PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Development of the Thymus, Epithelial Bodies, and Thyroid in the Marsupialia. Part II. Phascolarctos, Phascolomys, and Perameles

Elizabeth A. Fraser

Phil. Trans. R. Soc. Lond. B 1916 **207**, 87-112
doi: 10.1098/rstb.1916.0002

II. *The Development of the Thymus, Epithelial Bodies, and Thyroid in the Marsupialia.* Part II.—Phascolarctos, Phascolomys, *and* Perameles.

By Elizabeth A. Fraser, *B.Sc.* (*Lond.*).

Communicated by Prof. J. P. Hill, *F.R.S.*

(Received March 10,—Read May 13, 1915.)

[Plate 11.]

Introduction.

The occurrence of a cervical thymus gland in the Koala was first recorded by Symington (61)* in 1900. This investigator examined one example of *Phascolarctos cinereus* measuring 30 cm. in length and found two thymus lobes in the neck lying in close contact with one another in the median plane, the right lobe slightly overlapping the left. The larger lobules were made up of cortical and medullary substance, in the last of which were Hassall's corpuscles. No thoracic thymus was present. Johnstone (26), in 1898, had previously examined two specimens of the same species measuring 20 cm. from the snout to the root of the tail, but in neither did he find any trace of a thymus either in the thorax or the neck.

As regards the Wombat, Symington (60), in 1898, dissected three adult examples of Phascolomys, in all of which a cervical thymus was observed. It consisted of two lateral lobes placed just below the skin and the platysma, parallel with the median plane and overlapped by the submaxillary gland. On the right side of the thorax of one specimen an irregularly shaped thymic lobule, about 18 mm. long, was seen in some loose fat in front of the large vessels and the upper part of the pericardium, but no corresponding structure was present on the left. Symington at the same time remarked that the study of the development of the thymus ought to yield interesting results.

From the following observations it will be seen that both Phascolarctos and Phascolomys always possess a well-developed cervical thymus but normally no thoracic thymus is present. In only one fœtus of Pharcolarctos (Stage IX) was a small poorly developed thymus gland observed on both sides immediately behind epithelial body III. Considering the constancy of the cervical gland it seems probable that Johnstone (26) overlooked this structure in the neck of his two specimens of the Koala. This author himself suggested that, his example being a young one, he might perhaps have missed a small thymus rudiment which would have grown larger in later life.

* These numbers refer in all cases to the List of References in Part I.

The material available for examination consisted of ten stages (8 embryos and 4 pouch-young) of the Koala (*Phascolarctos cinereus*) and three stages (5 embryos) of the Wombat (*Phascolomys mitchelli*), all of which were obtained from Prof. J. P. Hill's excellent collection of young stages of Marsupials. My investigations were carried out under the supervision and with the constant help of Prof. Hill, to whom I wish to express my gratitude. I am also indebted to Mr. F. Pittock, of the Zoological Department, University College, for the microphotograph of text-fig. 1.

Description of Material.

Phascolarctos cinereus.

Stage I.—G.L. 4·5 mm. Plate 11, fig. 1.

The body of this embryo is greatly curved, the head being bent round parallel to the trunk, and it measures 4·5 mm. from the rounded hinder end to the margin of the body above the fore-limb. The eye is rounded and distinct and the jaw processes are very prominent; the fore-limb is differentiated into arm and manus and there is no indication of digits. The hind-limbs are undifferentiated ridges. In external characters this embryo may be compared with Stage I of Trichosurus, but the body here is perhaps more sharply flexed and the tail more curved round.

The *cervical sinus* (fig. 1, *s.c.*) forms a bay widely open to the exterior; it is bounded cranially by the well developed hyoid arch, dorsally by a raised rim, the retrobranchial ridge (His), and caudally by another ridge which lies above the heart swelling. Behind the hyoid in the floor of the sinus lie three smaller branchial arches: the first is the largest and its upper end is situated outside the sinus, the remaining portion sloping inwards behind the hyoid; the second arch is also well marked but rests wholly within the sinus, whilst the third, in the caudal region of the latter, though smaller, is better developed than in Stage I of Trichosurus (fig. 1, *br.a.* 3).

Ectodermal grooves are present opposite the pharyngeal pouches; the second, running up between the hyoid and the first branchial arch, being far the most extensive. Besides the third and fourth grooves, behind the third branchial arch is a fifth ectodermal groove which is more marked than the fourth and more distinct than in Trichosurus. The caudal wall of the sinus is slightly thickened to form the placode of the tenth nerve.

The *second gill-pouch* communicates with the pharynx by a wide cavity, and runs outwards as a narrow tubular passage. A distinct dorsal diverticulum is present and also a ventral one. The closing membrane, which consists of fused ectoderm and entoderm, is much thinner than in Trichosurus, and, as in the latter, its upper portion lies above the dorsal margin of the sinus. Within the sinus on the left side, it is broken open, and ventrally disappears altogether, so that the pouch communicates with the exterior for a distance of approximately 0·20 mm.; on the

right, however, it appears to be open along the whole lateral extent, that is, for 0·36 mm. Considering the fact that the closing membrane of this pouch is completely closed throughout its extent in the next stage, the opening here is very probably an artificial one.

The *third pouch* is shorter than the second, is cranio-caudally flattened and possesses a small ventral diverticulum, but no dorsal one. The closing membrane has a dorso-ventral measurement of 0·20 mm. on the right and 0·11 mm. on the left, and involves the whole lateral margin of the pouch; at its extreme ventral end, however, it is open for a distance of 0·03 mm. on the right side and 0·02 mm. on the left, the pouch thus apparently communicating with the exterior, but here again the aperture has most probably been artificially made.

The *fourth pouch*, which runs directly outwards from the anterior side of the pharyngeal bay, has a short transverse portion and a thick-walled ventral prolongation. The distal wall of the pouch (fig. 1, *cl.* 4) adjoins the ectoderm, but entoderm and ectoderm are never fused, no definite closing membrane being formed.

The posterior pharyngeal complex passes out from the hinder region of the pharyngeal bay (fig. 1, *ph.b.*), and runs ventrally and caudally for a length of 0·12 mm. The proximal portion of the complex is of greater transverse diameter than the distal portion, and probably represents the remains of the fifth gill-pouch, though it is less well developed here than at Stage I (*c*) of Trichosurus, being separated from the fifth ectodermal groove by a distance of 0·08 mm., compared with a measurement of 0·025 mm. in Trichosurus. Here also there is a small fifth aortic arch, which runs up the third branchial arch (fig. 1, *aort.a.* 5).

The *median thyroid* has already lost its connection with the ventral surface of the pharynx, and forms a short tube, measuring 0·10 mm. in length, with thickened walls and very small central cavity, lying on a level with the second gill-pouch (fig. 1, *th.*).

Stage II.—G.L. 7·5 mm.

The embryo is slightly less bent than the preceding one, and measures 7·5 mm. from the neck protuberance, which is here well marked, to the hinder end of the body. The eye is oval, and a depression at the lower end of the hyoid cleft indicates the primordium of the external auditory meatus; in the fore-limb the position of the digits is faintly indicated. This stage resembles Stage III of Trichosurus, but it is more curved, and, on the whole, younger; the sinus is also wider, notwithstanding the reduction of the posterior portion described below.

The *cervical sinus* is still widely open, but is reduced on the posterior side, where, owing to the forward growth of its caudo-dorsal margin, it is converted into a passage which, with a considerably thickened caudal wall, runs inwards and dorsally. Its cavity, at first wide, quickly diminishes towards the inner end, where the coalesced walls form a solid, deeply staining epithelial structure, in close proximity to and apparently in proliferative continuity with the ganglion nodosum. On the

floor of the sinus lie the first and second branchial arches; although the first is now situated entirely within the sinus, both are very well marked and only slightly reduced since the last stage. The third arch has completely disappeared, having evidently become incorporated into the wall of the passage formed by the forward growth of the posterior region of the sinus.

The *second pouch* runs outwards from the wide pharyngeal opening, and possesses a short, narrow ventral diverticulum. The closing membrane, which no longer extends above the dorsal margin of the sinus, involves the entire lateral extent of the pouch, including the ventral diverticulum, and has a dorso-ventral length of 0·14 mm. on the right side and 0·19 mm. on the left. The second ectodermal groove has begun to close in at its upper end as a result of the growth backwards, as yet not very marked, of the hyoid arch. Beyond the termination of the lumen the epithelium of the pouch is now continued on for a short distance as a solid prolongation, fused with the bottom of the second ectodermal groove exactly as in Trichosurus.

The *third pouch*, with its ventral diverticulum, is again very flattened in a cranio-caudal direction. The closing membrane, measuring 0·18 mm. in length, still stretches along the whole lateral side of both pouch and diverticulum. The ectodermal groove is only slightly reduced.

The pharyngeal bay has narrowed considerably and the conditions are similar to Stage III of Trichosurus. The *fourth pouch*, which is shorter than the third and very narrow, communicates by a contracted opening with the cranial side of the bay, and runs ventrally outwards to reach the ectoderm anterior and ventral to the inward prolongation of the postero-dorsal side of the sinus. Ectoderm and entoderm are now fused along the ventral part of the pouch to form a thick closing membrane, and the ectodermal groove is well marked.

The *ultimobranchial body* arises from the bay posterior and dorsal to the fourth pouch, and there is no longer any trace of a fifth pouch, the ultimobranchial body throughout its course being far removed from the ectoderm. It runs directly back as a tube with thick walls and prominent cavity which ends some distance cranial to the pericardium after a course of 0·19 mm. on the right side and 0·21 mm. on the left.

The *median thyroid* forms a short circular tube, 0·13 mm. in length, with a distinct central cavity, lying far removed from the floor of the pharynx between the second and third gill-pouches, just cranial to the ventral aorta.

Stage III.—G.L. 9 mm. Plate 11, fig. 2.

This embryo shows a considerable advance on Stage II. The body is much less curved than the two earlier stages and measures 9 mm. from the neck bend to the rounded hinder end; the head is raised but still forms an acute angle with the trunk, and the neck protuberance is less prominent. The digits of the fore-limb are also more distinctly marked and the hind-limb is in the form of a flattened oblong outgrowth

in which differentiation into leg and foot is just indicated. Although much less curved this embryo is similar to Stage V of Trichosurus, but it is at the same time slightly older.

