  7) Blood-circulation-promoting endocrine organ ...... MITOLO (1938)
c) On anatomical and experimental bases
  8) "Medulla of adrenal gland" ...... TAKI (1940, 1943)

B. Morphological Characteristics of Branchial Gland

a. Size (or weight)

It has been confirmed that in three species of *Octopus* examined that the branchial gland is heavier in female than in male. The sexual difference in organs except the gonad is hitherto little known in this group. I have formerly (TAKI 1937) showed that in *O. minor variabilis* the posterior salivary gland is larger in male than in female, and that in *O. ocellatus* the liver is larger in female than in male. It is expected that these morphological difference has some physiological significance. For example, in *O. ocellatus* the matured ovary occupies an extremely large volume in the viscera and each ovum is enlarged with voluminous yolk, and the liver seems to play an important role in this yolk production. As already described, in *O. vulgaris* the ovary degenerates by branchialectomy, and it is surmised that the branchial gland seems to have an important bearing on the maintenace and development of the ovary.

b. Innervation and Secretory Activity

The nervous supply, at least in so far as the dissection has shown, is not found in the glandular part of the branchial gland, though the capsule is abundantly innervated. Thus the secretory function does not seem to be regulated by the nervous control. However, there are two possibilities that the activity of the gland is controlled by other agencies: (1) the gland may become active when the animal is well-fed, (2) if the branchial nerve is stimulated, the capsule of the gland, which contains abundant muscular fibres, may contract and temporarily press out the secretion into the efferent vessel. However, this may occur instantly, as the contraction of the capsule on the one hand suppresses the entrance of blood and injures the secretion. Anyhow, the regulation of secretory activity seems to occur very slowly.

It should be noted that the the secretion of the branchial gland is indirectly regulateed by the nervous system, quite unlike the case of the adrenal gland in higher vertebrates. It is likely that the gland secretes an active substance evenly throughout life, irrespective of its age, daily activity, etc. It is also possible, however, that it is activated by the function of the ovary, because a mutual relationship between these two organs seems to exist in the breeding season.

c. Vascular System

As to the vascular system in the branchial gland, the endocrine nature is well shown, and the following may be worthy of notice:

i. The large supply of venous blood,
ii. The small supply of arterial blood,
iii. The venous and arterial blood mix together in the branchial gland,
iv. The great development of capillary,
v. The thinness of endothelium,
vi. The broadness of efferent vessels.

d. Histology and Cytology

It should be noted that the branchial gland is, except its capsule, composed of both glandular cells and vascular system, and the connective tissue does not take part in its construction. This fact may be thought that it is differentiated in the highest level.

The glandular cell is characterised by its basophily and extreme softness of its cytoplasm. Each gland cell is protected in a mesh of a reticular stroma. As observed from both the vital and fixed materials, a remarkable physico-chemical change occurs in the formation of the secretory globule, not seen in other tissues of *Octopus*, at least. Also it is worthy of notice that the secretion is actually poured into the vein, and this is the direct proof of the endocrine organ.

C. Physiological Characteristics of Branchial Gland

a. Suppression of Function of Branchial Gland

i, Ligature of Branchio-lienal Vein

Even if the branchio-lienal vein, which is the efferent vessel of the secretion, is ligated, the secretion can be distributed by the capillary of the capsule of the branchial gland, and no symptoms of branchial insufficiency ensues. This is also the direct proof that the active principle is distributed through the vascular system. MITOLO's (1938) opinion that the ligature gives as much ill effect as branchialectomy is not substantiated by my experiments.

ii. Unilateral Branchialectomy

So far as I have been able to ascertain, unilateral branchialectomy gives no particular ill effect. HUTCHINSON (1928), SERENI (1930) and MITOLO (1938) observed the hypertrophy of the remaining gland, and my results also agree with it. But the compensatory hypertrophy is a phenomenon universally seen in various organs of the body. So that this can not be the direct proof of the endocrine function of the branchial gland.

iii. Bilateral Branchialectomy

SERENI (1930) carried out branchialectomy for the first time. He noticed that high mortality of the operated animal, which even if survived the operation, became very weak, especially the resistance to narcotics decreased, and the animal prefers to stay out of water, and emaciation. However, he did not give any concrete data on the growth rate of the operated animal, except for a single example, which "in 15 days between the first and second unilateral extirpations of the branchial gland, grew from 115 g to 210 g, while in 13 days following the second operation it remained in 200–210 g" (translated from Italian original). He concluded that "such a suppressing effect on growth is absolutely constant, permanent and definite," but this view is unwarranted, since various lesions appear in the body besides the growth. He also found that, no example which had survived the total extirpation of the branchial gland; if at all, two months were the longest. This result has been confirmed by my experiment.

SERENI points out that the power of cicatrisation and regeneration is not suppressed by the extirpation of the branchial gland. In my experiment also it was impossible to suppress completely the regeneration of the excised arm tip in a branchialectomised octopus. But (1) the regeneration took place only during the operation, and practically never after the total branchialectomy, (2) since the desquamated wound in the dorsal mantle was not healed during that time; it clear that, the tip of the arm alone has a special regenerating power (cf. LANGE 1920). Therefore this fact can not be any serious objection to the bad effect of abranchialism.

All the results of SERENI have been substantiated by my experiments, but he missed the following points : He did not notice that (1) the branchialectomy invariably results death of the operated animal, and also (2) anorexia and (3) edema of the body.