The hyoid arch is much less prominent and the external auditory meatus better marked. A great change has taken place in the *cervical sinus*, which now appears from the exterior as a deep cleft-like depression just behind and below the primordium of the external auditory meatus. The forward growth of the caudo-dorsal margin has progressed still further so that the passage, mentioned in the last stage, has become transformed into a very narrow vesicle duct, which runs up into a definite cervical vesicle with a central cavity, closely approximated to the ganglion nodosum (fig. 2, *v.c.*). Owing to the backward extension of the hyoid arch the cranio-dorsal side of the sinus has also closed in, converting the second ectodermal groove into a *ductus ectobranchialis* II, which on the left side still retains a minute lumen in its inner part, but which is already practically solid on the right. Posterior to the ventral end of the *ductus ectobranchialis* and immediately in front of the cervical vesicle and the vesicle duct, the walls of the sinus have grown together and form a bulbous mass, which lies opposite the connection of third pouch with the ectoderm and extends down to the ectodermal connection of the fourth pouch (fig. 2, *s.tm.*). This mass is the primordium of the superficial cervical thymus. Further ventrally the sinus is still open and consists of a shallow depression, on the floor of which the ventral part of the first and second branchial arches are still visible, though the former is greatly reduced, and the latter very flat and only just apparent. On comparison with Trichosurus, Stage V, a difference in the method of closure of the sinus is at once apparent. In Trichosurus, the growth of the cranial (hyoid) and caudal margins towards each other is more extensive and involves the whole sinus, so that the latter becomes reduced to a small central part, the sinus proper, which is united with the cervical vesicle by means of the vesicle duct and communicates with the exterior by an oblique slit-like cervical duct (*see* fig. 11); thus all traces of the branchial arches have necessarily disappeared. In Phascolarctos, on the other hand, the dorsal portion of the sinus completely closes, whilst the ventral still remains quite open to the exterior, two low branchial arches still persisting on its floor; no cervical duct is formed, and the very narrow vesicle duct runs down directly into the open sinus. It is evidently owing to these differences, which are therefore especially interesting, that the primordium of the cervical thymus in Phascolarctos does not appear to have any connection with the *ductus ento-ecto-branchialis* II, but lies behind the latter, opposite the third pouch; although here again the thymus is in greater part an ectodermal formation, its close association with the *ductus ecto-entobranchialis* III, and to a less extent with *ductus ecto-ento-branchialis* IV, affords strong support to the view that the entoderm may also to some extent participate in forming the epithelial basis of the cervical thymus.

The *second pouch* communicates with the pharynx by a narrow opening, and runs outwards and downwards as a much elongated and attenuated tube with a gradually

diminishing lumen, which finally disappears after a course of 0·19 mm. on the left side and 0·12 mm. on the right, where the pouch then forms a solid cord fused with the distal portion of the *ductus ectobranchialis* II. These conditions of the second pouch and *ductus ecto-entobranchialis* II are almost identical with Stage V of Trichosurus.

The *third pouch*, which is still connected with the pharynx by a narrow duct-like portion with a very minute cavity, extends laterally and ventrally as a tubular structure with thick walls, and is connected by a broad, solid cell plate, constituting a *ductus ecto-entobranchialis* III, with the closed and expanded area of the sinus destined to become the future cervical thymus (fig. 2, *d.e.b.* III). The third ectodermal groove is very insignificant.

The pharyngeal bay has become still narrower and we may now speak of a pharyngeal duct. The *fourth pouch* is connected with the duct by an almost closed aperture; it consists of a more expanded proximal portion with thickened walls and a distal stalk-like part, which is joined to the lower end of the primordium of the cervical thymus by a short, solid and thickened *ductus ecto-entobranchialis* IV.

The *ultimobranchial body* arises from the pharyngeal duct behind and dorsal to the fourth pouch as a tube of very small diameter and with a small central cavity. It passes caudally and ventrally, gradually increasing in size, and terminates with thickened walls dorsal to the cranial end of the pericardium after a course of approximately 0·24 mm.

The *median thyroid* has begun to flatten out at its ventral end, but the dorsal portion is still in the form of a circular tube. It has a length of 0·20 mm.

Stage IV.—G.L. 11 mm. Plate 11, fig. 3.

There is a considerable interval between this stage and the preceding one. The head is strongly flexed and lies parallel with the trunk, the neck protuberance is still present but is not very marked, and the triangular ear pinna is free. The snout is flattened and the lips are largely free, fusion having taken place only for a short distance; the elbow is just indicated, and the five digits are furnished with the primordia of claws, whilst the hind-limb is differentiated into leg and foot, and the digits are being liberated. This embryo may be compared with Stage XI of Trichosurus, although, on the whole, it is somewhat further developed.

As regards internal structure a great change has taken place. The cervical sinus, including the cervical vesicle, has completely disappeared. The *cervical thymus* is now a well-developed gland lying on a level with the cervical groove, but quite separated from the ectoderm; it has a length of 0·15 mm. on the right side and 0·13 mm. on the left, and a maximum diameter of 0·25 × 0·14 mm. It is surrounded by a connective tissue capsule, and composed of a cellular reticulum with closely packed nuclei, among which only a few lymphocytes are present. Blood vessels have penetrated within the capsule, and even into the edge of the gland itself.

Epithelial body III is already advanced in structure and is now well vascularised. It lies in the typical position far forwards dorsal to the common carotid artery, just behind the point of bifurcation of the latter into external and internal carotids.

The *ultimobranchial body*, which has a length of 0·31 mm. on the right side and 0·36 mm. on the left, is found on the dorsal side of the lateral lobe of the thyroid on each side. It consists of a mass of loosely arranged cells, which surround a wide and conspicuous cavity, and which is distinct from the thyroid except for a few sections, where its ventral surface is continuous with the tissue of the dorsal surface of the lateral lobe (fig. 3, *u.b.*). On the left a definite sprout extends out from the ventro-medial wall of the body, but this does not appear to have a connection with the thyroid tissue.

The lateral lobes of the *thyroid* are now well developed, especially on the right side, where they reach a length of 0·57 mm., compared with a measurement of only 0·48 mm. on the left.

They extend upwards and forwards on each side of the trachea, below which they are connected by a slender bridge, and the tissue of the gland is composed of irregular cell-cords, the majority of which contain a central lumen (fig. 3, *l.th.*). There are as yet no vascular connective tissue ingrowths.

There are no traces of a thoracic thymus, nor any epithelial structures corresponding to epithelial body IV. A small group of vesicles of unknown significance, extending from the cranial end of the pericardium for a length of 0·22 mm., is found dorso-laterally to the œsophagus on the left side. The vesicles are lined by a definite epithelium, and, running into each other, terminate caudally as a single tube.

Stage V.—G.L. 12·25 mm.

Apart from increase in size, no very important changes have occurred in this embryo. The head is slightly raised and there is no neck protuberance, the external auditory meatus is becoming filled up and the pinna soldered down. Lip-fusion has now progressed to about midway between the eye and the snout, and the grooved tongue protrudes; the arm is flexed, with a distinct elbow, and the digits of the manus have definitely pointed and slightly recurved claws. In the hind-limb the digits are more free.

The *cervical thymus* is about the same size as in the last stage, measuring approximately 0·24 × 0·14 mm. in diameter, with an average length of 0·15 mm., and consists of two conspicuous glands situated close together in the middle line, on a level with the cervical groove. They do not differ essentially from the last stage, lymphocytes being hardly more numerous.

Epithelial body III is very large, and is of a more compact structure than in the preceding embryo, the interpenetration of blood vessels being less extensive.

The *ultimobranchial body* lies in the same position, but its connection with the thyroid is perhaps more intimate. It is made up of deeply staining and compact

walls surrounding a well marked cavity; on the right side a narrow, elongated cell-cord stretches down from it for some distance along the medial side of the lateral lobe.

Considerable growth has taken place in the lateral lobes of the *thyroid*, this being especially marked on the left side, where the gland now attains a length of 0·69 mm., whilst that on the right, although shorter, with a measurement of 0·50 mm., has increased appreciably in breadth. Its structure is more compact than at our last stage, the component cells forming a more solid mass with few cavities, and no blood vessels have as yet penetrated into the tissue. There is no median bridge.

Stage VI (*a*) *and* (*b*).—G.L. 13·5 mm. Plate 11, fig. 4.

In this stage are placed two embryos, both measuring 13·5 mm., of which (*a*) is cut transversely and (*b*) longitudinally. The head is considerably more raised and the mid-brain protuberance is less marked; the pinna is soldered down and almost completely covered by epitrichium, the snout is more prominent, and lip-fusion has progressed further. The elbow is more distinct and the digits of the hind-limb are more free.

The *cervical thymus* resembles that of the last stage, lymphocytes being still very few in number. In (*b*) it is circular in form, and has an average length of 0·25 mm. and an average diameter of 0·37 × 0·26 mm.

Epithelial body III is large and conspicuous in (*a*), and lies in the usual position dorsal to the common carotid artery. Owing to the interpenetration of connective tissue ingrowths carrying capillaries, the gland has now come to be formed of cellular cords, most of which are composed of two layers of cells; the body has thus almost attained its adult condition. In (*b*) epithelial body III is not well seen; on the left it is situated immediately cranial to the bifurcation of the common carotid artery, and on the right is on a level with this bifurcation.

The lateral lobes of the *thyroid* are very asymmetrical in both embryos, and there is no connecting bridge. On the right, in Stage (*a*), the gland is well developed and has a length of 0·52 mm., and much vascular connective tissue has penetrated between the irregular cell-cords (fig. 4, *l.th.*), many of which contain a central lumen. Dorsal to it lies a group of loosely connected cells, the ventral side of which is united with the thyroid in several sections (fig. 4, *u.b.*); I have no hesitation in regarding these cells as representing the ultimobranchial body of this side, which has lost its cavity and is undergoing degeneration. On the left the lateral lobe is small, measuring only 0·28 mm. in length, and no ultimobranchial body is recognisable.

In (*b*) a stout bridge is present which, on the left, extends up into a well-developed lateral lobe, 0·4 mm. in length, but which towards the right only enlarges very slightly and then terminates, there being no lateral lobe on this side. The ultimobranchial body is, however, to be found equally developed on both sides; its walls, which surround a conspicuous lumen, are extensive and composed of loosely arranged cells,

the arrangement recalling the condition in Stage IV. On the right it stands isolated, but on the left it lies so closely approximated to the dorsal surface of the cranial portion of the lateral lobe of the thyroid that it is almost impossible to determine whether the two glands are only in contact or whether there is a more intimate connection.

On each side in Stage (*a*), a second epithelial body is observed for the first time in this series of embryos. On the right, it is small and is found laterally to the right ultimobranchial body (fig. 4, *e.p.b.* IV) and on the left it is placed much further caudally, just cranial to the pericardium, between the posterior end of the common carotid artery and the jugular vein; the latter gland has an irregular structure and appears paler in the centre. These two epithelial structures may very possibly be derivatives of the fourth gill-pouch (epithelial body IV), the one on the left having retained the position in which it is usually found in Trichosurus, whilst that on the right has remained attached to the ultimobranchial body and consequently has moved forwards with the latter, a phenomenon which is the usual one in many of the higher mammals.

In (*b*) an epithelial gland is present on each side dorsal to the carotid artery, not far caudal to epithelial body III and to the ganglion nodosum. It is smaller than epithelial body III and is fairly compact, although apparently vascularised round the edges; it is more elongated on the left side. There is here no evidence of the origin of these extra bodies and if they correspond to epithelial body IV, they lie in a more anterior position than is usual.

Stage VII.—(*a*) G.L. 16·5 mm.; (*b*) G.L. 17 mm.