MITOLO (1938) extirpated the branchial gland with scissors, and noticed the death of animal mostly within 24–36 hours, at most in two days. But this operative technique can not be appropriate, as it brings about variable amount of hemorrhage. The branchial gland is abundantly vascularised, so that a mere excision of it causes a serious hemorrhage. SERENI (1930) did not show the operative technique precisely, but the branchialectomised animal survived it for up to two months. The cauterisation seems to be a more suitable technique than excision.

MITOLO is of an opinion that the length of the survival of the branchialectomised animal is independent of the body weight. But his experiments were carried out in the summer season when the mortality of the operated animal is very high. In my experiments larger animals usually have much greater endurance for the branchial deficiency than smaller ones, and can survive severer operative shock.

b. Administration of Branchial Gland Extract

The injection of the branchial gland extract was first tried by MITOLO (1938). The injection, however, does not prolong the life of the branchialectomised animals, as confirmed by experiments. He gives precise data on the suppression of growth in uni- or bilaterally branchialectomised animal, but the animal he experimented on was very young (at beginning 30–190 g in weight) and did not live long.

The same author has stated on the effect of the branchial gland on the circulatory system, that "the decrease or cessation of growth is not the direct cause of the death of the animal." He found in the heart of branchialectomised octopus both the negative inotropic and chronotrophic effects and the disturbance in the rhythmic movement. Furthermore, he confirmed a positive effect to the cardiac function of the extract of the branchial gland injected into the intact or branchialectomised animal, and that the extract of the gill, mantle or arm also has a similar slightly positive effect. I have observed a similar effect of the extracts of these organs to the cutaneous contraction (including the expansion of chromatophores) of octopus.

MITOLO's work on the branchial gland extract may be summarised as follows:

(1) the extract accelerates and strengthens the function of the circulatory organ,

(2) its effect is not species-specific,

(3) it has no effect on vertebrates (*Rana*).

The non-specificity of the effect of the branchial gland is quite probable, but in my experiments the scope of its applicability seems to be confined within the Cephalopods, tested by the contraction of the integument, and the experiment is rather difficult in other groups of animals.

I have found that ineffectiveness of the alcoholic extract of the branchial gland to the heart of a toad (*Bufo*), and also of its salt water extract on the growth of the mouse, although a slight accelerating effect on the growth of a tadpole has been noticed.

It is to be noted that the effect of the extract of the branchial gland appears rather slowly, in contrast to that of the adrenal gland which appears instantly. Probably the efficiency of the active principle of the branchial gland is so low that it can give effect only to the larval animal (for example, a tadpole of frogs) and not to the adults of higher vertebrates.

The repeated injection of the branchial gland extract into the branchialectomised octopus seems to save the animal from the degeneration of body organs to a certain degree, especially from the edema formation. But judging from the activation of chromatophores and particular degeneration of the white body, the animal seems to suffer from an intoxication to a certain degree. Also the injection of an extract into an intact octopus brings about intoxication but no "hyperbranchialism."

The active principle stands boiling and can be extracted with alcohol.

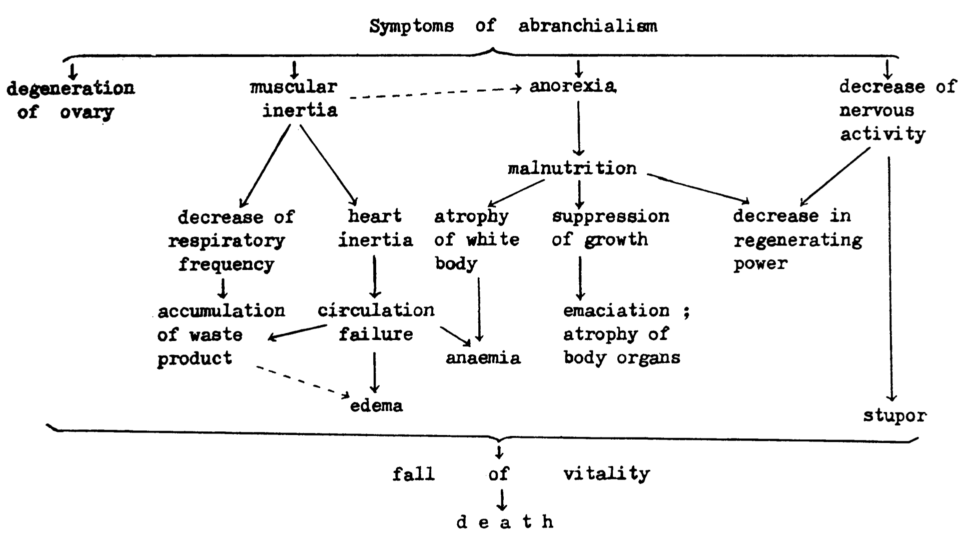
D. Pathology of Branchial Gland

The pathology of Cephalopods is only very imperfectly known (PETTIT 1904, JULLIEN 1928, 1928a, RAABE 1933, 1934). The size of the branchial gland is variable, but if it comes below the normal limit (say,half the normal) it should be thought as pathological. Undoubtedly it is the quantity of an active glandular part and not the total bulk of the gland, that controls the vitality of the octopus.

The occurrence of an infarct has never been recorded, except by myself (TAKI 1943). This has an important bearing on the vitality of the animal ; it seems to give bad effect on the animal not only by the reduction in quantity of the active tissue, but also by its autointoxication. It is surmised that the active principle of the branchial gland, if stagnant, turns toxic and injures the health of the animal.

E. Nature of Branchial Gland

The symptoms of branchial deficiency may be divided into four groups, namely failure in (1) the nutrition, (2) the muscular tonus, (3) nervous system and (4) gonad. The interrelationship of each symptom may be shown in the annexed diagram:



Text-fig. 28. Diagram showing the interrelationship among the symptoms of abranchialism ; broken lines show an indirect relation.