A new-born fœtus measuring 16·5 mm. and an embryo of 17 mm. are placed together in this stage. The head still lies at an acute angle to the trunk, the snout is rounded and the eye covered by epitrichium. The lip is nearly complete, especially in 16·5 mm., where there is a definite sucking mouth; the tongue no longer protrudes and is distinctly V-shaped in (*a*), but in (*b*) it is very slightly hollowed and only slightly bifid. The claws are strongly recurved and the digits of the hind-limb are free and distinct.

The *cervical thymus* has increased considerably in length, and in (*a*) measures 0·27 mm. in length with a diameter of 0·33 × 0·26 mm. on the left side. The two glands lie some distance apart in the middle line and are situated close to the skin at the bend of the neck. Vascular ingrowths from the capsule are penetrating into its substance, and as a result we have a lobular formation round the edge of the gland. Lymphocytes are more abundant than in Stage VI but they are not yet more numerous than the epithelial elements. Thymic cavities are present in both glands, especially in that of the right side; they occur several grouped together, or singly.

Epithelial body III in both examples occupies the typical position and has now reached the adult condition. In (*a*) the gland appears to be better vascularised than in (*b*).

In both (*a*) and (*b*) the lateral lobes of the *thyroid* are well developed and very vascular, and they are also divided into several portions, especially in (*b*); the cords are becoming broken up into cell-masses, many of which are luminated and invested by connective tissue. In (*a*) there is no median bridge, and besides the two main lobes, which have a length of 0·73 mm. and 0·63 mm. respectively, is one small, isolated, more darkly staining thyroid segment (0·16 mm. in length) lying further caudally on the left side. In (*b*) there is one lateral lobe on the right side, which measures 0·72 mm., whilst on the left, some distance dorsally to the main lobe, which has a length of 0·37 mm., and beginning at the caudal end of the latter, lies a smaller but more elongated portion, 0·48 mm. in length, quite separated from the first. This smaller portion continues further back and, passing ventrally, is alone joined by a slender connecting bridge with the lateral thyroid of the opposite side. Again on the left side, another isolated fragment (0·1 mm. in length) occurs behind the bridge.

The *ultimobranchial body* cannot be determined with certainty. In (*a*), on the dorsal side of the right lateral lobe, occurs a region in which the cells stain a little more deeply and in which an indefinite cavity is present in one section; we may quite possibly have here a last vestige of the ultimobranchial body. In (*b*), the dorsal portion of the two main lobes is triangular in shape, with the more pointed end lying dorsally, and appears to be more or less marked off from the rest. At its cranial end, this part is more deeply staining, but farther back it is identical with the remainder of the lobe, though at the same time marked off from the latter for a long way caudally, especially on the right side. Its exact posterior limits, however, are impossible to decide. Whether this darker portion of the thyroid has any significance or not, it is impossible to say, and we have no proof that it is in any way connected with the ultimobranchial body, but if it has any relation to the latter, then the ultimobranchial body must furnish a considerable portion of the thyroid tissue; but such speculation is hardly warranted with the meagre evidence before us.

An extra epithelial body is present in (*b*). It is deeply staining and very small in cross section but is very elongated, extending back from the point of origin of the two common carotid arteries, dorso-medially to the truncus arteriosus for a length of 0·24 mm.

Stage VIII.—G.L. 18 mm.

This fœtus has a maximum length of 18 mm. The head still forms an acute angle with the axis of the trunk but the hinder end of the body is more straightened out than in Stage VII. The snout now presents the flattened form characteristic of the adult and the external nares are also flattened; lip fusion is complete, the vertical height of the mouth-opening has increased and the tongue is distinctly hollowed out above. The arms are more raised and meet in the median line across the chest.

The *cervical thymus* is made up of three separate vascular glands, all of which have an irregular lobed outline and show indications of a cortex and a medulla, lymphocytes being more numerous round the edges than in the centre. The largest of the glands

is elongated in the transverse plane, and measures 0·35 × 0·12 × 0·13 mm.; it is situated ventro-laterally close to the skin on the right side. A short distance farther back are two much smaller glands with a length of 0·21 mm. on the left and 0·15 mm. on the right and an average diameter of only 0·15 × 0·14 mm., lying close together in the middle line near the ventral surface. There are no thymic cavities. It seems likely that the unusual occurrence of an extra superficial cervical thymus on one side is simply due to a secondary division of the normally single gland of that side.

Epithelial body III is well developed on each side and is situated dorso-laterally to the common carotid at its point of bifurcation.

The *ultimobranchial body* of the right side is no longer recognisable, but on the left an irregular mass of cells lying dorsal to the lateral lobe of the thyroid is suggestive of an ultimobranchial body, chiefly in its posterior portion, where it is separated from the lateral lobe; further anteriorly, however, it takes the form of luminated cords or cells exactly like those of the thyroid tissue, and becomes united indistinguishably with the latter.

The structure of the *thyroid* is very similar to that of the last stage and there is no median bridge. It is well developed on the left side, having a length of 0·73 mm., but is very short on the right, being only about half as long (0·39 mm.).

Stage IX.—G.L. 20 mm. H.L. 9 mm. Longitudinal Series.

The *cervical thymus* rather resembles that of Stage VII (*a*), although lymphocytes are less abundant, and contains thymic cavities, which occur singly or in groups. It is larger on the right side, where a maximum length of 0·39 mm. and a diameter of 0·18 × 0·31 mm. is reached.

Epithelial body III is found in the usual position, and is somewhat smaller than at Stage VII (*a*).

A lateral *thyroid* lobe is present on each side, but is better developed on the left, where it measures 0·53 mm. in length, as compared with 0·34 mm. on the right. There is no change in its structure, and a median bridge is absent.

All traces of the ultimobranchial body have disappeared.

Three accessory epithelial bodies are to be seen in this fœtus. A small, solid and darkly staining body lies immediately cranial to the left thyroid lobe, and a second, which is exceedingly small, is situated dorsally to the caudal end of the right lobe. The position of these two glands points, as in the case of Stage VI (*a*), to their possible origin from the fourth pouch. The third body, which is again very small and solid, is found farther back, just cranial to the pericardium.

An extra thymus gland is present on each side dorsally to the common carotid artery, just caudal to epithelial body III, its position suggesting an origin from the third gill-pouch. Although it has a length of 0·35 mm., it is very narrow dorso-ventrally and is poorly developed, being composed of epithelial cells, among which

are a few lymphocytes. As already noted (p. 87), this is the only instance in which a possible thymus III has been observed in Phascolarctos.

Stage X.—G.L. 24 mm. Text-fig. 1.

Our last stage is a fœtus measuring in G.L. 24 mm.

The *cervical thymus* (text-fig. 1, *s.tm.*) now forms two large conspicuous glands with many lobes; on the right side they measure 0·63 mm. in length, with a

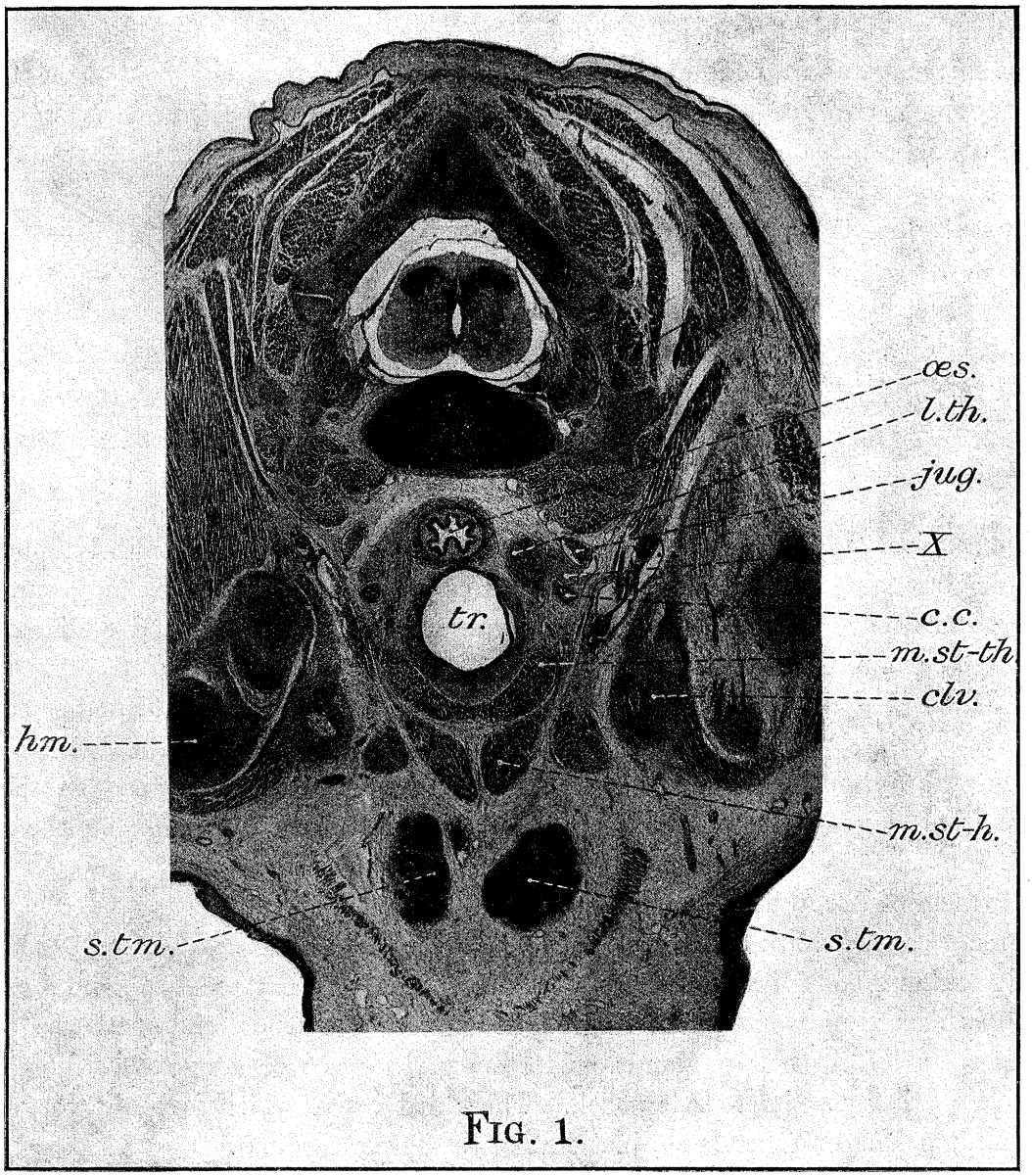


FIG. 1.

TEXT-FIG. 1.—Stage X. Phascolarctos, G.L. 24 mm. Microphotograph of a transverse section (S 11–2), showing the superficial cervical thymus and the main lateral thyroid lobe of the left side. × 20.

c.c. = common carotid artery; *clv.* = clavicle; *hm.* = humerus; *jug.* = jugular vein; *l.th.* = lateral thyroid lobe; *m.st.-h.* = sterno-hyoid muscle; *m.st.-th.* = sterno-thyroid muscle; *œs.* = œsophagus; *s.tm.* = superficial cervical thymus; *tr.* = trachea; X = vagus nerve.

diameter of 0·80 × 0·43 mm., but on the left have a length of only 0·46 mm., with a diameter of 0·77 × 0·70 mm., and lie close to one another in the middle line at the bend of the neck. Lymphocytes are very abundant, filling the greater part of the gland, differentiation into a cortex and a medulla being as yet not very distinctly indicated. There is a thick capsule, which apparently forms a matrix in which the thymus is imbedded. No Hassall's corpuscles or epithelial bodies are developed, but thymic cavities are very plentiful, and occur mainly as groups of vesicles or tubules ramifying throughout the length of the gland. A conspicuous branching epithelial cord in the centre of each thymus appears to be connected with the vesicles.

Epithelial body III is situated medial to the division of the common carotid. On the right side it appears to be sending out sprouts, one of which extends out for some distance in a cranio-dorsal direction.

The lateral *thyroid* lobes are well developed, and are again very much broken up (compare Stage VII, *b*). They consist of irregular tubes, some of which have divided up into definite vesicles, though vesicle formation is not far advanced, and there is no colloid. On the right side there are two main lobes quite isolated from one another, a cranial one with a length of 0·34 mm., in which lies the ultimobranchial body, and a caudal one with a length of 0·55 mm., placed just lateral to the œsophagus and more dorsal in position. Besides these, three small fragments are found on the same side, one far behind the main lobes and lateral to the œsophagus, and two still further caudally, more ventrally situated. The ultimobranchial body of this side has a large cavity surrounded by comparatively thin walls, from which thin, hollow sprouts run outwards, and it is a question whether it has not given rise to the whole of the main cranial lobe in which it lies. On the left side there is only one main lobe (text-fig. 1, *l.th.*), measuring 0·70 mm. in length, in which is situated a small and apparently more quiescent ultimobranchial body; far caudally is one small accessory gland measuring 0·16 mm. No median bridge connects the lobes below the trachea.

A small, solid epithelial body is present on the right side dorso-medially to the carotid artery and cranial to the first small thyroid fragment, just behind the accessory thyroid lobe of the opposite side.

Phascolomys mitchelli.

Stage I a.—G.L. 9 mm.

Our youngest stage of the Wombat has a maximum length of 9 mm., and is comparable to Stage III of the Koala of the same length, and similar to Stage VI of Trichosurus. The axis of the head forms an acute angle with that of the trunk, the neck protuberance is large and projecting, and the mid-brain prominent. The eye has a very small lens, and the external auditory meatus is wide below and narrow above, and in front of it is a triangular thickening, the apex of which points

anteriorly. The hyoid is of large size, and shows the rudiment of the ear pinna, the arch being produced backwards to a blunt point, and forwards, above the wide lower part of the meatus, into a distinct projection, behind which is a second excrescence, the whole recalling the hyoid in Stage VII, Trichosurus. The heart forms a cushion on which the head rests. The fore-limb is directed parallel with the trunk, and is differentiated into an arm and a manus, with the five digits indicated as lobes; there are no digits on the hind-foot.

The *cervical sinus* is much reduced, and is visible from the exterior as a distinct depression along the postero-inferior margin of the hyoid arch. The sinus proper forms an almost closed chamber, triangular in cross section, which is united with the cervical vesicle by means of a very short vesicle duct, and communicates with the exterior by the last vestige of the cervical duct, which has not yet quite closed. The cervical vesicle is closely adjacent to the ganglion nodosum. The second ectodermal groove is completely closed, and the walls of the cranio-dorsal margin of the sinus have already fused and thickened to form the primordium of the superficial cervical thymus, the thickening extending from the base of the solid *ductus ectobranchialis* II down to the connection of the third pouch with the sinus. The description of the sinus of Phascolomys at this stage shows that its mode of closure resembles the conditions found in Trichosurus (compare Stage VII) rather than those in Phascolarctos. As in Trichosurus, we have here the formation of a distinct cervical duct, and the same relations prevail between the first origin of the cervical thymus and *ductus ecto-entobranchialis* II.

The *second pouch* runs out from the pharynx as a tubular outgrowth, which, soon becoming solid, extends outwards and downwards as an attenuated solid cord, the lower end of which is indistinguishably fused with the upper end of the *ductus ectobranchialis*. The *ductus ecto-entobranchialis* II, which is thus already formed, increases in diameter at its ventral end, where it passes into the cervical thymus.

The *third pouch* leaves the pharynx as a short tubular prolongation, the distal portion of which has considerably thickened walls. It is joined by a solid cellular cord with the walls of the sinus just at the base of the developing cervical thymus.

The *fourth pouch* is quite disconnected from the pharyngeal duct, but is still joined with the ultimobranchial body on the right side by a degenerating strand, though quite separated from it on the left. The pouch forms a partly hollow, partly solid, insignificant group of cells, which is connected with the wall of the sinus by a long, drawn-out *ductus ecto-entobranchialis* IV, running close to the medial side of the ganglion nodosum and immediately in front of and parallel to the vesicle duct.

The *ultimobranchial body* extends backwards and ventrally from the pharyngeal duct as a narrow, hollow tube, the walls of which gradually increase in thickness towards the distal end, where the two bodies become slightly approximated in the middle line. It has at this stage a length of 0·20 mm.

The *thyroid* has a length of 0·12 mm.; its cranial end is rather irregular in form,

and apparently asymmetrical, lying to the left of the middle line, whilst the hinder portion is a flattened plate-like structure containing a transversely flattened central cavity.

Three very small epithelial glands, two on the left side and one on the right, are found in this embryo, medially to the outer end of the second pouch.

Stage I b.—G.L. 8·5 mm. Plate 11, fig. 5.

The external appearance of this embryo, which measures 8·5 mm. at its greatest length, does not differ essentially from the preceding one, though it is slightly older. The neck protuberance is more marked and the digits of the fore-limb more free. The sections are longitudinal.

Further progress has been made in the formation of the *cervical thymus*, which now forms a solid swollen mass, surrounding the reduced cavity of the cervical sinus and tapering upwards into the *ductus ecto-entobranchialis* II. The sinus is thus smaller than at our last stage, but still communicates with the exterior by a narrow cervical duct. The cervical vesicle lies at the extreme ventro-lateral side of the ganglion nodosum, but it is small and only contains a minute cavity which extends into the vesicle duct for a short distance, the duct being apparently solid at the end where it joins the wall of the sinus.

The *second pouch* does not differ from Stage I (*a*) and resembles Stage VIII (*a*) or Trichosurus (*see* fig. 21).

The *third pouch* is still connected with the pharynx by a narrow tube, which is difficult to see, on the left side of the embryo; it runs outwards and ventrally for an average length of 0·25 mm. and is joined with the ectoderm of the sinus by a fine cord. The proximal dorsal portion of the pouch (fig. 5, *cl.* 3) has thickened walls and contains a small central cavity which soon becomes obliterated, the distal ventral portion, which lies on the cranial side of the ganglion nodosum just dorsal to the cervical vesicle, being solid and of smaller diameter. There is here no differentiation between the cells of the dorsal and ventral portions of the pouch such as in Stage VII of Trichosurus, but the cells, especially at the solid ventral end, are somewhat more eosinophil in character, suggesting the first transformation into an epithelial body.

All that remains of the *fourth pouch* is a small mass of cells having no connection with either pharynx or sinus, situated in the fork of the vagus and hypoglossal nerves posterior to the third pouch (fig. 5, *cl.* 4). On the right side it is visible through three sections and contains a very small lumen, but on the left it is still more insignificant and consists of a degenerating remnant only recognisable in two sections.

The *ultimobranchial body* is still joined with the pharyngeal duct. Its ventral end, however, is apparently beginning to move in a cranial direction towards the thyroid, though the movement is as yet very slight; it lies dorsal to the hinder end of the ventral aorta.

The *thyroid* possesses a well marked central cavity which breaks up into several smaller lumina on each side, where the gland, especially towards the left, enlarges to form the primordium of the lateral lobe. The whole structure is very asymmetrical, being placed very much to the right side, the larger left lateral lobe lying almost immediately below the trachea.

Stage II a and b.—G.L. 15·5 mm. Plate 11, figs. 6 and 7.

There is a great interval between the embryos of this stage, which have a length of 15·5 mm., and the last one. The head is raised, but not completely, the eye is slit-like, the eyelids being not yet completely united, and there is a triangular raised pinna, covered like the eye with epitrichium. Distinct lip-fusion is not yet developed, and the tongue is slightly bifid and projects from the mouth. The elbow is formed and the five digits have marked curved claws. In (*b*), the pinna is not filled up and not covered by epitrichium, and the foot has five digits.

In both specimens the *superficial cervical thymus* consists of two glands situated one on each side of the neck, just cranio-ventrally to the submaxillary gland and widely separated from each other in the middle line. Each gland is well vascularised and surrounded by a connective tissue capsule ; lymphocytes are very few in number.

Epithelial body III is a large and well developed gland lying dorsal to the common carotid artery, immediately caudal to the point of bifurcation of the latter. In (*a*) (fig. 6, *ep.b.* III) it has, for the most part, the form of a compact mass of cells, but on the right the interpenetration of connective tissue carrying blood vessels is already conspicuous, giving the gland a lobed appearance. In (*b*) the ingrowth of capillaries has proceeded further, and the cells form branching walls of varying thickness surrounding sinusoids.

The lateral lobes of the *thyroid* are well developed and very vascular ; they consist of branching cords of cells, most of which contain a central cavity. In (*a*), the right lobe is larger than the left and there is a slender median bridge ; in (*b*), the bridge is more substantial.

It is impossible definitely to determine the *ultimobranchial body.* In (*a*), situated on the dorsal side of the cranial end of the lateral lobes, there are some more darkly staining and more solid cords of cells, which probably correspond to that body, but no cavity is present. In (*b*), on the left side, the cranial end of the lobe stretches anteriorly and dorsally for some distance, close to the medial side of the common carotid artery, as a slender solid strand.

Epithelial body IV is present in both examples on each side. In (*a*) (fig. 7, *ep.b.* IV) it is a small but conspicuous gland of closely packed cells, placed some distance behind the thyroid between the common carotid artery and the jugular vein, just behind the union of the latter with the subclavian vein. In (*b*), it lies a trifle further anteriorly, and consists of columnar cells surrounding a clear central space ; on the left side it presents a branching appearance.

Stage III.—G.L. 17·5 mm.

This embryo probably represents a stage just before birth. It measures 17·5 mm., but the difference in length is mainly due to a longer neck and longer abdomen, though the body is less curved than at Stage II. The head lies practically at right angles to the trunk, the eyelids are united, and both eye and ear are covered by epitrichium; lip-fusion is almost completed and the tongue is bilobed and no longer protrudes. The elbow is more distinct, the arms raised and approximated in the middle line, and the digits of the hind-limb are more free.

The *cervical thymus* is very similar to that of the last stage and has much the same length, though smaller in diameter. Lymphocytes are hardly more abundant.