As abranchialism causes the suppression of the animal's vitality in many directions, it seems convenient to compare the symptom with that in vertebrates. The symptoms of branchialectomy remind one of those of adrenalectomy more than those of removal of the anterior pituitary or thyroid gland. The adrenalectomy causes the muscular inertia, suppression of respiration and growth, fall of blood pressure and metabolic rate, atrophy of bodily organs, and finally death of the animal (TRENDELENBURG 1929, p. 192; MORI 1955, II, p. 48); these are also seen in branchialectomy.

At first sight, as SERENI (1930) has thought, the branchial gland appears to have an important bearing on growth, so that it recalls one of the thyroid or anterior pituitary of vertebrates (cf. SCHAEFER 1904, TURCHINI 1923). But extirpation of these organs is not fatal. In vertebrates, there are only two endocrine organs, whose total extirpation results death, namely the adrenal and parathyroid. But the nature of the latter widely differs from the branchial gland.

The most striking difference between the symptoms of adrenalectomy and branchielectomy lies in the edema formation in the latter. JULLIEN (1928a) observed edema in the connective tissue near the artificially inflamed part, in the mantle of *Sepia*; there, the connective tissue fibres were swollen and scarce, the nucleus became pycnotic and the tissue liquefied. But in this case the edema was in a small area. Edema formation is a symptom of the failure in circulation and nutrition. The death comes rapidly after adrenalectomy, even if it is carried out gradually. In Cephalopods death comes rather slowly, if gradually branchialectomised. If the effect of the active principle of the branchial gland is slow, and if the gland is gradually extirpated, the formation of such an edema seems to be probable.

It is interesting to note that the branchial gland resembles morphologically the medulla of the adrenal gland, for instance, it is larger in female than in male, the cytoplasm of component cell is extremely soft and basophilic, and the tissue is rich in lipid substance. Also the difficulty of transplantation of an excised piece, the presence of an active physiological renewal of component cells, and the fact that a lesion in the gland often becomes fatal to the animal, may be added to this inference.

Thus, the branchial gland of Cephalopods coincides morphologically and physiologically to the medulla of the adrenal gland in vertebrates. In vertebrates, the adrenal gland is composed of both the cortex and medulla, each having different embryological origin, anatomical construction and physiological function respectively. Therefore, it can be not concluded, by the coincidence given above, that the branchial gland in Cephalopods plays the same physiological role as in adrenal medulla. As will be seen later the endocrine organs in Cephalopods which have been widely confirmed are as yet few in number, and moreover the interrelation among these organs is little known. We can at present only say that the striking similarity exists between these two organs.

F. Other Endocrine Organs in Cephalopods

a) Posterior salivary gland. SERENI (1930) is of the opinion that the chromatophore in the integument of Cephalopods is activated by the humoral agency (tyramine)[1] produced by this gland. Formerly this view was taken by myself also (TAKI 1935). Later, however, it has been discarded, because, (1) the extirpation of this gland gives no effect on the activity of chromatophores, contrary to SERENI's findings ; (2) the extract of this gland can expand chromatophores, but that of some other organs shows a similar effect, (3) morphologically the gland has no feature characteristic of the endocrine organ. VERNE (1922) and HOLZLÖHNER & NIESSING (1938) reported respectively the histology of this gland in detail. A crucial examination is desired on this line.

b) Branchial heart. MARCEAU (1905, p. 570) supposes that this is an organ of internal secretion, but without giving any good evidence for the view.

c) Pericardial gland. KESTNER (1931) thinks that it is endocrine. The extirpation of this gland from *Sepia* brings about death within a short period, otherwise there is no evidence for the endocrine nature of the organ. MITOLO (1938a) reported in detail that this is a pulsating organ, but did not deal with the problem whether this is an endocrine organ or not.

b) Epistellar body. YOUNG (1936) found a small neurocrine organ within the stellate ganglion, which in Octopoda forms a particular organ. By its extirpation muscular weakness results.

e) Corpus subpedunculatum. THORE (1936) has found a small organ attached to the optic ganglion, which resembles the white body histologically, but the cell is smaller and more intensively basophilic, and vascularised. In a later work he (THORE 1938) supposes this organ to have some influence on the activity of the chromatophore. But WELLS & WELLS (1959) reported that there is a mutual relationship between this and the optic gland.

f) Optic gland. The same authors (1959) found that this has an inhibitory function on the maturation of gonads. "Maturation of the gonad is determined by secretion from the optic glands which is normally held in check by an inhibitory nerve supply from the subpedunculate/dorsal basal lobe area. The action of this region in turn dependent upon the integrity of the optic nerve and thus, presumably, upon light." (p. 31)

g) UNGAR & ZERLING (1935, 1936 ; UNGAR 1937) have found that, if the visceral nerve is electrically stimulated, a heart-activating substance is produced in the vascular system. They repeated the same experiment after the removal of the posterior salivary gland or the ink sac, but there was no change. It is not known whether this activation is due to the heart-activating hormone produced from the heart in Mollusca (HABERLANDT 1930, and others) or to the hormone of the branchial gland.

h) Gonad. I have expressed the view (TAKI 1941, 1944) that both the testis and ovary may be endocrine in nature, on the basis of the observations in anatomical changes caused by gonadectomy. Extirpation of the testis in *Octopus* caused the histolysis of the gonoduct system except the penis, and ovariotomy the

1) HENZE (1913) reported that the venomous substance of the posterior salivary gland is tyramine.

decrease of growth rate of body and degeneration of the branchial gland to a certain degree, but further confirmation is desired.