The lateral lobes of the *thyroid* are well developed on each side, and consist of hollow cords, whose cavities are more conspicuous than in 15·5 mm. There is a very slender median bridge, which is almost broken near the lobe on each side and also in the centre below the trachea. The dorsal side of the cranial end of the lobes may correspond to the ultimobranchial body, but the exact position of the latter is doubtful.

Epithelial body IV is present on both sides. It is similar in structure and position to Stage II (*b*) of Phascolomys and Stage XVII (*b*) of Trichosurus, having on the left side a vesicular appearance, though it does not branch.

A very small accessory epithelial body occurs on each side behind and medial to the larger one.

Summary.

The cervical sinus at our first stage of Phascolarctos (fig. 1, *s.c.*) forms a wide bay, on the floor of which lie three branchial arches; it resembles in all essential respects the sinus of Stage I of Trichosurus except that in Phascolarctos, although the fifth pouch is less well marked, the third branchial arch is more prominent. At Stage II we find the caudo-dorsal margin of the sinus has already grown forwards, with the result that a vesicle duct, running up to the solid placode in connection with the ganglion nodosum, is now formed, although we cannot as yet speak of a definite cervical vesicle. The rest of the sinus is widely open and two branchial arches are present within it, the third having become obliterated in the formation of the vesicle duct. By Stage III the dorsal portion of the sinus has closed with the exception of a cervical vesicle and vesicle duct, whilst the lower ventral portion is still open, but at the same time now more shallow, and two reduced and flattened branchial arches are still visible on its floor. Owing to the backward growth of the hyoid arch, an almost solid *ductus ectobranchialis* II has developed and the coalesced walls of the upper portion of the sinus posterior to the ductus form a swollen mass of cells opposite to the third pouch (fig. 2, *s.tm.*) and extending down opposite to the fourth pouch. This is the first indication of the superficial cervical thymus, which does not appear in Phascolarctos to have any relation to the *ductus ecto-entobranchialis* II as in

Trichosurus, but is closely associated with the *ductus ecto-entobranchialis* III (fig. 2, *d.e.b.* III), which at this stage has the form of a broad solid cell-plate, the *ductus ecto-entobranchialis* IV also apparently taking some share in its formation. This distinction is probably the consequence of a different mode of closure of the sinus in the two genera.

From examination of our two earliest embryos of Phascolomys, it is evident that the closing of the sinus takes place there in the same manner as in Trichosurus, a cervical duct being developed, and the primordium of the cervical thymus occurring as a swollen mass surrounding the sinus and tapering upwards into the *ductus ecto-entobranchialis* II.

The constitution of the cervical thymus is therefore the same in both cases in so far as we cannot exclude the participation of entoderm in its formation. Whereas in all three genera it is mainly composed of the ectoderm of the sinus, in the case of Trichosurus and Phascolomys it has a close connection with the *ductus ecto-entobranchialis* II, whilst in that of Phascolarctos a similar association is to be found with *ductus ecto-entobranchialis* III and probably also with the *ductus ecto-entobranchialis* IV. As the *ductus ecto-entobranchialis* III in the latter case is particularly thickened and solid, the probability of the entoderm taking a share in the building up of the thymus seems here to have more weight.

Returning to Phascolarctos, we see in Stage IV a considerable advance. Here the cervical thymus is completely separated from the ectoderm and already encapsulated; vascularisation has set in, but only a few lymphocytes are present within the tissue of the gland. The cervical vesicle is no longer visible, and thus disappears at an earlier stage than in Trichosurus. The formation of lobes has begun at Stage VII, and the first indications of cortical and medullary regions occur at Stage VIII. By Stage X (text-fig. 1, *s.tm.*) the gland is of large size and is composed of many lobes, in which lymphocytes are abundant. No epithelial bodies or Hassall's corpuscles were observed in any of our specimens, but thymic cavities, occurring singly or in groups, are present in the older stages of Phascolarctos. These have a distinctive appearance in Stage X, where they are connected with a conspicuous branching epithelial cord.

No thoracic thymus normally occurs in either Phascolarctos or Phascolomys. In only one case (Stage IX) was a fairly long but narrow thymus gland found on each side immediately caudal to epithelial body III, and therefore more cranially situated than a typical thoracic thymus, but its position suggests an origin from the third pouch.

In possessing only one pair of thymus glands, which are superficially situated at the bend of the neck, Phascolarctos and Phascolomys show a remarkable parallelism with Talpa, where similar conditions are found. According to Rabl (46) a thymus III is developed in the Mole, but its caudal part, which he terms the thoracic thymus, early undergoes complete atrophy, only a remnant of its cranial part remaining. The cervical glands, which are well developed, thus form practically alone the functional

thymus of the adult. The latter here arises from the whole of the closed-off sinus and is therefore a purely ectodermal structure. If, as was suggested in Part I, the ectodermal cervical gland arose from a purely entodermal thymus II by a gradual substitution of ectoderm from the walls of the cervical sinus for the original purely entodermal basis, then both Phascolarctos and Phascolomys show, like Trichosurus, a more primitive condition than Talpa in the probable retention of some entoderm in the structure of the cervical thymus.

Although the thoracic thymus is usually a purely entodermal gland, in several cases, for example in the Pig (Zotterman, 69) and in the Guinea-pig (Anikiew, 1; Ruben, 50; Rabl, 49), ectoderm has been found to take a part in its construction. In Cavia (Rabl, 49), it is developed from the lateral segment of the third pouch and the adjacent ectoderm of the sinus, when fully formed, however, it lies in the neck at the side of the trachea and is not superficial. The thoracic thymus of Tarsius (Nierstrasz, 39) appears to be of the same nature, and probably also of Nycticebus (Nierstrasz, 39), where it is also similarly related to the fourth pouch. In these forms, therefore, we have possibly the first step in the transformation of an entodermal into an ectodermal gland. In Phascolarctos, the process of substitution has advanced further, and thymus III and IV are completely absent, owing to their having been replaced by the cervical gland, which, although mainly ectodermal in origin, still retains some entoderm in its composition.

The condition of the *second pouch* need not detain us, as its development is almost identical with that of Trichosurus. The early development of the pouch is similar, and later a corresponding solid elongated *ductus ecto-entobranchialis* II is formed. Instead of gradually increasing in diameter at its ventral end to run into the primordium of the cervical thymus, as in Phascolomys and Trichosurus, the lower end of the ductus in Phascolarctos is simply a solid fairly substantial cord joined with the cranio-dorsal margin of the sinus.

As in the younger embryos of Trichosurus, the *third pouch* in Stages I and II of Phascolarctos is shorter than the second and flattened cranio-caudally, whilst the closing membrane involves the whole lateral extent of the pouch. At Stage III its walls are somewhat thickened, and it is connected with the primordium of the cervical thymus by a remarkable thick and solid cell-plate, forming a *ductus ecto-entobranchialis* III, the significance of which has already been discussed. In Phascolomys (Stage I) the *ductus ecto-entobranchialis* III is much narrower and joins the sinus at the base of the developing cervical thymus, thus resembling Trichosurus. In Stage I *b* of Phascolomys the thickened walls of the pouch stain slightly more deeply with eosin and suggest the primordium of an epithelial body (fig. 5, *cl.* 3). In later stages an epithelial body with the typical structure is always found in the usual position dorsal to the bifurcation of the common carotid artery. From our observations in Phascolomys we are justified in concluding that this body arises from the third pouch as in Trichosurus, and corresponds to epithelial body III. It will be

remembered that, in the latter genus, it develops from the cranial wall of the dorsal luminated portion of the pouch, whilst in many other mammals it is said to originate from a dorsal diverticulum of the same, although in the human embryo, as Grosser (16) has pointed out, it arises as a proliferation of the oral and lateral walls of the dorsal diverticulum and also from the pouch itself; in the Wombat and probably also in the Koala, practically the entire pouch appears to take part in its formation. In this connection we may again refer to Rabl's (46) description of the Mole, where he says (p. 50): "So komme ich zum Ergebnis, das das Epithelkörperchen aus der ganzen sekundären Tasche und nicht blos aus einem dorsalen Diverticulum derselben hervorgeht." This is probably correlated with the absence of a thymus from this pouch.

The *fourth pouch*, which runs out from the pharyngeal bay, is very short and narrow. By Stage III, Phascolarctos, the pharyngeal bay has become converted in the pharyngeal duct, with which the pouch is still connected; it now consists of a thick-walled proximal and stalk-like distal portion, and is united with the lower end of the cervical thymus by a short and thick *ductus ecto-entobranchialis* IV. Turning to Phascolomys, Stage I *a*, we find that the fourth pouch is an insignificant structure, already disconnected from the pharyngeal duct and almost separated off from the ultimobranchial body, whilst the *ductus ecto-entobranchialis* IV has become thin and elongated, recalling Stage VI of Trichosurus. At Stage I *b*, it is evidently undergoing degeneration and is represented by a small group of cells, only just recognisable on the left side, but still containing a minute lumen on the right (fig. 5, *cl.* 4), lying posterior to the third pouch. From the examination of this stage one would come to the conclusion that this pouch completely degenerates without giving rise to either thymus or epithelial body; in our three older embryos of Phascolomys, however, we find a second epithelial body on both sides, situated some distance caudally to the thyroid gland and therefore in the typical position of epithelial body III of Trichosurus.

In Phascolarctos, a second epithelial body is altogether absent in four cases, namely in Stages IV, V, VII *a*, and VIII, but in five cases, Stages VI *a* and *b*, VII *b*, IX and X, one is present on one or both sides. These extra epithelial bodies vary in position, but are usually placed cranially to the pericardium; in two instances, however, at Stage VI *a* (fig. 4, *ep.b.* IV) and IX, they are found lateral to the ultimobranchial body and thyroid lobe, on the right side in VI *a* and on each side in IX. This latter situation accentuates the probability of their having been derived from the fourth pouch, the primitive connection with the ultimobranchial body having been retained, and consequently they have moved together with the latter in its forward migration towards the lateral lobe of the thyroid. This relation to the ultimobranchial body is the usual one in higher mammals and is possibly facilitated by the absence of a functional thymus from the fourth pouch. From the foregoing facts we may conclude that in Phascolomys and Phascolarctos the fourth pouch may develop into an epithelial body or may, on the other hand, altogether degenerate.

The *ultimobranchial body* at Stage I, Phascolarctos, leaves the pharynx together with the fifth gill-pouch, which is here less well developed than in Trichosurus (Stage I *c*), and which has quite disappeared by Stage II, where the body passes back from the pharynx as a tube with thickened walls, which attains a length of 0·24 mm. at Stage III. At Stage IV it forms a mass of loosely connected cells surrounding a wide cavity and lying on the dorsal side of the lateral lobe of the thyroid, its ventral surface being in contact with the latter for a very short distance (fig. 3, *u.b.*). A similar connection exists in Stage VI *b*, but in VI *a* it has lost its central cavity and, though definitely recognisable on the right side, is obviously breaking down. In later stages it cannot be detected with certainty, although possible vestiges are present within or forming part of the thyroid lobes; there is an exception, however, in the case of Stage X, where it is visible on both sides within the lateral lobe and on the right has actively sprouting walls. In Stage I *b* of Phascolomys the ultimobranchial body, although still joined with the pharyngeal duct, appears at its ventral end on the one side to have begun to shift forwards towards the thyroid. In the older embryos, the determination of the body is again not easy. With the single exception of the fœtus of 24 mm., the ultimobranchial body disappears at an earlier stage than in Trichosurus, or at all events earlier loses its central cavity, a circumstance which makes its identification more difficult. Although it seems probable that it does participate in the formation of thyroid tissue, taken as a whole, the evidence here does not appear to be so convincing as in Trichosurus.