Thus, there might be found some more endocrine organs in Cephalopods, and of those given above, the epistellar body, subpedunculate organ and optic gland seem to be most plausible ones, and the latter two have been well studied. However, they are generally small, so that it is concluded that the branchial gland is one of the largest endocrine organs in Cephalopods, as well as in invertebrates.

(VI) SUMMARY

1. This paper deals with the morphological and physiological studies on the branchial gland of *Octopus*, especially *O. vulgaris*.

2. Since it seems safe to assign endocrine function to the branchial gland, new terminology like branchialectomy, abranchialism, branchial deficiency etc. has been introduced.

3. The branchial gland is slightly heavier in female than in male in the three species of *Octopus* examined.

4. There are two veins (afferent and efferent) and an artery supplying blood to the branchial gland; the efferent vein (vena branchio-lienalis) serves to transport the secretion of the gland.

5. No nervous supply is present in the glandular part of the organ.

6. The branchial gland is an organ of mesodermal origin ; in the embryonic stage it appears in the axis of the gill.

7. Histologically observed, the distribution of the veins favours the transport of the secreted substance.

8. The cytoplasm of the gland cell is characterised by its basophily and extreme softness, and great liability to shrinkage by fixatives.

9. The secretory globule is acidophilic.

10. The ligature of the efferent vein does not give much injury on the animal, because the active principle can be transported by other vessels.

11. Unilateral extirpation of the branchial gland does not give a noticeable ill effect on the animal.

12 Total extirpation of the branchial gland is always fatal to the animal.

13. Symptoms of deficiency of the branchial gland are as follows:

   a. Loss of appetite,
   b. suppression of growth,
   c. decrease of regenerating power,
   d. decrease of respiratory frequency,
   e. muscular inertia,
   f. injury in the integument,
   g. anaemia,
   h. fall of blood pressure,

i. edema formation in the connective tissue,

j. degeneration of the ovary,

k. decrease of nervous activity, resulting stupor,

l. low general vitality.

14. Histological examination of the operated animal shows the conspicuous atrophy of all tissues.

15. So long as the branchial gland is not injured, the effect of inanition, castration or artificial anaemia is rather slight.

16. Repeated injection of the extract of the branchial gland into branchialectomised animals saves to some extent the lesion of branchial deficiency.

17. Repeated injection of the extract of the branchial gland into intact animals causes intoxication.

18. An alcoholic extract of the branchial gland activates the function of the circulatory system of an octopus.

19. Administration of the extract of the branchial gland into a mouse is ineffective.

20. Administration of the extract of the branchial gland is slightly effective on the growth of a frog tadpole.

21. The active principle of the branchial gland stands boiling and can be extracted with alcohol.

22. Transplantantion of an excised piece of the branchial gland was unsuccessful.

23. The animal having an abnormally small branchial gland shows low vitality.

24. Local anaemic infarcts are often found in the branchial gland; when such infarcts are numerous, the animal is weakened.

25. The part of an infarct is in the state of necrosis.

26. The infarct appears also in the branchial heart, possibly caused by the same lesion in the branchial gland.

27. The infarct can be recovered by the absorption of a necrotic tissue and regeneration.

28. The cause of the infarct is due to the morphological and physiological characteristics of the branchial gland.

29. Colouration in life, and post-mortem discolouration of the branchial gland are described, which seem to be due to the oxidation of the secretion.

30. The branchial gland in Cephalopods has probably a function somewhat similar to that of the medulla of the adrenal gland in vertebrates.

31. So far as our present knowledge goes, the branchial gland is one of the largest endocrine organs in Cephalopods, as well as in all invertebrates.

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頭足類における鰓腺の形態と生理について

滝　　　巌

(本篇の原稿は昭和17年に作成したが，当時出版が困難であったのでその要項を和文で公表し（滝 1943)，この度欧文で公表する次第である．そのため前報を僅か修整した箇所がある.）

形態：鰓腺は軟体動物中，頭足類のみに見られるがオオムガイ類では組織が分化していない．イカ類及びタコ類ではよく分化し，その組織は発生を見ると中胚葉起源で，その原基は鰓の中軸部に生ずる．マダコ属の3種についてその重量を調べたが，雌のものが雄のものより僅かに重い．この腺は鰓の背側にこれに密着して存在し，血管分布は鰓入血管の分枝したものが腺中に入り，毛細管に分かれ再び集って鰓腺輸出静脈として腺外に出て鰓心臓に注ぎ，動脈は細いものが内部に入っている．神経は鰓腺皮膜に多く分布しているが腺組織にははいっていない．腺は主として腺細胞から成るが，これは切片で多角形に現われ，生時細胞質は柔軟で，固定液で固定すると著しく収縮する性質があり，塩基性色素によく染まる．分泌物の形成過程は複雑で，最後に生じた分泌物は大形で球状を呈し，酸性色素でよく染まるものとなる．その分泌物は静脈中に小片となって移行する状態が切片で認められ，内分泌器官であることが証された．

鰓腺に環状・帯状・点状その他種々の形の半透明あるいは灰白色部が見られることがあるが，これは局所的貧血性梗塞を生じたもので，切片によるとその部分は組織融解をしている．このようなタコは生活力が低下しているが，飼育してみるとある程度自然に治癒する．即ち血液中の変形細胞がその部分に集合して新組織を作ってその空洞部を埋める．又時に鰓腺の全体又は一部分が淡黄褐色ないし褐色を呈していることがあるが，これを変色と呼ぶことにする．これは分泌物の酸化によって生じたものと考えられる．この組織には細胞分裂も見られ，又壊死(えし)部もあることから，常時生理的更新が行なわれているものと思われる．

生理．マダコを材料として鰓出血管の結紮と鰓腺輸出静脈の結紮を行なったが，動物には著しい欠除症状のような影響は与えられなかった．それは分泌物が他の血管によって運ばれるからで，これも内分泌器官の証となる．

鰓腺摘出実験は赤熱した白金線を鰓腺皮膜にあて，腺部の壊滅を待って取除くと出血なく完全に摘出することができた．この方法は片側ずつ，しかも徐々に行なうのがよい．片側の鰓腺を除去すると残りの鰓腺が補償的肥大成長をする．この摘出を左右完全に行なうと，タコは食慾減退し，成長は遅くなり，腕先の再生力，呼吸・筋肉その他内臓諸器官は総べて機能が低下し，体の各所に水腫を生じて遂に斃死する．

鰓腺の抽出物を鰓腺摘出したタコに反復して注射すると，いくらか生活力を持続し皮膚の色彩も正常に近くなるが，その生命を救うことはできない．又正常なタコに注射すると中毒症状を起こす．この抽出物はタコやイカの皮膚に注射してそれを収縮させ，色素胞を拡張させる性質があるが，ハツカネズミの腹腔内に注射してもその動物の成長には効果なく，唯カエルのオタマジャクシに僅かに成長促進効果が見られた．

以上の事実は鰓腺は明らかに内分泌器官であることを証し，その機能は高等脊椎動物における副腎の髄質部に類するものであることを知るのである．

PLATE 1

Figs. 1-11, 15-16, *Octopus vulgaris* ; Fig. 12, *O. minor variabilis* ; Figs. 13-14, *O. ocellatus.*

1-8. Secretory stages of the gland cell of the branchial gland (body weight 500 g, female, Nov. 7, 1935; Flemming's strong, Delafield's haematoxylin and eosin). × 1500. Cf. Text-fig. 18. (p. 361). 1-6. formation of secretory globule ; 7, globule formation completed and ready to be secreted; 8, after the secretion the nucleus returns to the centre of the cell.

9. A gland cell of the branchial gland, stained with Feulgen's nucleal staining method.

10. The same, vital staining with Nile blue sulphate solution.

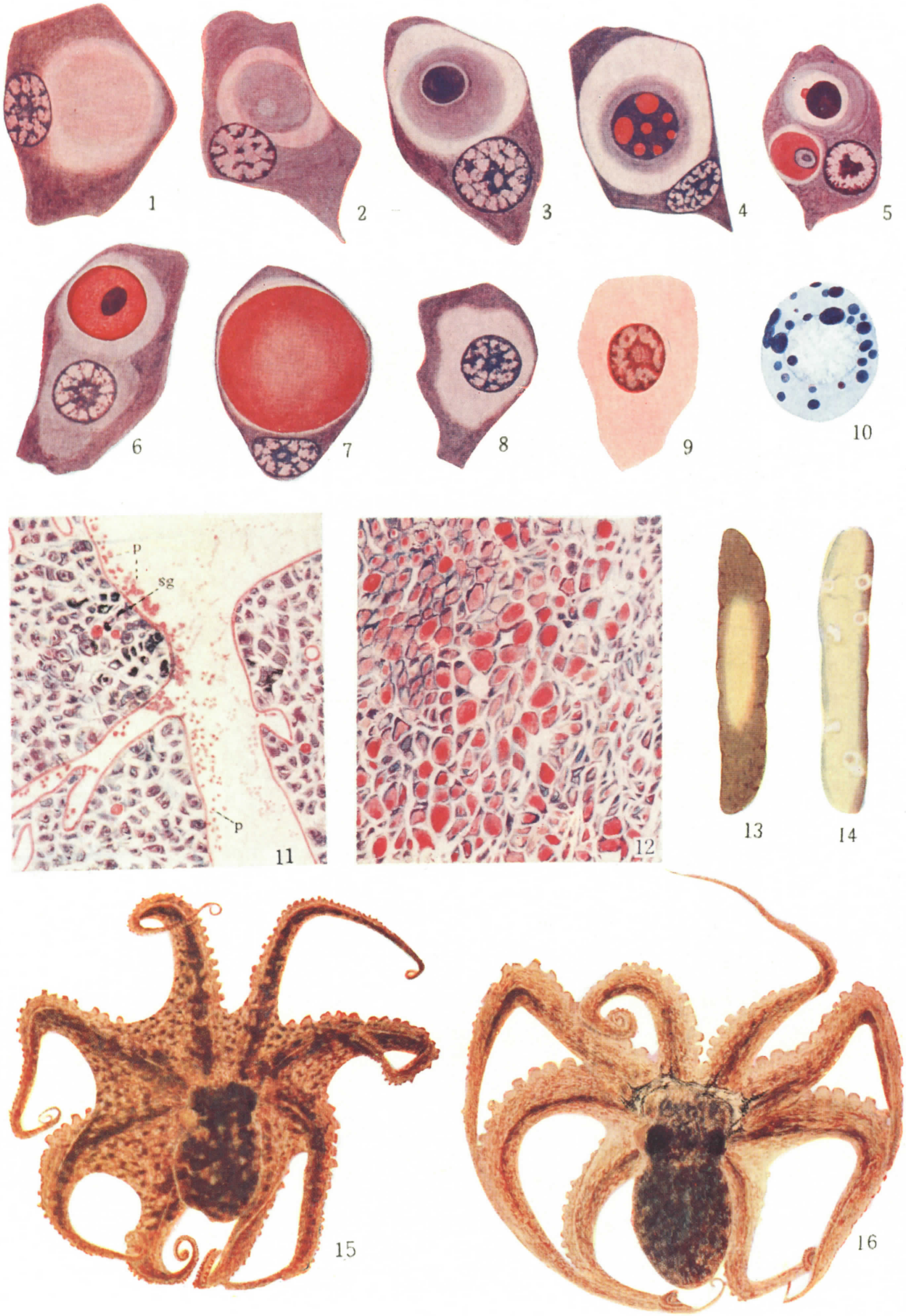
11. Diminished particles (p) of secretory globule (sg) of the branchial gland poured into a large vein. × 230.

12. Cross section of the branchial gland of *O. minor variabilis*, which contains numerous gland cells filled with secretory globules stained deep red with eosin. × 230.

13-14. Branchial gland of *O. ocellatus*, male, April 29, 1939. 13, Left branchial gland, deeply discoloured at the peripheral part but the central part is intact ; 14, outer surface of the right branchial gland, in which several anaemic infarcts are seen.

15. *O. vulgaris.* Repeated injection with the extract of the branchial gland into branchialectomised animal (No. J H 2) ; photographed before the animal became weakened ; notice the conspicuous colour pattern on the integument.

16. Control animal of the same (No. J H + 1). See Table 9. Notice that the colour pattern did not appear.



TAKI : Branchial Gland in Cephalopoda

PLATE 2

1. Ligature of left branchial vein, No. 1, male, body weight at the end of experiment, 1016 g (see Table 3). Two arrows show the spots where the connection between the gill and auricle was lost. Notice the degeneration of the left gill (lg) and the normal state of the right gill (rg).

2. Injection of sea water into branchialectomised octopus, No. J + H2, male, body weight at the end of experiment, 595 g (see Table 9). Notice that the mantle as a whole is very swollen.

3. An octopus at the end of inanition experiment, duration 130 days; body weight 296 g, female, No. H 1 (see Table 8). The animal is conspicuously emaciated and inactive, entirely pale in colouration.

4. The animal the same as in Fig. 2; the mantle is cut open from the ventral side. Notice the prominent edematisation of the hypodermis of the mantle.

5. A control animal of that in Fig. 3; body weight 678 g, female, No. H + 1. It is well-fed, active and dark in colouration.

6. Transverse section of the branchial gland of an octopus of inanition experiment (Fig. 3), in which the gland cell is emaciated, weakly stainable, with only small quantity of secretion.

7. Control of the above (Fig. 5), which is normal in size and secretion.

TAKI : Branchial Gland in Cephalopoda

PLATE 3

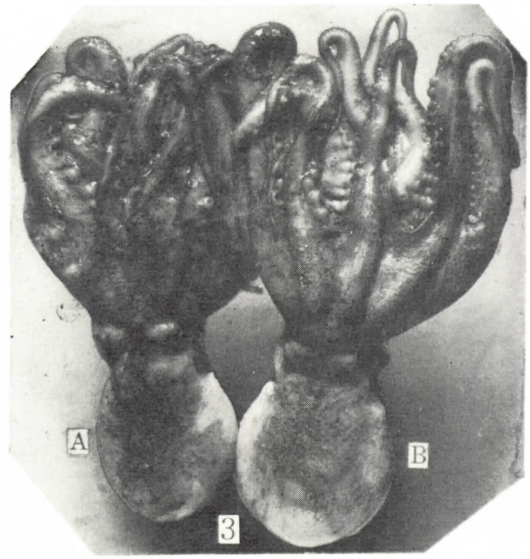
1. An octopus in the third period of abranchialism ; attaching the wall of an aquarium, with its arms hanging down : the integument is thoroughly pale.

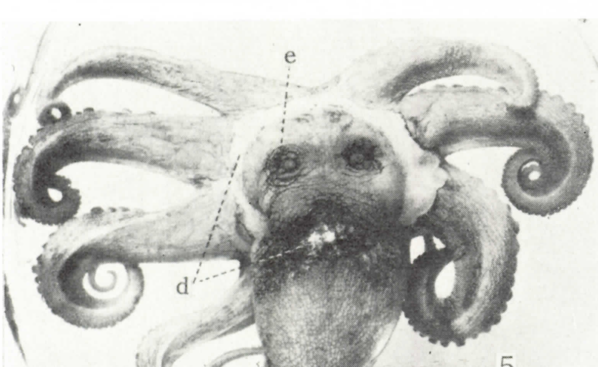
2–3. Showing the difference in growth of body between a branchialectomised octopus (No. C 44 ; Fig. 2) and its control (No. C + 28, C + 29 ; Fig. 3, A, B). See Table 5.

4. A. An axial contraction of the gill in a branchialectomised octopus (No. C U 2), and B, no such contraction in its control (No. C + U 2). Also notice the hypodermic edema of the mantle in the former specimen. White dotted lines show the outline or trace of the branchial gland.

5. A dead specimen of a branchialectomised octopus (No. Ca ; died on 12. VII. 1939 ; body weight 777 g), showing a conspicuous edema in the mantle and arms. The eyes (e) are completely buried under the edematous integument, and its surface is desquamated (d).

6. A female genital organ of the same individual. The whole organ is as large as that of a normal mature female, but the ovary is degenerated in a small brownish piece (ov) and the ovarian capsule (c) is filled with body fluid. ob, oviducal ball; od, oviduct.





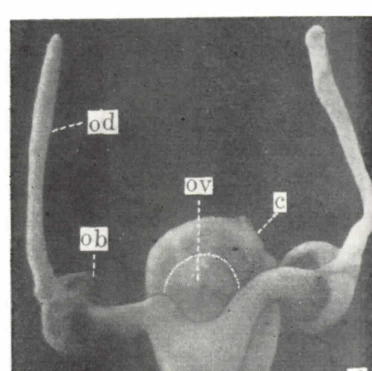


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PLATE 4

1. Cross section of the axis of the gill, showing a part of the gill supporter (g) and the surrounding connective tissue (c) of *O. minor variabilis* (body weight 72 g).

2. Regenerated pieces of the tip of an arm in A, a branchialectomised octopus (No. C S 1) and B, its control (C + S 1). See Table 6.

3. Cross section of the edematous capsule of the branchial heart in a branchialectomised octopus (No. C U 2; Table 7), showing the voluminous thickening of the hypodermic connective tissue, which is not found in the normal condition. e, epithelial layer ; c, edematous connective tissue ; h, branchial heart.

4. Section of the integument of a branchialectomised octopus (No. C K 3), showing the desquamation of the epithelium. (Experiment begun on 28. IV. 1938, body weight 200 g; killed on 9. VII. 1938, 404 g ; female). e, a clump of disintegrated epithelial layer ; cr, chromatophore; c, connective tissue containing muscular fibrilles.

5. Control animal of the preceding (No. C + K 1); the surface of the integument is conspicuously shrunken, blood vessels and muscular fibrilles are well developed in the connective tissue. ep, epithelial layer; m, mucous gland cell. (Experiment begun on 28. IV. 1938, body weight 364 g; killed on 9. VII. 1938, female).

6. Section of the kidney of a branchialectomised octopus (No. C U 2a).

7. The same of the control animal (No. C + U 1 ; Table 7)

8. Section of the pancreas of the branchialectomised octopus (No. C U 2).

9. The same of the control animal (No. C + U 2). See Table 7. The component cell is tall and its distal surface is vacuolated, showing in a state of high activity, while in Fig. 8, each cell is low and not vacuolated.

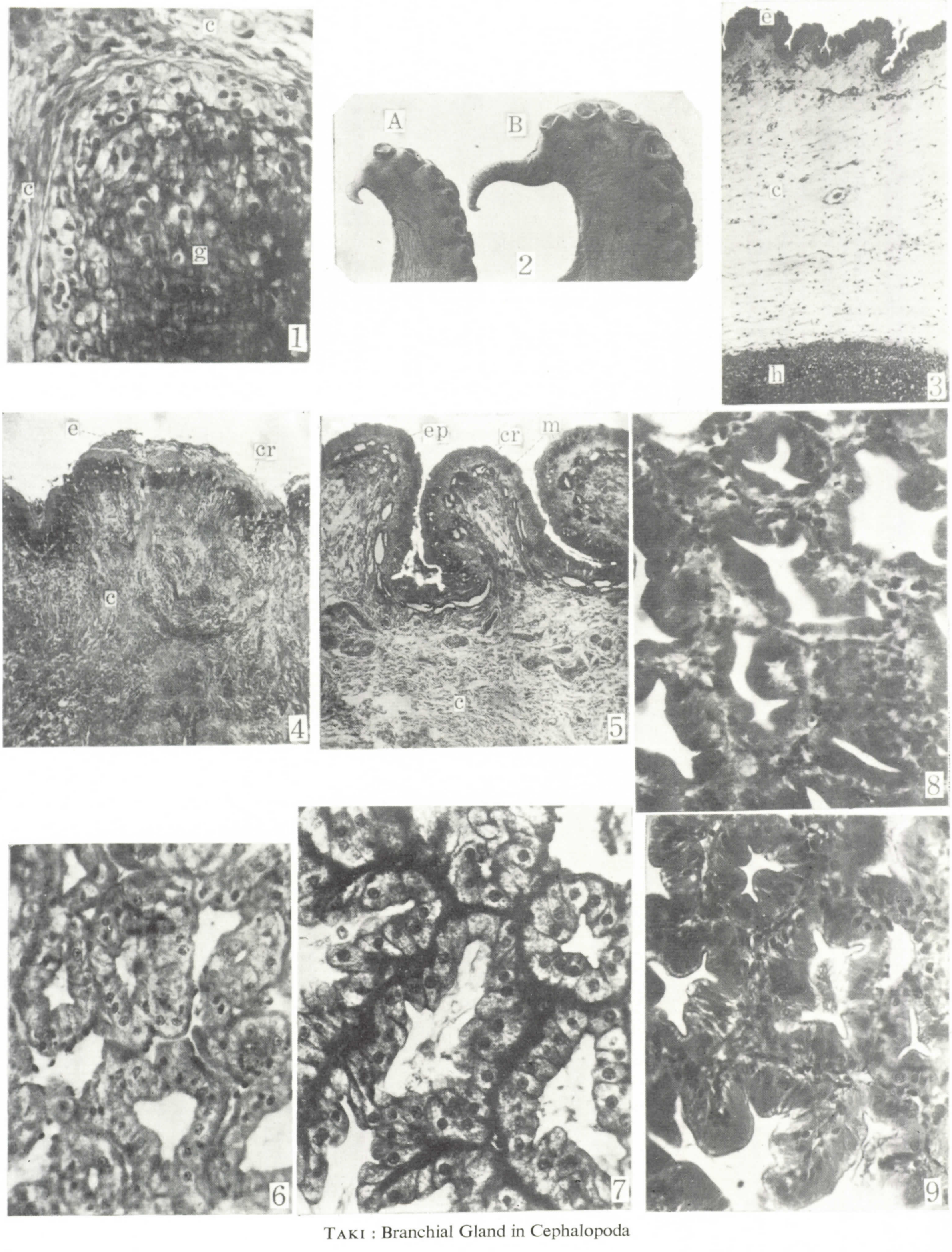

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PLATE 5

1. Section of a thrombus formed in the blood lacuna of the branchial heart of an intact octopus (No. J E 19), after the extract of the branchial gland has been injected repeatedly. bh, tissue of branchial heart; t, thrombus formed with amoebocytes and colloidal substance; x, xanthin concrement.

2. Section of a thrombus (t) formed in the centre of the pericardial gland (p) of the same individual as the preceding.

3. Section of the branchial gland of *Octopus vulgaris* (body weight 404 g; female), showing the irregularly-shaped, variously sized and dissociated component cell, and the pycnotic and karyolytic nuclei.

4. The same of another individual (515 g; female). The component cells are distinctly separated from each other, some of them being extremely large and spherical, others quite small and apparently devoid of nucleus. However, the blood vessels alone hold their original form.

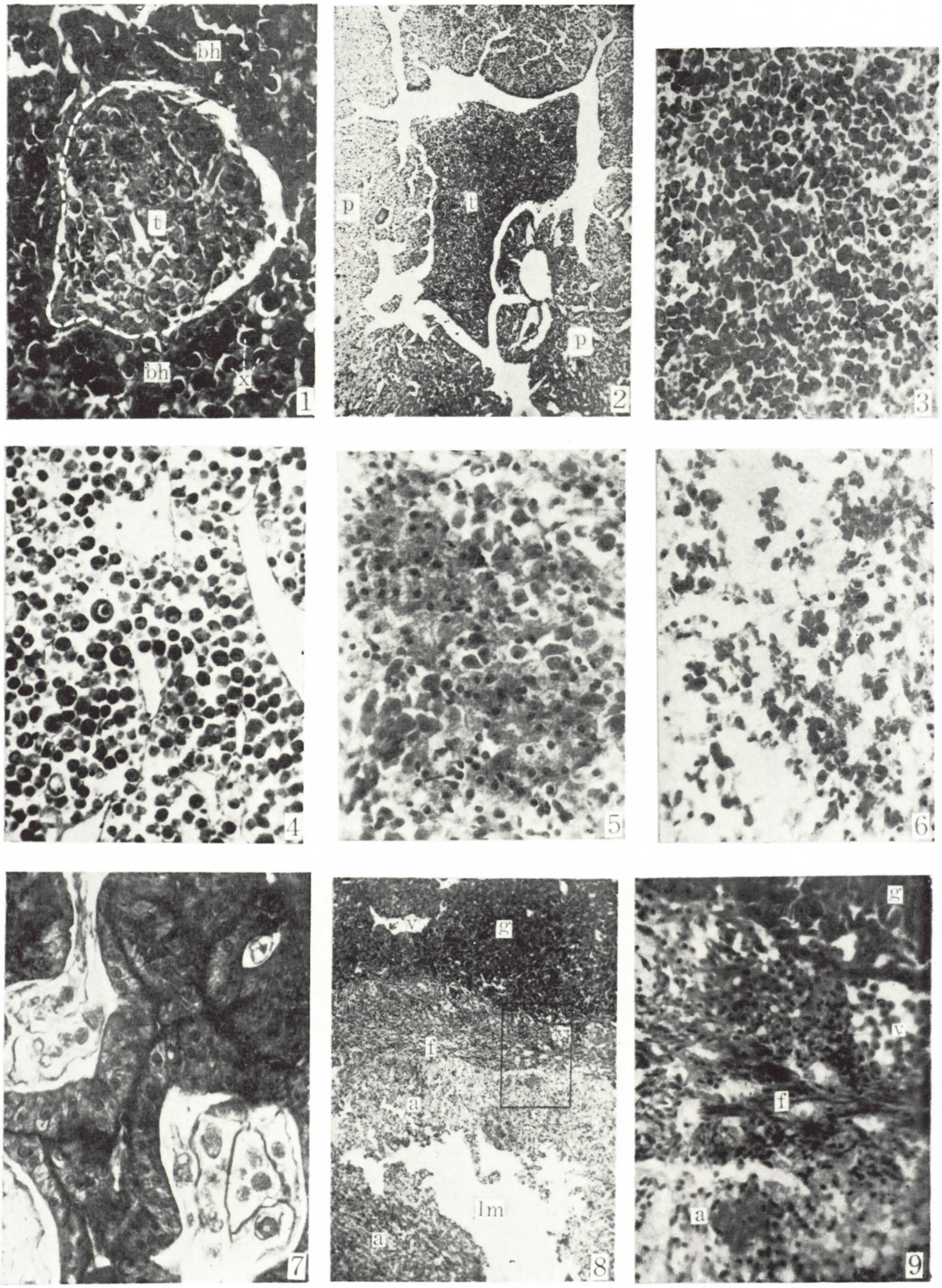
5. The same of *O. minor variabilis* (62 g; female), showing a complete cytolysis of the component cell.

6. Section of the branchial gland of *O. vulgaris* (900 g; male), showing a part which suffers from a local anaemic infarct. The component cells are sparsely scattered, irregularly shaped, dissociated and having pycnotic nuclei.

7. Section of the kidney of *O. vulgaris* (348 g; male), whose branchial gland and branchial heart suffer from a local anaemic infarct. In the lumen are seen a number of the disintegrated cells transported from both the branchial gland and the branchial heart.

8. Section of the branchial gland of *O. vulgaris*; the lumen which suffered from a local anaemic infarct is being recovered with the assemblage of amoebocytes. The area of rectangle is enlarged in Fig. 9. g, normal gland cell; f, fibroblasts changed from amoebocytes; a, parenchymes composed of amoebocytes; v, blood vessel; lm, lumen not yet recovered.

9. Enlarged figure of the preceding.



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