The development of the *thyroid* is like that of Trichosurus. At Stage I of Phascolarctos the median thyroid primordium has already lost its connection with the pharynx, and forms a short tubular structure with thickened walls and central cavity, lying on a level with the second gill-pouch. By Stage III it has begun to flatten out at its ventral end, and at Stage IV (fig. 3, *l.th.*) the lateral lobes are well developed and composed of irregular cell-cords, and the median bridge has disappeared. This bridge in older stages is wanting in five out of eight examples, and in two it is very slender. The gland is well vascularised at Stage VI (fig. 4, *l.th.*), and the formation of vesicles has begun at our next stage. The lateral lobes in all have a tendency to be asymmetrical, for example, in VI *b* the left lobe is present, but the right is completely lacking, and in several cases the lobe is much smaller on one side than the other. Again, from Stage VII onwards, it is quite usual for smaller or larger portions of the lateral lobes to be found isolated from the rest, this condition being especially marked in Stage X, where, on the right side, we find two main lobes and no less than three smaller fragments. In this last respect Phascolarctos differs from Trichosurus. In the older embryos of Phascolomys the lateral lobes are well developed and very vascular, and a slender connecting bridge is present, although broken at Stage III.

The chief features in Phascolarctos and Phascolomys may be summarised as follows :—

1. A well developed superficial cervical thymus is normally the only thymus gland in the Koala and the Wombat. It arises mainly from the ectoderm of the sinus, but entoderm, either from the second pouch (Wombat) or the third and fourth pouches (Koala) may take part in its formation.

2. Epithelial body III is always present.

3. A second epithelial body, presumably epithelial body IV, is usually found in the Wombat, but may or may not be present in the Koala.

4. The median thyroid primordium differentiates to form a bridge and lateral lobes, but the median bridge usually disappears in older stages. In the Koala the lateral lobes tend to be asymmetrical, and to divide up into several isolated portions.

5. The ultimobranchial body early loses its identity on reaching the lateral lobe of the thyroid, and, though sprouts are given off, the part taken by these in the formation of thyroid tissue is difficult to ascertain definitely.

6. A thoracic thymus is not usually developed, being found only in one case (Stage IX of Phascolarctos).

Perameles. Plate 11, fig. 8.

In 1898, JOHNSTONE (26) dissected a full-grown specimen of *Perameles Gunni*, and found a thoracic thymus of two well developed lobes extending from a little below the first rib to about half-way between the second and third. The right lobe was thicker than the left, and there was no external sign of division into secondary lobules. He also examined a fœtus of the same species with a head-length of 1·75 cm. Here again there was no cervical thymus, but the thoracic gland was especially large and lobulated, and extended from about the anterior limit of the sternum to the apex of the heart.

My observations on Perameles were made on two embryos of *P. nasuta*, having a length of 7 mm. and 14·25 mm., and a fœtal specimen of G.L. 17 mm., and three embryos of *P. obesula*, one measuring 8·75 mm. and two measuring 12·25 mm. A thoracic thymus is always found, but a cervical gland is never developed. These results thus confirm those of JOHNSTONE.

Cervical Sinus and Second Gill Pouch.—In *P. nasuta*, 7 mm., the *cervical sinus* is already partially closed owing to the forward growth of the cranio-dorsal margin and the backward growth of the hyoid arch, the branchial arches having quite disappeared. It consists of a central portion, the sinus proper, which on its posterior side is connected by a narrow vesicle duct with the cervical vesicle, and on its anterior side runs up into the *ductus ectobranchialis* II, and which communicates with the exterior by a fairly wide cervical duct. The *ductus ectobranchialis* II is elongated and narrow throughout its extent, and becomes quite solid at its inner end, and the cervical vesicle, which lies in close connection with the ganglion nodosum, has thickened walls and a small transversely elongated lumen.

The *second pouch* leaves the pharynx as a narrow tube which passes outwards

and downwards, still retaining its well marked central cavity until it becomes united with the inner end of the *ductus ectobranchialis* II. Although the pouch is luminated throughout the greater portion, the ventral end is perhaps solid, for it is impossible to be certain where the pouch ends and the ductus begins. By 8·75 mm., both second pouch and sinus have completely disappeared.

Third Pouch.—In our youngest embryo of 7 mm. the *third pouch* is still joined with the pharynx by an almost closed tube, but has lost all connection with the ectoderm of the sinus. It consists of a proximal dorsal half with thickened walls, and at first wide but gradually diminishing lumen, and a distal ventral half, which is quite solid and projects down ventrally and cranially. The dorsal portion retains the columnar arrangement of its cells, and at the same time appears to stain a little more deeply with eosin, recalling the very first development of the epithelial body in Trichosurus, whilst the cells of the ventral part, which furnish the primordium of the thymus, have an irregular arrangement. The difference in staining is so slight that it is impossible to make a distinction between the cranial and caudal walls.

Fourth Pouch.—The *fourth pouch* at 7 mm. arises from the lateral side of the pharyngeal duct just in front of the ultimobranchial body, as an exceedingly long and thin tube with a very small central cavity. Passing ventrally, it runs into the main part of the pouch, which is composed of a luminated dorsal and a solid ventral portion, the latter, which is the primordium of thymus IV, extending down ventro-laterally as far as the anterior end of the pericardium on each side. The differentiation of the two portions is even less marked than in the case of the third pouch, and the lumen penetrates further down, so that the solid part is less extensive. These conditions may be closely compared with Stage VIII of Trichosurus. On the left side a small remnant of the former connection with the ectoderm is still present as a fine strand running outwards from the lateral side of the pouch for a short distance.

Thymus III *and* IV.—In Perameles *thymus* III and IV are always present, but, as in Trichosurus, they may form either two separate glands or may be united together on one or both sides. At 8·75 mm., there is only one thoracic thymus on the left side, which extends from the outer side of the cranial end of the ultimobranchial body down to the anterior end of the pericardium, whilst on the right are apparently two glands, each equal in the length to the single one of the opposite side. They are surrounded by a connective tissue capsule and lymphocytes are as yet very scarce. At 12·25 mm. (transverse series), thymus III forms two large and conspicuous structures lying very close together in the median plane, the one on the left side stretching from just behind the union of the two carotid arteries to posterior to the first rib, that of the right being placed slightly further caudally. They are made up of a reticulum of epithelial cells amongst which are lymphocytes, although these are not so numerous as the epithelial elements. The glands are now vascularised. Thymus IV is similar in structure but rather shorter in length. Their anterior ends overlap the hinder side of thymus III and are situated on the lateral side of the latter, but they reach back on

each side beyond the cranial end of the pericardium. In the longitudinal series, the glands have an undulating margin, indicating the early formation of lobules. At 14·25 mm., although it is possible to distinguish two thymus glands on each side, they overlap and lie so close together that it is difficult to completely separate them from each other; besides this, the anterior ends of thymus III may possibly be joined together across the middle line, in which case there is only one very large single thymus in the thorax. At the posterior end they are divided by the pericardium and end at the cranial side of the heart. The glands are composed of many lobes but as yet no cortical and medullary regions are recognisable, lymphocytes being equally scattered throughout. At 17 mm., one exceedingly large thymus is found on each side, beginning just cranial to the union of the common carotid arteries and extending on each side of the pericardium to the level of the cranial end of the ventricle of the heart. Lymphocytes are abundant, and we have at this stage the first formation of Hassall's corpuscles. At the anterior end again, the two glands may actually join together, but are for the most part separated by a thin layer of connective tissue.

Epithelial body III, whose origin from the third pouch was intimated at 7 mm., is difficult to distinguish at 8·75 mm., but is well developed in all the later stages. At 12·25 mm., it is found in the usual position near the division of the common carotid artery; the gland is well vascularised and is composed of cellular cords of varying thickness surrounding sinusoids. No change is observed at 14·25 mm., and even at 17 mm. the adult condition is not yet reached, the body being still made up of irregular cords or masses of cells.

Epithelial body IV was only observed on the left side in 8·75 mm., and was apparently absent in one of the examples measuring 12·25 mm.; in the other specimens it was found on both sides. The body may lie dorsally or medially to thymus IV as in 12·25 mm., or at about the middle region of the conjoint thoracic thymus as in 8·75 mm. and 14·25 mm. At 17 mm., it is a transversely elongated mass of epithelial cells, placed dorsally to the single thymus on the left side at the anterior end of the pericardium, but on the right it is larger and better developed and situated between the lobes on the dorso-medial side of the thymus. Epithelial body IV is a smaller structure than epithelial body III, and is best developed in the oldest embryo where interpenetration of capillaries has progressed further.

At 17 mm. there is a small solid extra epithelial body towards the left side dorsal to the truncus arteriosus, and another, which is still more insignificant, dorso-laterally to the left common carotid artery just before it joins with the corresponding artery of the opposite side.

Thyroid.—The median thyroid at 7 mm. forms a short circular tube, lying below the ventral aorta between the second and third gill pouches. It contains a distinct central cavity and at its ventral end has begun to flatten out towards the left side. At our next stage (8·75 mm.), the lateral lobe on the right side is well marked and circular in cross section, while that of the left is only feebly developed. The median

portion is narrow and contains a few irregular cavities. At 12·25 mm., the lobes have grown considerably and now stretch up on either side of the trachea. They are composed of irregular masses of cells, some solid and others, especially at the cranial ends, with a central lumen; vascular connective tissue ingrowths have already penetrated into the gland. There is a connecting bridge in both embryos. At 14·25 mm., the lateral thyroid (fig. 8, *l.th.*) still consists of irregular cords and the bridge is absent. At our last stage there is little advance in structure, although the gland is very vascular and many of the cords are tubular in form; the median thyroid is again present. The band-like pointed anterior end of the lateral lobe of the left side stretches cranially along the side of the trachea for a long distance.

Ultimobranchial Body.—The ultimobranchial body at 7 mm. passes off from the pharyngeal duct posterior to the fourth pouch, and runs down on the medial side of the latter as a narrow tube, whose walls increase slightly in thickness towards the distal end, which lies at the anterior end of the pericardium. By 8·75 mm. it has become independent, and has moved forwards close to the dorsal side of the lateral lobes of the thyroid. It here forms a closed tube, elongated in a cranio-caudal direction, its walls being especially thickened at the cranial end. At 12·25 mm. it is no longer possible to recognise the ultimobranchial body, although in one embryo a portion of the lateral thyroid appears to be marked off from the rest at the cranial end. At 14·25 mm., however, it is again found on the dorsal side of the lateral lobe on each side, and is very conspicuous, consisting of a central cavity surrounded by thick darkly staining walls which are in an active condition. Long sprout-like processes extend out from the ventral wall to become joined with the cords of the thyroid lobe, the two glands being intimately united. This is especially well seen on the right side (fig. 8, *u.b.* and *spr.*). We consider the evidence in favour of the participation of the ultimobranchial body in the formation of thyroid tissue is more convincing in this embryo than in any other marsupial so far examined. At 17 mm. the body is still present on the medial side of the cranial end of the lobes, and contains a smaller but distinct cavity which is surrounded by a comparatively thin wall of two or three layers of cells. Although less active, the walls still branch out at a few places into the thyroid lobes.

Summary.

The main features in Perameles may be summarised as follows:—

1. There is no superficial cervical thymus at any stage of development.
2. Well developed thoracic thymus glands, arising from the third and fourth pouches, are always present. They attain a remarkably large size, and may unite to form a single thymus on each side or may remain quite separated from each other.
3. Epithelial body III is constant.
4. Epithelial body IV is usually present.

5. The median thyroid primordium differentiates to form a bridge and lateral lobes, the bridge usually persisting in older stages.

6. The ultimobranchial body moves forwards close to the lateral lobes of the thyroid, and most probably takes part in the formation of thyroid tissue.

EXPLANATION OF PLATE.

For list of reference letters see Part I.

Fig. 1.—Stage I, Phascolarctos, G.L. 4 mm. Transverse section (S. 2–2–3), right side, showing the cervical sinus (*s.c.*) with the three branchial arches (*br.a.* 1, 2, 3) on its floor. The pharyngeal bay (*ph.b.*) and the fifth aortic arch (*aort.a.* 5) are also seen, and the median thyroid (*th.*) lies below the pharynx. ×75.

Fig. 2.—Stage III, Phascolarctos, G.L. 9 mm. Transverse section (S. 5–4–7), showing the primordium of the cervical thymus (*s.tm.*), the ductus ecto-entobranchialis III (*d.e.b.* III), and the distal end of the third gill-pouch (*cl.* 3) of the right side. The cervical vesicle (*v.c.*) is also seen. ×75.

Fig. 3.—Stage IV, Phascolarctos, G.L. 11 mm. Transverse section (S. 7–1–9) through the lateral lobe of the thyroid (*l.th.*) and the ultimobranchial body (*u.b.*) of the right side. ×200.

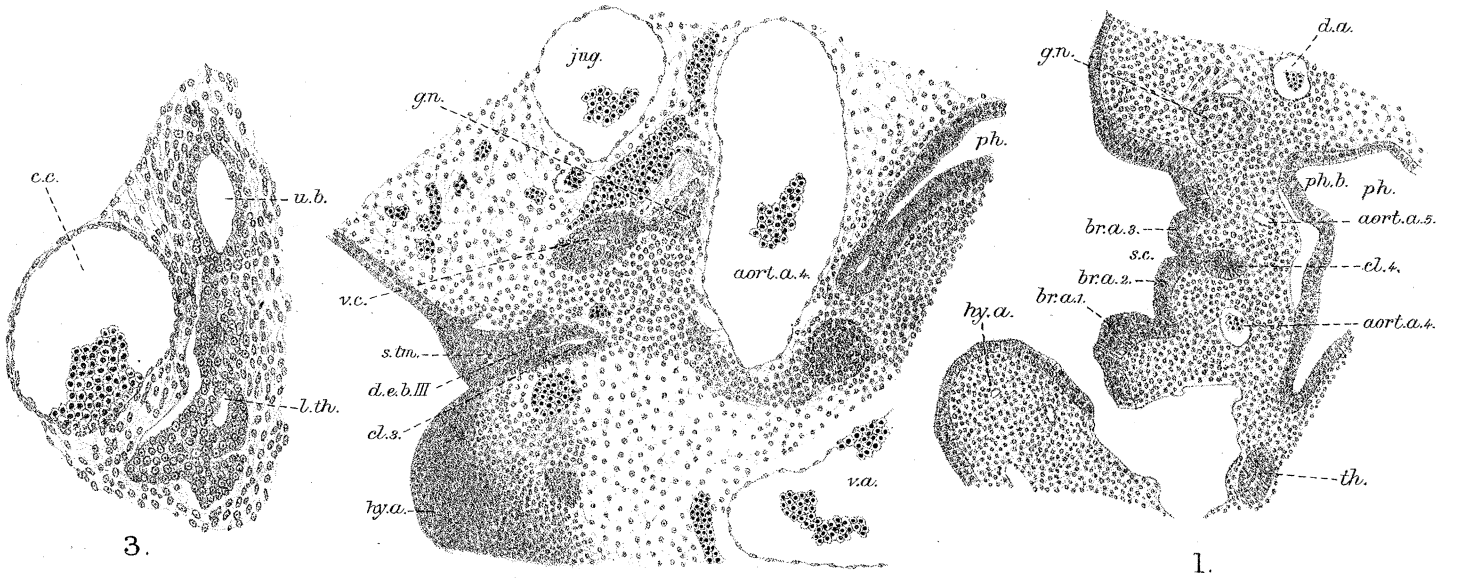
Fig. 4.—Stage VI *a*, Phascolarctos, G.L. 13·5 mm. Transverse section (S. 12–1–3 and 4) through the lateral lobe of the thyroid (*l.th.*) and the degenerating ultimobranchial body (*u.b.*) of the right side. Epithelial body IV (*ep.b.* IV) is seen near the lateral side of the ultimobranchial body. ×120.

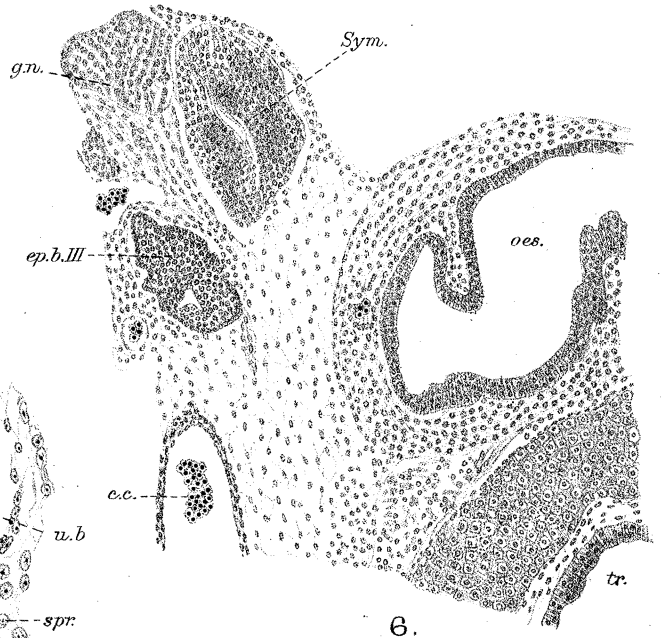
Fig. 5.—Stage I *b*, Phascolomys, G.L. 8·5 mm. Longitudinal section (S. 10–1–3), showing the dorsal portion of the third pouch (*cl.* 3) with its central cavity and thickened walls, and the small fourth pouch (*cl.* 4), which reaches its maximum size in this section. Right side. ×300.

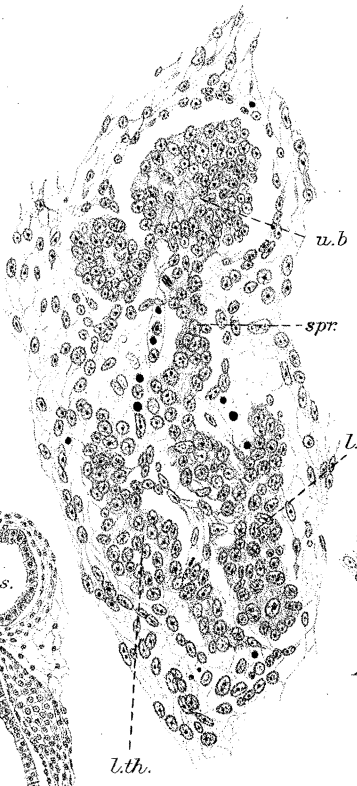
Fig. 6.—Stage II *a*, Phascolomys, G.L. 15·5 mm. Transverse section (S. 10–3–8), showing epithelial body III (*ep.b.* III) of the right side. ×75.
Sym. = cervical sympathetic.

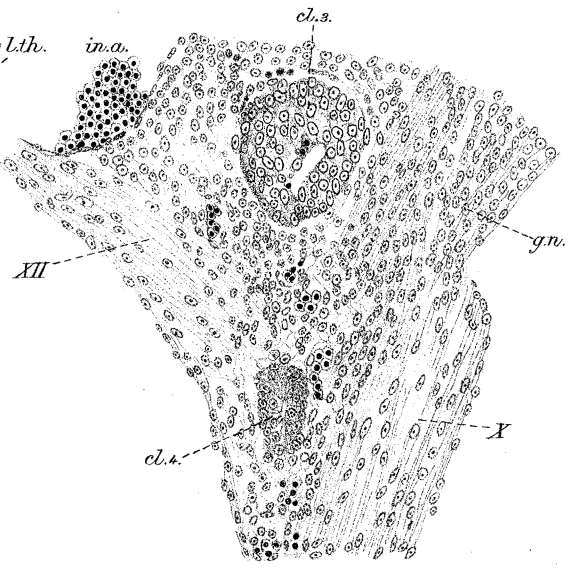
Fig. 7.—The same. Transverse section (S. 19–3–4), showing epithelial body IV (*ep.b.* IV) of the same side. ×75.

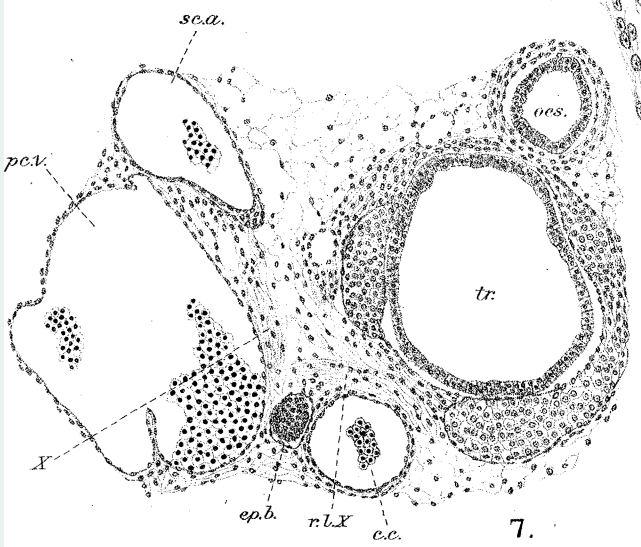
Fig. 8.—*Perameles nasuta*, G.L. 14·25 mm. Transverse section (S. 9–1–9), showing the ultimobranchial body (*u.b.*) and the lateral lobe of the thyroid (*l.th.*) of the right side. The ultimobranchial body is joined with the tissue of the thyroid lobe by an elongated prolongation (*spr.*). ×300.











E.A.F. del.

Huth, London.

DEVELOPMENT OF THYMUS etc..

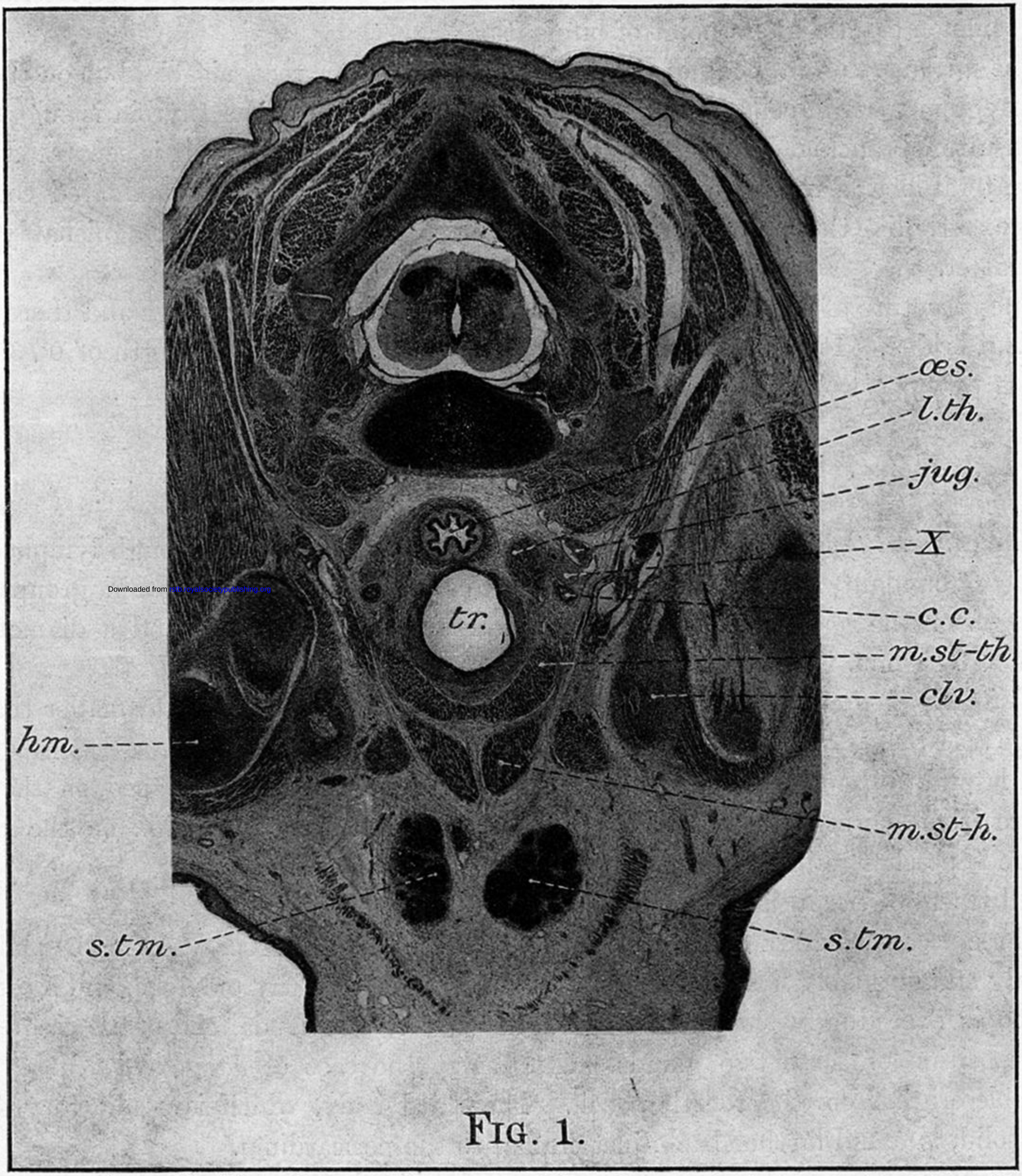
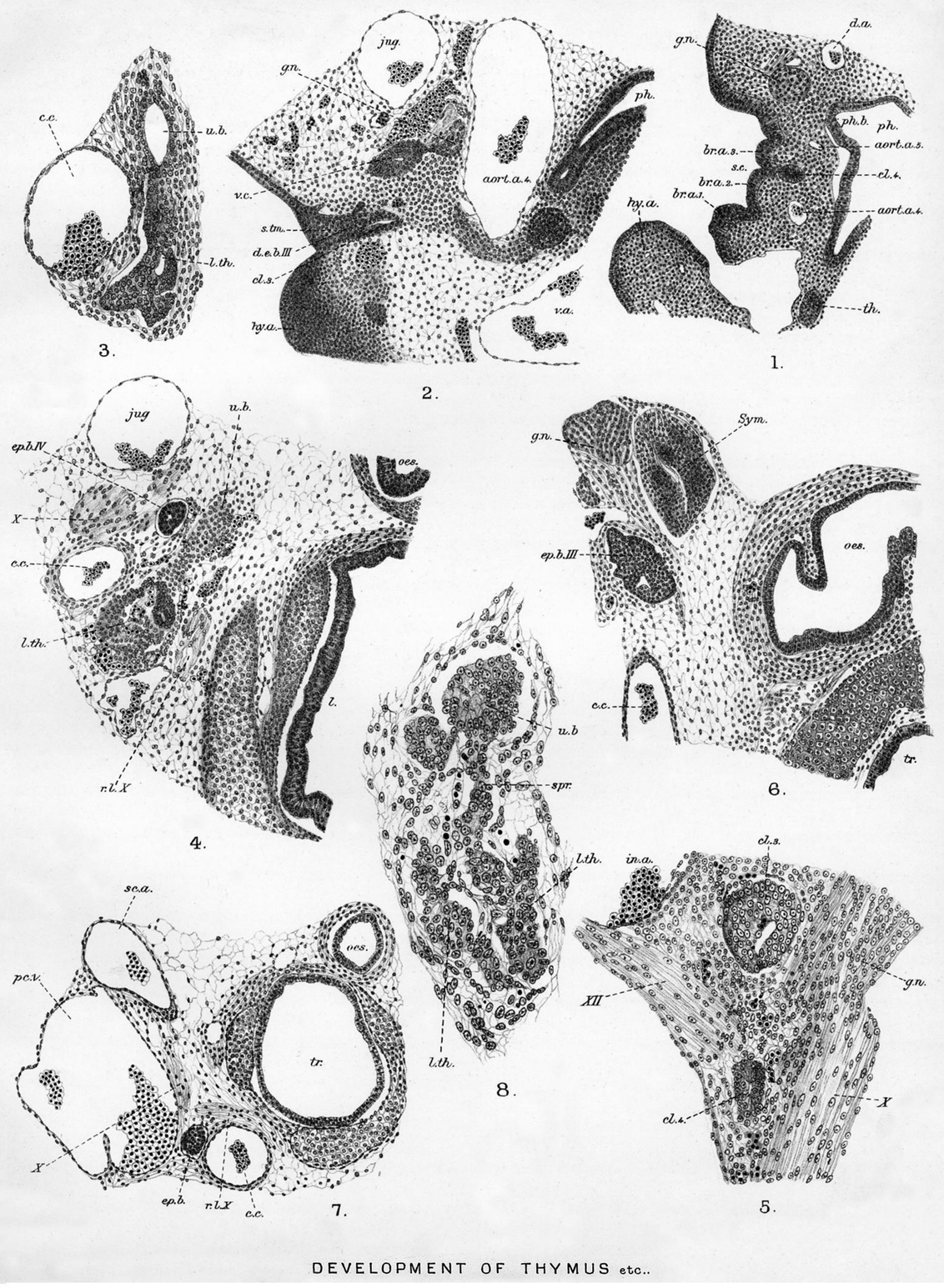


FIG. 1.

TEXT-FIG. 1.—Stage X. Phascolarctos, G.L. 24 mm. Microphotograph of a transverse section (S 11–2), showing the superficial cervical thymus and the main lateral thyroid lobe of the left side. × 20.

c. = common carotid artery; *clv.* = clavicle; *hm.* = humerus; *jug.* = jugular vein; *l.th.* = lateral thyroid lobe; *m.st.-h.* = sterno-hyoid muscle; *m.st.-th.* = sterno-thyroid muscle; *œs.* = œsophagus; *s.tm.* = superficial cervical thymus; *tr.* = trachea; X = vagus nerve.



DEVELOPMENT OF THYMUS etc..

EXPLANATION OF PLATE.

For list of reference letters see Part I.

Fig. 1.—Stage I, Phascolarctos, G.L. 4 mm. Transverse section (S. 2–2–3), right side, showing the cervical sinus (*s.c.*) with the three branchial arches (*br.a.* 1, 2, 3) on its floor. The pharyngeal bay (*ph.b.*) and the fifth aortic arch (*aort.a.* 5) are also seen, and the median thyroid (*th.*) lies below the pharynx. ×75.

Fig. 2.—Stage III, Phascolarctos, G.L. 9 mm. Transverse section (S. 5–4–7), showing the primordium of the cervical thymus (*s.tm.*), the ductus ecto-entobranchialis III (*d.e.b.* III), and the distal end of the third gill-pouch (*cl.* 3) of the right side. The cervical vesicle (*v.c.*) is also seen. ×75.

Fig. 3.—Stage IV, Phascolarctos, G.L. 11 mm. Transverse section (S. 7–1–9) through the lateral lobe of the thyroid (*l.th.*) and the ultimobranchial body (*u.b.*) of the right side. ×200.

Fig. 4.—Stage VI *a*, Phascolarctos, G.L. 13·5 mm. Transverse section (S. 12–1–3 and 4) through the lateral lobe of the thyroid (*l.th.*) and the degenerating ultimobranchial body (*u.b.*) of the right side. Epithelial body IV (*ep.b.* IV) is seen near the lateral side of the ultimobranchial body. ×120.

Fig. 5.—Stage I *b*, Phascolomys, G.L. 8·5 mm. Longitudinal section (S. 10–1–3), showing the dorsal portion of the third pouch (*cl.* 3) with its central cavity and thickened walls, and the small fourth pouch (*cl.* 4), which reaches its maximum size in this section. Right side. ×300.

Fig. 6.—Stage II *a*, Phascolomys, G.L. 15·5 mm. Transverse section (S. 10–3–8), showing epithelial body III (*ep.b.* III) of the right side. ×75.
Sym. = cervical sympathetic.

Fig. 7.—The same. Transverse section (S. 19–3–4), showing epithelial body IV (*ep.b.* IV) of the same side. ×75.

Fig. 8.—*Perameles nasuta*, G.L. 14·25 mm. Transverse section (S. 9–1–9), showing the ultimobranchial body (*u.b.*) and the lateral lobe of the thyroid (*l.th.*) of the right side. The ultimobranchial body is joined with the tissue of the thyroid lobe by an elongated prolongation (*spr.*). ×300.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES