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Phil. Trans. R. Soc. Lond. B 1998 **353**, 1101-1114
doi: 10.1098/rstb.1998.0269

The Royal Society

Early development and embryology of the platypus

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Information on the pre-hatching development of the platypus, *Ornithorhynchus anatinus*, is reliant on a small number of specimens, whose precise age is unknown. Material collected for J. P. Hill and now housed in the Hubrecht International Embryological Laboratory, Utrecht, contributes a major source of specimens. This paper presents new observations on developmental stages from the Hill collection, which allow for a more complete description of pre-hatching development.

A feature of the pre-embryonic development of the platypus is the incomplete meroblastic cleavage. A column of fine yolk spheres extends from beneath the embryonic blastodisc towards the centre of a yolky vitellus, as seen in birds.

The major expansion of extra-embryonic membranes occurs after the formation of the primitive streak. The primitive streak develops within an embryonal area as part of the superficial wall of the yolk-sac, a feature also shared with marsupials, birds and reptiles.

The full-term, subspheroidal, intrauterine egg of the platypus has a major axis of about 17 mm and contains a flat, 19–20 somite, neurula-stage embryo which has prominent trigeminal ganglion primordia. The embryo at this stage is in a period of rapid modelling of the major early organ primordia of the nervous system, cardiovascular system, excretory system, and somite-derived components of the body wall.

Soon after laying, five primary brain vesicles are present, the trigeminal ganglia CN5 as well as CN7, CN8, CN9, CN10, CN11 and CN12 are well developed. The alimentary system has an expanded stomach, pancreatic primordia and a gall bladder. Mesonephric tubules are associated with patent mesonephric ducts, which empty laterally into the cloaca. Extra-embryonic membranes at this stage show an extensive chorioamniotic connection that extends through the greater part of the caudal half of fused amniotic folds. The vascularized yolk-sac consists of a superficial yolk-sac omphalopleura and a deep yolk-sac splanchnopleure. The non-vascularized yolk-sac comprises one-quarter of the abembryonal pole.

Some distinctive monotreme features have developed by the mid-incubation period. The head is bent at an acute angle to the main body axis. The blunt upturned snout marks the site of the future oscaruncle and on the maxilla there is a median primordial papilla representing the egg tooth. The eye is open with a partly pigmented retinal ring. The forelimbs have partly separated digits, and the hindfeet are paddles. Just before hatching the upturned snout contains an oscaruncle and a sharp recurved median egg tooth. Forelimbs are pronated with separate digits possessing claw primordia. Portions of the highly vascularized extra-embryonic membranes are attached to the umbilical region and the flattened vesicular allantois has a distal region fused with the chorion.

Prominent features of the hatchling are the presence of a bluntly conical oscaruncle and a translucent, horn-like egg tooth. These structures are thought to enable the hatchling to extricate itself from the egg shell. At hatching, the forelimbs exhibit clawed digits and are capable of digitopalmar prehension. Hindlimbs are still paddles with digital rays. A prominent yolk-sac navel is present. The newly hatched platypus has an external form similar to that of a new-born marsupial.

The early development of the platypus has many major differences to the developmental sequence for humans, which has been categorized by the use of Carnegie Stages. The rate of somitogenesis of the platypus is faster in relation to the central nervous system morphogenesis than seen in humans, and the size of the early platypus embryonal area is massive in relation to that of humans.

The unique morphology and function of extra-embryonic membranes in the platypus defies comparative staging with human development. Structures adapted for altricial survival of the platypus hatchling require the acquisition of functional competence at an earlier stage of organogenesis than seen in eutherians, although they are reminiscent of those found in new-born marsupials.

Keywords: platypus; *Ornithorhynchus*; embryology; early development

1. INTRODUCTION

Since the independent but almost simultaneous discovery of oviparity in monotremes by Caldwell and Haake (separately reported on the same day in 1884), many people with a professional or simply a general interest in natural history have been intrigued with platypus developmental biology. The scientific community, and particularly evolutionary biologists, have been fascinated by the blending of avian, mammalian and uniquely monotreme developmental profiles that are characteristic of all stages in the developmental progression from egg to embryo to hatchling, and their eventual incorporation as conspicuous features of adult form.

The classical accounts of the embryology of the platypus (*Ornithorhynchus anatinus*) have been reviewed by Luckett (1977), Griffiths (1978), Hughes (1984), Hughes (1993) and Semon (1894). It is not generally appreciated that this information has been derived from a surprisingly small number of specimens and relies heavily on interpolation with echidna (*Tachyglossus aculeatus*) material to fill in gaps. For example, in the paper of Flynn & Hill (1942), concerned with the monotreme embryonic developmental stages from later cleavage to the establishment of the fully formed pre-primitive streak bilaminar blastoderm, a total of 42 monotreme developmental stages were reviewed. It is of interest that of these only two stages were from the platypus, with the remaining 40 stages derived from *Tachyglossus*. Flynn & Hill (1942) regarded the formation of the primary embryonic germ layers in the platypus and *Tachyglossus* to be sufficiently parallel as to justify a combined description for the monotreme taxon. The material covered by Flynn & Hill (1942) is again reviewed in a broad comparative context by Flynn & Hill (1947). These papers also overlap with data presented by Wilson & Hill (1908). All monotreme specimens are of unknown timing in relation to major events in the reproductive cycle and embryogenetic process, and almost all were collected from wild-caught animals during the late nineteenth century. This material is thus of very limited value in comparison with the detailed studies on the development of accurately staged mammalian species.

A significant proportion of the more important of these platypus embryo stages, as well as important unpublished data, remain as part of the Hill collection curated by the Dutch Academy of Science at the Hubrecht International Embryological Laboratory, Utrecht, The Netherlands.

This paper is a synopsis of the development of the platypus from early cleavage through to egg laying and eventual hatching. The paper has three primary objectives. The first is to provide a brief perspective of the early development that compliments the paper by Manger, Hall & Pettigrew (this issue), which describes the post-hatching development of external features of the platypus. The second is to outline the salient structural features of the unique pre-hatching developmental component of the monotreme life cycle. The third aspect is to formulate a comparative development profile of staging of the platypus with that of the Carnegie Stages used for describing early human development. This comparison emphasizes the striking nature of monotreme development.

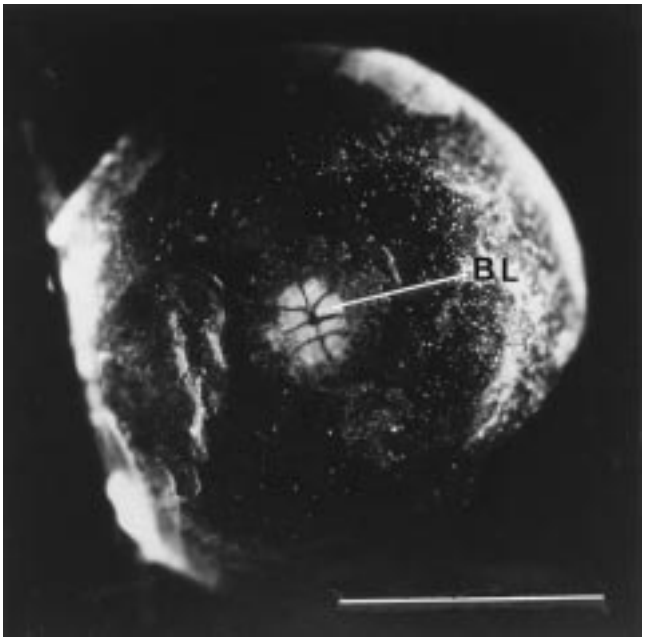

Figure 1. Photomicrograph of a uterine egg (4 mm diameter) of *O. anatinus* showing an ellipsoidal blastodisc with eight blastomeres (BL) exhibiting meroblastic cleavage. Scale bar, 2 mm.

2. INTRAUTERINE DEVELOPMENT

(a) *Pre-embryonic stage*

The initial cleavage stages of the monotreme zygote generate blastomeres with incomplete cell walls through the meroblastic furrowing of the superficial and thickened embryonic disc (figure 1). Subsequent mitotic divisions generate a tiny cellular blastodisc that commences to invest the yolky and uncleaved vitellus (Flynn & Hill 1939, 1942, 1947). The incomplete meroblastic cleavage (Caldwell 1887) is a feature that is not only found in monotreme mammalian taxa, but is also shared with submammalian amniote vertebrates, as evidenced in avian and reptilian species. In monotremes, as in avian species, a latebral column of finer yolk spheres extends from beneath the embryonic blastodisc toward the centre of the yolky vitellus. The earliest of the two platypus stages of Flynn & Hill (1942) marked the attainment of the blastodisc at the culmination of cleavage. The planoconvex surface of the blastodisc measured 41 μm × 368 μm and was up to four cells thick in its central region.

The entire embryonic blastodisc remains mitotically active as it spreads peripherally to superficially invest the underlying yolky vitellus. In this way, the central and older region of the blastodisc exhibits multicellular thickening and the small blastomeres acquire complete cell membranes. Blastomeres at the peripheral progress zone of spreading continue to exhibit incomplete cell membranes. In this way, the blastodisc becomes a lenticular-thickened structure of some six to seven blastomeres in the thickness centrally. Yolk spheres are incorporated into the cytoplasm of the deeper cells of the blastodisc as well as sub-disc migratory vitellocytes that form a marginal germ ring of the blastodisc progress zone.

The early embryonic disc commences to give rise to a discrete population of smaller prospective endodermal

cells by both mitosis and migration within the main body of the thickened embryonic disc. In this way, a deeper population of prospective endodermal cells begins to be generated and is subsequently incorporated into the superficial embryonic blastoderm as it thins to form a mixed unilaminar layer of both prospective ectodermal and endodermal cells. The proliferating blastodermic disc continues to occupy an extremely small proportion of the vitelline surface and the vitellus has yet to expand beyond its original diameter. Absorption of uterine endometrial nutrients through the shell is not as yet expressed by dimensional expansion of the developing egg.

The almost circular peripheral outline of the blastoderm has now expanded and exhibits a well-defined blastodermic rim. The blastoderm at this stage is unilaminar over its entire extent and is closely associated with the under surface of the zona-albumen layer that is presumably formed by the fusion of the zona pellucida with the overlying glycoprotein mucoid coat. The unilaminar blastoderm at this stage consists of prospective ectodermal cells as well as increasing numbers of primitive endodermal cells that have not yet commenced the formation of an inner endodermal layer. The marginal region of the expanding blastodisc consists of a small number of vitellocytes that contain small yolk spheres in their cytoplasm, as well as an actively expanding germ ring. The diameter of the shell of the egg at this stage is 5.5 mm.

The bilaminar blastoderm is now about to commence its segregation from its established unilaminar predecessor and, according to Flynn & Hill (1947), the ontogenetic mitotic and migratory events leading to the establishment of the bilaminar embryonic disc of ectoderm and endoderm are strikingly similar in both monotremes and marsupials. In monotremes, the superficial layer of blastodermic ectoderm and subsequently segregated deeper layer of blastodermic endoderm both completely achieve migratory enclosure of the yolk mass; the overall diameter of the bilaminar blastodermic vesicle in *Tachyglossus* measures about 5.4 mm (Luckett 1977). The most advanced of the two platypus stages of Flynn & Hill (1942) was a pre-primitive streak terminal bilaminar disc stage with primary germ layers enclosing all but the yolk-navel at the lower pole of the egg. Small, isolated areas existed where there was incomplete separation of the endoderm from the overlying ectoderm. The formation of the completed blastodermic vesicle marks the beginning of a period of accelerated absorption of uterine endometrial nutrients as evidenced by marked subsequent expansion of the overall diameter of the vitellus. In both monotremes and marsupials, the major expansion in the extra-embryonic membranes occurs after the formation of the primitive streak stage.

The generation in monotremes of the third primary embryonic germ layer, the mesoderm, is marked by the formation of a central linear thickening of primitive streak cells on the surface of the embryonic disc. The overall length of the fully formed monotreme (*Tachyglossus*) primitive streak is 6.86 mm (Hughes 1993), and is much longer than that found in humans (0.7 mm: O'Rahilly & Müller 1987). The fully formed primitive streak stage is as yet unavailable for study in the platypus but the expectation is for a close resemblance to that of *Tachyglossus* (Hughes 1993).

Uterine endometrial nutrients are now being rapidly absorbed through the shell membrane, unique amongst amniote vertebrates for its capacity to stretch. The primitive streak is the site of generation of bilateral wings of mesodermal cells that migrate, by terminal intrauterine gestation in both *Tachyglossus* and the platypus, between the ectodermal and endodermal layers (with the exception of the presumptive proamnion rostral to the future head region, and the presumptive cloacal membrane at the caudal extremity of the future embryonic tail region). The primitive streak stage in monotremes is attained at an unknown time during intrauterine gestation, but is provisionally presumed to be at about three days before the developing intrauterine egg is laid (Hughes 1993). A thickening, the primitive node, at the rostral end of the primitive streak specifies the future head end of the embryonic area and in this manner designates the rostrocaudal embryonic axis. The medial positioning of the primitive streak within a slipper-shaped definitive embryonic area delineates the right and left sides of the future bilateral organogenetic fields. The primitive streak consequently initiates axial embryonic positioning as well as specifying and beginning the programming of definitive embryonic structures. It is of interest to note that the primitive streak in monotremes, like that of marsupials, develops within an embryonal area as part of the superficial wall of the yolk-sac, a feature also shared with birds and reptiles. In monotremes, a yolk navel, characterized by endodermal infolding, marks the abembryonal pole of the yolk-sac and is described by Flynn & Hill (1947).

At a very early primitive streak stage, the notochordal primordium, with a focal role in future organogenetic programming, forms as a rostrally proliferating pit that arises from the deeper aspects of the primitive node. As in other amniote vertebrates, the primordia of the neural folds and the concurrently developed paraxial somites are among the first definitive organ primordia of the monotreme embryo that differentiate immediately rostral to the primitive node region of the primitive streak.

(b) *Embryonic stage*

The early definitive embryo stage is known only for *Tachyglossus*. This is situated within an oval-shaped embryonal area of about 22 mm × 11 mm and the embryo curves around the surface of the distended spherical yolk-sac for a distance of about 13.7 mm. A relatively narrow, pear-shaped crescentic region of translucent yolk-sac, within the wider embryonal area, surrounds the brain plate and somite region of the embryo proper. This is the site of formation of the proamnion. The embryo proper consists of a flattened spatulate brain plate with a length and a width of 4.7 mm and 4.0 mm, respectively, and is as yet not divided into the primordia of the three primary brain vesicles. Two small lateral conical protrusions, a little in front of the middle of the brain plate, constitute the prospective future trigeminal ganglionic primordia later to be expressed as trigeminal brain plates that at this stage immediately abut the expanded prospective pons region of the hindbrain. The early expression of these trigeminal ganglionic primordia facilitate a long developmental profile needed to achieve a rudimentary degree of sensory and motor competence associated with the immediate pre-hatching period. The early formation of

trigeminal ganglionic primordia is also characteristic of marsupial species (Hughes *et al*. 1989). The brain plate narrows in its caudal extremity, so that at the level of the first pair of the five somites, the width of the embryo is about 2.2 mm. A prominent primitive streak of 5.6 mm in length is located within the mesoderm distal to the last pair of somites.

The full-term intrauterine egg of the platypus has expanded by the absorption of endometrial secretions to form a spheroidal or a subspheroidal shelled egg with axial dimensions of 17 mm in diameter or 16 mm × 18 mm, respectively. The period of rapid expansion marks the deposition of the outer matrix layer of the tripartite egg-shell membrane. The definitive embryo contained within term intrauterine eggs of monotremes constitutes a flat, superficial, 19–20 somite, neurula stage that extends around about two-thirds of the peripheral surface of the yolk-sac. A term platypus embryo (figure 2) occupies the uterus. The overall dimensions of the oval-shelled egg from which this embryo was obtained were 17 mm and 15 mm, respectively. The major longitudinal axis of the embryonic disc was 18 mm and that of the definitive embryonic area was 14 mm as measured from the rostral extremity of the head fold to Hensen's node. The total number of somites in this neurula stage was estimated to be at least 18 to about 20. The primitive streak at the caudal extremity of the embryo, originally about 7 mm in length, now occurs as a shrunken remnant of about 1.6 mm in length. The trigeminal ganglionic primordia have now expanded into enlarged semicircular plates, and ectodermal auditory placodes, which are more caudally superficial and thickened, are underlain by the stalked early ganglionic primordia of the future cranial nerve seven and eight complex that are adjacent to the thickened auditory placodes.

The small primordial notochord is now associated with a medial keel immediately beneath the axial and deepest portion of the fused neural folds. Blood islands, some exhibiting endothelial lined cavities, are without nucleated foetal erythrocytes and are present in the peripheral regions of the definitive embryonal areas as well as in the adjacent extra-embryonic wall of the trilaminar yolk-sac. The primordia of the functionless and bilaterally separated heart tubes are at an early stage of formation and are most completely formed lateral to the hindbrain primordium. According to Hill & Martin (1895) and Wilson & Hill (1908), the earliest nephric anlagen are represented within the intermediate mesoderm lateral to the somites. The anlagen of the forebrain, midbrain and hindbrain are present. Hill & Martin (1895) described the primordia of optic grooves that project laterally from the open forebrain primordium. The extremely rostral component of the cephalic head fold is at an early stage of differentiation. Forelimb buds are not yet evident. The full-term intrauterine embryo (figure 2) is at an equivalent stage of development to that reported by Hill & Martin (1895) and again by Wilson & Hill (1908).

In regard to the extra-embryonic membranes at full-term intrauterine development in the platypus, only the yolk-sac is present. The abembryonal third of the yolk-sac peripheral to the embryonic disc constitutes the bilaminar yolk-sac where superficial ectoderm is underlaid by endoderm cells. In regard to the cellular development of the

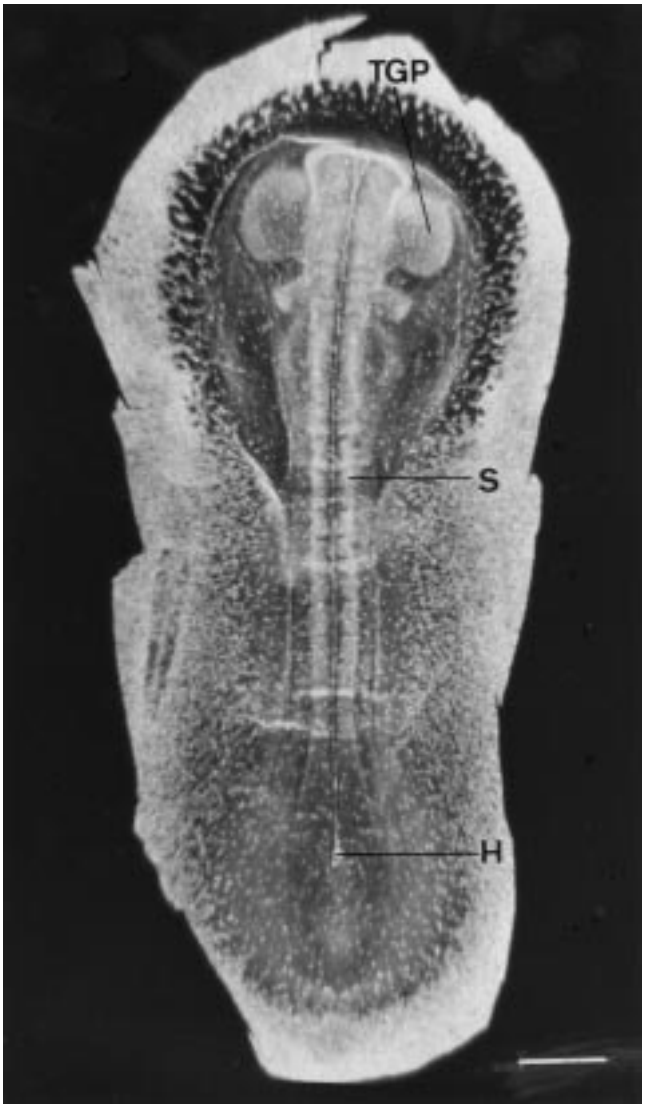

Figure 2. Photomicrograph of a terminal intrauterine embryo of *O. anatinus* collected at Yass, NSW, on 28 September 1973, by R. L. Hughes and F. N. Carrick. The overall dimensions of the oval shelled egg were 17 mm and 15 mm. The major longitudinal axis of the embryonic disc was 18 mm and that of the definitive embryonic area was 14 mm measured from the rostral extremity of the head fold to Hensen's node (H). The total number of somites (S) in this neurula stage was estimated to be at least 18 to about 20. The trigeminal ganglionic primordia (TGP) are prominent expanded semicircular plates. Scale bar, 2 mm.

ectoderm and endoderm layers of the bilaminar region of the yolk-sac, it should be noted that the superficial ectodermal cells are a tightly linked and slightly thickened, constituting a simple squamous epithelium of mostly polygonal cells. In the immediate extra-embryonal area, ectodermal cells are cuboidal. The deeper endodermal layer is of irregular thickness. Some endoderm cells active in the absorption of yolk spheres exhibit distended cytoplasm that bulges into the yolk-sac. However, between these distensions, the endodermal layer is relatively thinner than that of the overlying ectoderm.

The embryonic trilaminar pole of the yolk-sac is as yet unvascularized. However, lateral to the embryonic somites, the narrow slit-like rudiment of an intra-embryonic

Table 1. *Carnegie (human) embryo staging of major features of the full-term intrauterine platypus embryo*

(Data taken predominantly from O'Rahilly & Müller (1987), with occasional supplementation from O'Rahilly & Müller (1992).)

major structural features of full-term platypus embryo	equivalent Carnegie Stage in human development	estimated post-ovulatory age (days)
neural folds	8	18±1
prefolding flat embryo	9	20±1
18–19 somites	11	24±1
bilaterally separated heart tubes	9	20±1
expanded trigeminal primordium	9	20±1
primordia CN VII and VIII	10	22±1
otic plate	10	22±1
rostral neural plate flat with neural folds fused between somites	10	22±1
greatest rostrocaudal length of definitive embryo = 14 mm	18	44

coelom is bounded by somatic and splanchnic layers of mesoderm. A small, extra-embryonic proamnion, which consists of a bilaminar layer of superficial ectoderm underlaid by endoderm, immediately abuts and surrounds the rostral extremity of the head process and represents an area where the lateral wings of mesoderm generated from the primitive streak have failed to penetrate. Likewise, at the extreme distal extremity of the embryo, the thickened apposed ectodermal and endodermal primordia of the anal membrane also lack the primitive-streak-derived interposing mesodermal layer (Wilson & Hill 1908). In the full-term intrauterine embryo of the monotreme, the amnion, pure chorion and allantois have yet to exhibit identifiable primordia. In histological sections of the embryo (figure 2), it was shown that a high proportion of the endoderm cells in the trilaminar area of the yolk-sac immediately peripheral to the embryo exhibit cytoplasmic distension and very active incorporation and degradation of yolk spheres. Flynn & Hill (1947) reported that yolk-endoderm absorption was maximal within the extra-embryonic region of the blastoderm. The endoderm within the definitive embryonic areas of our full-term platypus specimen showed very little yolk-endoderm absorption and consisted of a single layer of closely packed flattened cells.

Posterior to the somites, the embryonic plate gradually increases in breadth to form a lanceolate terminal segment. It is presumed that the full-term intrauterine embryo of the platypus is attained at approximately three days after the formation of the primitive streak. This is consistent with the observations of Griffiths (1968), who suggested that the brevity of post-primitive streak intrauterine development was consistent with the relatively small proportion of post-primitive streak intrauterine embryos available for scientific study in an otherwise relatively extensive collection such as that curated at the Hubrecht Laboratory. This conclusion is also supported by the relatively primordial state of organogenesis in the flat, 19–20 somite stage of the monotreme egg at laying, together with the incompleteness of organogenesis at hatching as reported by Griffiths (1968) and Griffiths *et al.* (1969).

The terminal intrauterine stage of the platypus embryo is in the early stages of rapid modelling of major early organ primordia of the nervous system, cardiovascular system, excretory system, and somite-derived components of the body wall. In relation to human development this represents selected aspects of Carnegie Stages 8 to 18 that span human post-ovulatory ages of about 18±1 days to 44 days (see table 1).

In relation to human development, distinctive aspects of the definitive embryo of the platypus at full-term intrauterine development represent retardation of the formation of neural folds, and definitive embryonic folding as well as delayed heart development. There is acceleration in overall growth of the greatest rostrocaudal length of the definitive platypus embryo of 14 mm. An equivalent length is only achieved in human development at 44 post-ovulatory days, when Carnegie Stage 18 is attained.

The extra-embryonic membranes of the platypus at full intrauterine development are unique, so that comparable Carnegie Stages for human development cannot be appropriately assigned. Major features at this stage of platypus development are the ectoderm of the definitive flat embryonic plate, which grades laterally in yolk-sac ectoderm, and the lack of primordia for the amniotic folds and allantois. Structures equivalent to human chorionic villi are not a feature of platypus intrauterine development.

In order to highlight differences between the developmental profiles of platypus and human extra-embryonic membranes, the profile of the development of human extra-embryonic membranes to either Carnegie Stage 9 or 10 now follows. Data is from O'Rahilly & Müller (1987, 1992).

(c) *Profile of human chorionic development to Carnegie Stage 10 (ca. to 22 days)*

Towards the end of the first post-ovulatory week of human gestation, Carnegie Stage 4 is attained. The syncytiotrophoblast of the unilaminar blastocyst now begins its apposition, attachment and invasive implantational sequence of the uterine endometrium. Carnegie Stage 5 encompasses post-ovulatory days 7–12 and is the interstitially implanted pre-villous stage of the peripheral chorionic extra-embryonic membrane.

Carnegie Stage 6 (post-ovulatory days 13–15) is characterized by the establishment of chorionic villi from cytotrophoblastic clumps and mesoblastic crest cells of

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Stage 5. An endometrial decidual reaction becomes evident at the implantation site and maternal blood lacunae form primordial intervillous spaces. At Stage 6 the chorionic villi possess cores of mesoblastic crest cell. A cytotrophoblastic shell now forms as the most peripheral chorionic element of this extra-embryonic membrane. Syncytiotrophoblast remains as the internal lining of the intervillous space. The mesoblastic cores of the chorionic villi are vascularized by embryonic blood vessels by the mid-point of Carnegie Stage 6. The human embryonic heart begins to beat towards the end of the third week, at Carnegie Stages 9–10. At this stage placental chorionic tissue constitute an extremely high proportion of the tissues of the conceptus. It is quite unambiguous that the Carnegie staging relating to this profile of human chorionic development has no monotreme equivalent.

(d) *Profile of human amniotic development to Carnegie Stage 9*

In human development, the primordial amniotic cavity forms within the inner cell mass at Carnegie Stage 5a (estimated post-ovulatory age of 7–8 days). The conceptus is now at an early stage of implantation of the unilaminar blastocyst.

A well-formed vesicular amniotic cavity lined by squamous cells is present at about 13 post-ovulatory days (Carnegie Stage 6). Thickened epiblastic cells of the primordial embryonic disc now constitute the floor of the amniotic cavity.

At approximately 16 post-ovulatory days (Carnegie Stage 7), the amnion consists of two layers: an inner extra-embryonal ectodermal layer, and a peripheral extra-embryonal mesenchymal layer. The primitive streak is now a feature of the embryonic disc within the floor of the amnion. At Carnegie Stage 9 (approximately 20 ± 1 post-ovulatory days), the early somite (1–3 pairs) stage embryo is a prominent feature of the embryonic disc on the floor of the amnion. The amnion, embryonic disc and associated yolk-sac are suspended within the extra-embryonic coelom by a mesodermal connecting stalk. The extra-embryonic coelom is derived indirectly from the cavity of the unilaminar blastocyst. In the platypus, the amnion forms from the fusion of head and tail folds of the peripheral somatopleure immediately beneath the egg shell during the early stages of the first half of the post-laying incubation period. This contrasts with the human's profile of amnion formation, which occurs relatively early in the intrauterine development by the cavitation of the inner cell mass (a structure never formed in platypus development). It is obvious that no appropriate Carnegie staging can be assigned to platypus amnion formation.

(e) *Profile of human yolk-sac (umbilical vesicle) formation to Carnegie Stage 9*

The primitive yolk-sac forms from the hypoblast of the bilaminar embryonic disc during Carnegie Stage 5b, with an estimated post-ovulatory age of 9 days. Its placental function is trivial and although it is easily identifiable within the extra-embryonic coelom beyond Stage 9, it is excluded within the extra-embryonic coelom from uterine endometrial interchange. It becomes embedded as a rudiment within the base of the umbilical cord in the latter stages of gestation. In the platypus, the yolk-sac constitutes the almost exclusive nutritive gatherer throughout both intrauterine and post-laying development. In the platypus, pre-ovulatory vitellogenesis of the oocyte provides the structural scaffolding for the incorporation of yolk within the yolk-sac by the encroachment peripherally of the superficial embryonic blastodermic disc that inverts the yolk during the formation of the blastodermic vesicle. Yolk-sac morphogenesis is the major extra-embryonic event of intrauterine development of the platypus. This is a characteristic feature of monotreme development not amenable to meaningful comparative Carnegie staging equivalents within human development.

(f) *Profile of human allantois formation to Carnegie Stage 9 and beyond*

According to O'Rahilly & Müller (1987), the primordium of the human allantois is established with certainty at Carnegie Stage 7 (estimated post-ovulatory age of 16 days). The allantois then occurs as a hypoblastic evagination into the precociously mesodermalized embryonic connecting stalk. By Carnegie Stage 9 (estimated post-ovulatory age of 20 days) the allantois is an endodermal-lined diverticulum in contact with the embryonic hindgut and situated medial to the right and left umbilical arterial primordia. In subsequent development the allantoic vesicle becomes a rudiment within the umbilical stalk. Consequently in humans, unlike the platypus, a vascularized allantoic vesicle never expands to fuse peripherally with the chorion to form a chorioallantoic membrane. At the pharyngeal arch stage in human development at Carnegie Stage 12 (estimated post-ovulatory age of 26 days), the human chorionic placental villi are functionally vascularized by four blood vessels that proximally traverse the embryonic connecting stalk. These consist of right and left umbilical veins and right and left umbilical arteries. These four blood vessels in humans are the evolutionary homologues of the blood vessels of the allantoic stalk typical of amniote taxa including monotremes. In subsequent human development the right umbilical vein disappears to achieve the mature vascular pattern of the umbilical cord of right and left umbilical arteries and a left umbilical vein. In their description of the full-term intrauterine platypus embryo, Wilson & Hill (1908) describe a presumed rudiment of an allantoic plate. However, in the experience of the present authors, the earliest endodermally lined allantoic evagination of the embryonic hindgut of both monotremes and marsupials occurs at the early pharyngeal arch stage concurrent with the acquisition of functional mesonephric organogenesis. In both monotremes and marsupials, the outer wall of the allantois becomes vascularized and expands to overtake the axial dimensions of the definitive embryo. In the platypus and the echidna the distal vascularized wall of the large vesicular allantois displaces the yolk-sac from the chorion to become a fused chorioallantoic respiratory membrane with no human equivalence in the Carnegie system of staging.

The comparative developmental profiles of humans, marsupials and monotremes are summarized in table 2.

Table 2. *Comparative developmental profiles of humans, marsupials and monotremes*

human	marsupial and monotreme
pre-embryonic period	
occupies minor part of gestation (5%, week 1–week 2)	occupies major part of gestation (50–80%)
major event is establishment of invasive chorionic placental tissue	major event is establishment of non-invasive expanded and shelled yolk-sac
bilaminar embryonic plate forms on floor of amnion derived from inner cell mass	bilaminar embryonic plate forms on surface of yolk-sac; amnion unformed; no inner cell mass
implantation achieved	no implantation
embryonic period	
minor part of early gestation (15%, from week 3 to week 8)	minor part of end of gestation (10–25%, 32 h–6 d)
organ primordia ⬇ organ modelling ⬇ early organ histogenesis	organ primordia ⬇ organ modelling ⬇ very early organ histogenesis
no gestational equivalent in human embryonic development	specialized prenatal or pre-hatching period in marsupials, minor part of terminal gestation (10–25% 32 h–6 d); in monotremes, *ca*. two-thirds of incubation period; early organ histogenesis ⬇ precocious adaptations for survival at birth
foetal period	
major part of gestation (80%)	no gestational equivalent in marsupial and monotreme embryonic development
conceptus has adult likeness	
complex late histogenesis of organ systems	
adaptations for survival at birth in late organ profiles	

3. INCUBATION PERIOD

(a) *Pharyngeal arch stage*

The following specimen is from the Hill collection, Hubrecht International Embryological Laboratory, Utrecht.

Platypus 'J'. Greatest length of definitive embryo, 6.5 mm.

Collected by J. P. Kenny, Gayndah, Queensland, 8 September 1899.

Incubation stage from egg with shell dimensions of 17 mm × 14.5 mm.

The overall dimensions of the contracted extra-embryonic membranes after fixation were 11.5 mm × 10.75 mm. The definitive embryonic portion of specimen J is a 'C'-shaped pharyngeal arch stage. This was photographed and a drawing subsequently prepared (figure 3). Serial sections were prepared for descriptive analysis. The overall length of specimen J was 6.5 mm. These dimensions are attained in human development at Carnegie Stage 14 at approximately 32 post-ovulatory days.

The 'C'-shaped pharyngeal arch stage has long been recognized as a common feature of all vertebrate development and of considerable phylogenetic importance. In the Carnegie system the 'C'-shaped pharyngeal arch stage of human development is manifest between post-ovulatory days 24 and 37 and encompasses Carnegie Stages 11–16. The previously unpublished descriptive detail of platypus 'J' stage in the Hill Collection is based on a thorough analysis of serial sections by J. P. Hill and independently by his student G. S. Sanson. The extensive handwritten notes and associated diagrammatic reconstructions are in sufficient detail to show that platypus 'J' shares composite features with Carnegie Stages 13–15 as well as manifesting a wide spectrum of unique features. The descriptive detail that follows is an interpretative summary of the Hubrecht Laboratory data of the Hill Collection on platypus specimen 'J'.

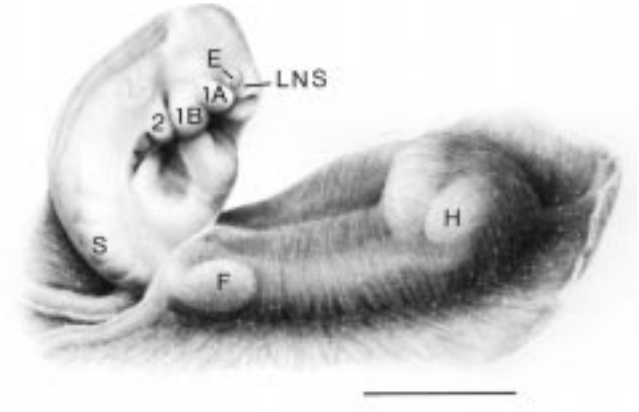

Figure 3. Drawing of platypus 'J' from the Hill collection. The greatest length of the definitive embryo was 6.5 mm. Evaginated eye cup of the diencephalon (E), forelimb (F), hindlimb (H), somites (S), lateral nasal swelling (LNS), maxillary and mandibular components of first pharyngeal arch (1A,1B), hyoid or second pharyngeal arch (2). Scale bar, 2 mm.

The facial plates are bounded by deep furrows with prominent maxillary and mandibular components. The frontonasal process is deeply furrowed medially. Deep lacrimal grooves are present. The prominent nasal pits are deep and separated. Medial and lateral nasal swellings are present at an early stage of formation. These olfactory primordia have a human equivalence of Carnegie Stage 15 (CS=15). The evaginated eye cup of the diencephalon manifests a ventral choroid fissure that can be seen externally (CS=14–15). The otic primordium consists of a detached otic vesicle with a dorsal endolymphatic projection (CS=13).

The first four pharyngeal arches are well marked externally (CS=13); however, the external bulging rudiment of a fifth arch is a unique feature. The second hyoid arch is very enlarged but marginally smaller than the mandibular component of the first arch. The early stages of cervical sinus formation of the second arch is in progress. The first pharyngeal pouch is well marked. The first pharyngeal membrane is thin and bilaminar. The second and third pharyngeal pouches are well formed and both exhibit a distinct ventral pocket.

There are four complete pairs of aortic arches. There are small remnants of the mandibular and hyoid aortic arches that connect with the ventral aorta. Three of the four complete pairs of aortic arches (3, 4 and 6) arise from the ventral aorta. The fifth aortic arch is a branch of the fourth arch. The third aortic arch is by far the largest. Dorsally the aortic arches connect with the paired carotid arteries extending into the head. Paired dorsal aortae extend into the embryonic body.

The embryonic trunk is cylindrical and 'C' shaped. Somites bulge from the external surface of the trunk. The forelimb buds exhibit axial elongation with an orientation parallel to the trunk (CS=14). The hindlimb primordia are body wall thickenings without structural differentiation (CS=13). The marked structural difference between the differentiation of forelimbs and hindlimbs is characteristic of limb organogenesis of both monotremes and marsupials and is expressed at this early stage of limb morphogenesis.

The heart consists of an undivided thickened ventricle and a thin-walled single atrial component (late CS=12 or early CS=13). The arterial system consists of dual dorsal aortae served by four prominent pairs of aortic arches (3, 4, 5 and 6). The dorsal aortae give off segmental arteries to the body. The dorsal aortae end as sinuses around the post-anal gut. Five allantoic arteries provide a rich vascularization of the rudimentary allantoic stalk. The arterial supply to the yolk-sac is by multiple arterial vessels. The two vitelline veins from the extra-embryonic yolk-sac are disproportionately enlarged and on each side pass caudally between the yolk-sac splanchnopleure and the amnion to enter the sinus venosus. The right vitelline vein (omphalomesenteric vein) is the larger and both vitelline veins pass through the liver primordium before entering the sinus venosus. This is a unique feature shared by monotremes and marsupials and is consequent on the yolk-sac being the principal nutritive organ. In the human, allantoic vasculature (umbilical veins) drain the chorionic villi very early in embryonic development, with the venous placental return proceeding in a caudorostral direction. The promixal components of the yolk-sac

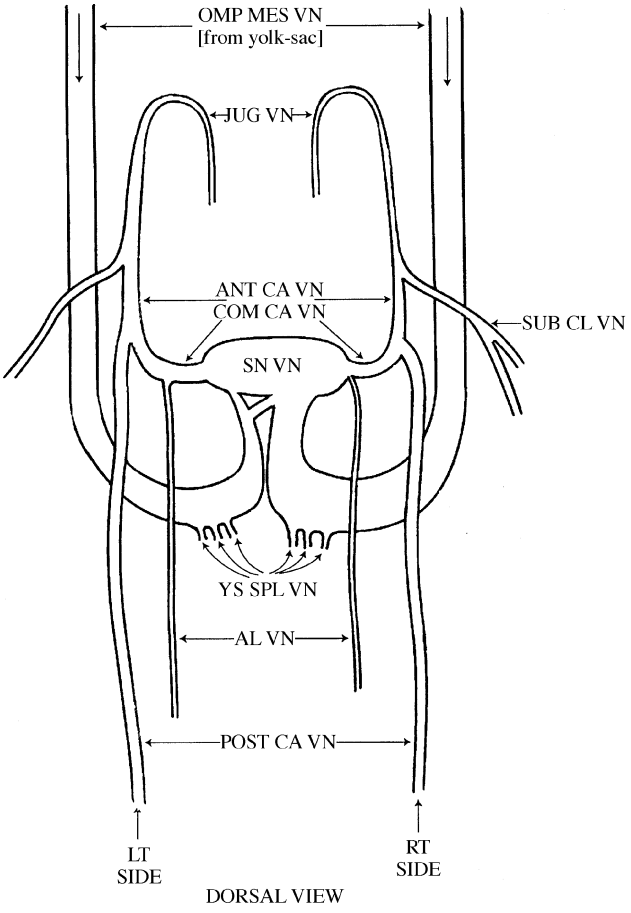

Figure 4. Unscaled diagram showing the arrangement of the venous system in the region of the sinus venosus of the heart. Arrows indicate the direction of blood flow. Allantoic vein (AL VN), anterior cardinal vein (ANT CA VN) draining rostral portion of body, common cardinal vein (COM CA VN), omphalomesenteric vein (OMP MES VN) from the yolk-sac, posterior cardinal vein (POST CA VN) draining posterior part of body, subclavian vein (SUB CL VN), sinus venosus (SN VN), jugular vein (JUG VN), veins draining the yolk-sac splanchnopleure (YS SPL VN).

vasculature in humans is modified owing to early yolk-sac regression, to serve intra-embryonic vascularization of internal abdominal organs. In humans, unlike monotremes and marsupials, the extra-embryonic yolk-sac serves no important placental function by this stage.

Details of the venous drainage of platypus J were reconstructed by G. S. Sanson (figure 4). This shows modifications of the anterior cardinal system to include paired inferior jugular veins and paired subclavian veins. These vessels are associated with wide anterior cardinal trunks that enter the common cardinal veins (Cuvierian ducts) that unite bilaterally with the sinus venosus. The bilateral posterior cardinal veins, which serve the trunk of the embryo, include a rich lateral vascularization of the mesonephros. The posterior cardinal veins also enter the heart by way of the bilateral common cardinal veins. The bilateral allantoic (umbilical veins) are small in the platypus J stage and are correlated with the rudimentary development of the allantois at this stage. (The allantoic rudiment appears in both monotremes and marsupials at the pharyngeal arch stage.) The left allantoic vein of the

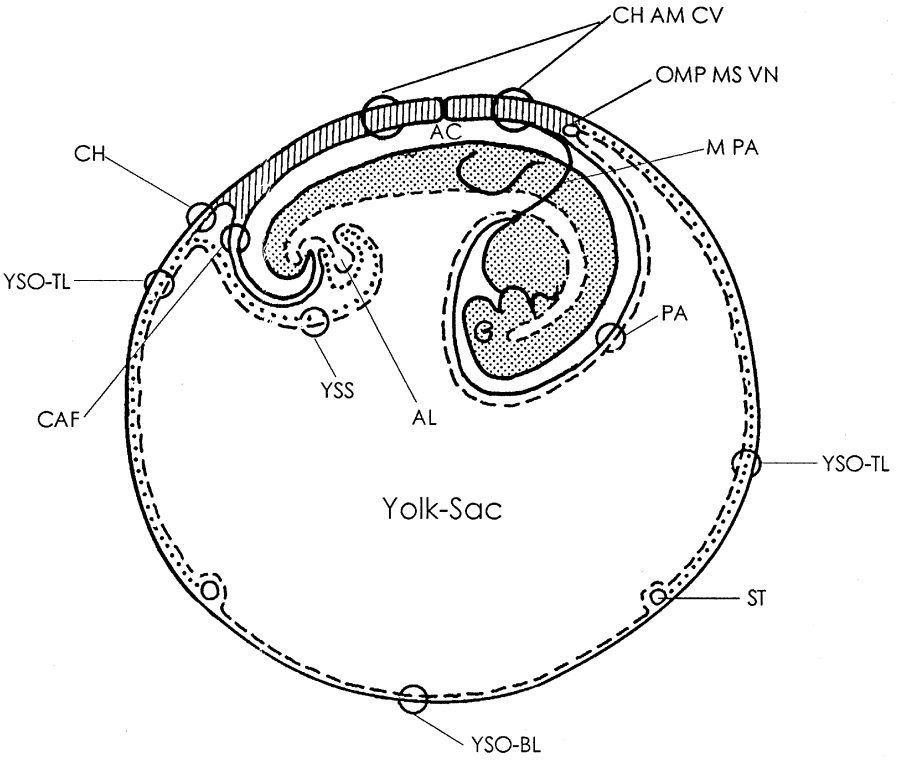

Figure 5. Unscaled diagram showing the arrangement of the extra-embryonic membranes in platypus 'J' at the pharyngeal arch stage. Allantois (AL), amniotic cavity (AC), caudal amniotic fold (CAF), chorio-amniotic connection (CH AM CV), pure chorion (CH), proamnion (PA), margin of proamnion (MPA), sinus terminalis (ST), yolk-sac omphalo-pleure bilaminar (YSO-BL). Non-vascularized portion of the yolk-sac: yolk-sac splanchnopleure (YSS), yolk-sac omphalopleure trilaminar (YSO-TL). Vascularized portion of the yolk-sac: the continuous line represents ectoderm or somatic mesoderm; the dashed line indicates endoderm; the dotted line indicates vascularized splanchnic mesoderm that invests the allantois and portion of the yolk-sac, branch of omphalo-mesenteric vein (OMP MS VN). Vertical hatching indicates the chorioamniotic connection. Circled labels indicate germ layer composition.

platypus J stage enters the mid-point of the common cardinal veins, whereas the right allantoic vein enters the sinus venosus near the junction of the common cardinal vein with the sinus venosus (figure 4).

It is clear that the unique dominance of the yolk-sac in embryonic nutrition in both monotremes and marsupials impacts on the arrangement of the intra-embryonic cardio-vascular pattern in a way that cannot appropriately be characterized in terms of equivalent Carnegie Stages in human development. Likewise, the late appearance of the allantoic-stalk-based umbilical circulation in both mono-tremes and marsupials reciprocally contributes to the development of the unique embryonic cardiovascular pattern.

The five primary brain vesicles are present: telence-phalon, diencephalon, mesencephalon, metencephalon and myelencephalon. A prominent midbrain flexure is a feature of the central nervous system. The thin roof of the hindbrain enables five neuromeres on its floor to be detected.

The trigeminal ganglion of cranial nerve five (CN5) is large, with the lateral portion of this ganglion derived from an ectodermal proliferation of considerable extent. The peripheral ophthalmic, maxillary and mandibular rami project to their target tissues. CN5 is centrally connected to the brain and exhibits development equiva-lent to human CS=15.

The acoustic-facial ganglion complex CN7 and CN8 is well formed. The auditory ganglion is divided into *pars superior* and *pars inferior*. The cochlear ganglion is as yet undeveloped. The geniculate ganglion of CN7 is well formed and exhibits nerve axons of the superior petrosal and chorda tympani nerves. The glossopharyngeal root ganglion (CN9) is well formed, with the petrosal ganglion conspicuous. The root ganglion of the vagus nerve (CN10) is poorly developed; however, the nodus ganglion is well formed. Forceps ganglion of the spinal accessory nerve (CN11) is conspicuous. The hypoglossal nerve (CN12) is well formed.

The respiratory system is at an early primary lung bud stage equivalent to human development CS=13. It consists of a short, median primordial trachea that is divided distally into two thick-walled primary bronchi with a length of 120 μm. The distal extremities of the primary bronchi are expanded into a thick-walled lung primor-dium of 384 μm × 160 μm in transverse section. The length of the lung buds is about 220 μm.

Alimentary primordia present include: an oesophagus of 430 μm in length; expanded stomach primordium as a wide tube bent slightly to the right; dorsal and ventral pancreatic primordia; and gall bladder (CS=14). The midgut is open to the yolk-sac over a distance of 278 μm; this is a characteristic of the extra-embryonic membranes of the platypus and is without equivalence in human development.

The proximal component of the embryonic nephric system on each side consists of a solid cord associated with a blind coelomic nephrostome. This presumably represents the remnants of the functionless pronephric system. More caudally, the bilateral mesonephric ducts are patent and enter the cloaca laterally. A total of 59 distinct bilateral mesonephric tubules were associated with the mesonephric ducts (CS=14). The rostral 30 mesonephric tubules open medially into the mesonephric duct; the remaining tubules are solid immature struc-tures. The most highly differentiated of the mesonephric tubules consist of a proximal limb associated with the mesonephric duct. A ventral limb is associated with a nephrostome and a medial limb exhibits primordia of a glomerulus and Bowman's capsule. The mesonephros is well vascularized by a plexus of the posterior cardinal veins.

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The structure of the extra-embryonic membranes was recorded in handwritten notes of J. P. Hill and summarized in a diagram (figure 5). An abstracted and interpretative account of the major features of the extra-embryonic membranes of platypus stage 'J' follows.

The head of the embryo is sunken into the yolk-sac with the proamnion of fused ectoderm and endoderm extending caudally to just anterior to the forelimbs (PA; figure 5). The head fold and tail fold of the amnion have fused to form a complete but relatively narrow amniotic cavity (AC, figure 5). The superficial component of the amniotic membrane extends for approximately one-quarter around the peripheral circumference of the extra-embryonic membranes. An extensive chorioamniotic connection is present and extends throughout the greater part of the caudal half of the now fused amniotic folds. Within the amniotic cavity the intact anal plate with a width of 240 µm is well marked with a thickened ectodermal component. The post-anal proctodeal gut has a length of 700 µm.

The vascularized yolk-sac consists of a superficial yolk-sac omphalopleure (YSO-TL, figure 5) and a deep yolk-sac splanchnopleure (YSS, figure 5). Both are richly vascularized. The superficial yolk-sac omphalopleure occupies approximately half the perimeter of the superficial extra-embryonic membranes and is bounded by a sinus terminalis (ST, figure 5) at its junction with the non-vascularized yolk-sac (YSO-BL, figure 5).

The non-vascularized yolk-sac (YSO-BL, figure 5) consists of approximately one-quarter of the abembryonal pole of the superficial extra-embryonic membrane. The yolk-sac cavity (Yolk-Sac, figure 5) contains a small dense mass of free yolk. A very narrow area of pure chorion (CH, figure 5) was present at the junctional region between the caudal amniotic fold (CAF, figure 5) and the peripheral vascularized yolk-sac omphalopleure (YSO-TL, figure 5). The allantoic vesicle (AL, figure 5) was small and measured about 1.4 mm × 0.8 mm in external dimension. Its everted thick wall from the hindgut was richly vascularized. The equivalent Carnegie Stage in human development was CS = 12.

(b) *Summary of the descriptive details of platypus 'J' (pharyngeal arch stage)*

The pharyngeal arch stage of mammalian embryonic development is a period when major organ systems of the future definitive body undergo rapid modelling. The major steps in the modelling sequence for given organ systems have long been known to follow very similar ordered sequences of structural expression within all amniote vertebrate taxa. On this basis, Butler & Juurlink (1987) produced an atlas for staging mammalian and chick embryos using the Carnegie staging system. However, between mammalian taxa the temporal rates of expression of structural modelling steps vary, both within and between organ systems, so that asynchronous organogenesis occurs. Authors such as Butler & Juurlink (1987) seek to discount such differences as trivial. Three primary factors, as illustrated by the platypus 'J' stage, contribute to this asynchrony within definitive mammalian embryos and are regarded as a sufficient basis for the rejection of the validity of formulating a universal equivalent staging system for mammalian embryos such as that based on the Carnegie system as proposed by Butler & Juurlink (1987).

First, fundamental taxon-specific variation occurs in the temporal rate of expression of morphogenetic modelling steps both within and between organ systems.

Second, taxon-specific variation occurs in the placement of either birth or, in the case of monotremes, hatching, within the profile of organogenesis. This necessitates that structural adaptations for survival be conferred irrespective of the completeness of the organogenetic profile.

Third, taxon-specific variations occur in both the functional and the structural expression of extra-embryonic structures. This impacts on the diversity of intra-embryonic profiles of organ systems including the cardiovascular system, the excretory system and the embryonic gut.

In this context, the descriptive detail of platypus 'J' pharyngeal arch stage is in sufficient detail to satisfy taxon-specific criteria for each of these three categories to demonstrate that comparative placement of platypus J in terms of a single, human Carnegie Stage, as outlined by Butler & Juurlink (1987), would be inappropriate. In the experience of the authors, platypus J is equivalent to the second quarter of marsupial organogenesis (Hughes *et al.* 1989). An analysis of organogenesis of serial sections of diverse stages of marsupial and monotreme organogenesis demonstrates that it is possible to formulate a relatively consistent staging system applicable to both monotremes' and marsupials' taxa (Hughes & Hall 1988; Hughes *et al.* 1989; Hughes 1993).

(c) *Mid-incubation period*

This specimen is from the Hill collection, Hubrecht International Embryological Laboratory, Utrecht.

Platypuses 'L' and 'LL'. Greatest length of definitive embryo, 9 mm.

Collected by J. P. Kenny, 8 October 1899.

Two incubation-stage eggs were adherent with peripheral shell dimensions of 16 mm × 15 mm and 17 mm × 14 mm. The definitive embryos obtained from these eggs were of equivalent stages of development.

The head was bent at an acute angle to the axis of the trunk. The hindbrain exhibited a thin roof and the cerebral hemispheres were well developed. The blunt upturned snout marked the site of the future oscaruncle. The narial openings were elongated in the direction of the snout. A median primordial papilla of the egg tooth was present in the maxilla. The eye was open with early eyelid primordia present. The pigmented retinal ring was incomplete. The external auditory meatus was open. Epitrichial coverings of the eye and the ear were as yet undeveloped.

The forelimbs exhibited partly separated digits on the slightly rotated manus plate. The slightly recurved digits lacked primordial epitrichial claws. In terms of human development, the manus plate was at CS = 20.

The hindlimb pes plates were as paddles and the margin of the separated pes plate exhibited smooth margins, with the earliest beginnings of digit ray modelling that was equivalent to human CS = 18. The tail was circular in cross-section and slightly bent forward. The plantar surfaces of the pes plate clasped the tail.

A prominent genital tubercle is present just rostral to the inner curvature of the base of the tail. Somites and spinal cord were visible through the body surface in the caudal abdominal region.

The definitive embryo was closely invested by an amnion. A chorioamniotic connection was present. This consisted of a 11.8 mm persistent portion of the proamnion that unites the amnion with the chorion. It begins at about the level of the middle portion of the head and terminates caudally at the rostral border of the forelimbs.

A vesicular allantois of about 12.5 mm in diameter expands from the naval without an appreciable stalk. The allantoic vesicle was situated on the right side of the embryo. The distal richly vascularized portion of the allantois was extensively fused peripherally with the chorion. The very short allantoic stalk contained a fine lumen continuous with the allantoic vesicle. At the origin of the allantois the blood vessels consisted of a single left allantoic vein and two arteries, with the left artery larger than the right. More distally, two arteries and two veins were present. The inner, unattached, concave surface of the allantoic vesicle carried the major arterial and venous connections to the outer, highly vascularized, attached chorioallantoic area. Marginal to the peripheral area of chorioallantoic attachment is a thin zone of pure chorion.

The peripheral yolk-sac (omphalpleure) had axial dimensions of 15 mm × 12 mm. The yolk-sac splanchnopleure had axial dimensions of 14 mm × 16 mm when flattened after dissection. The vitelline artery on emerging from the yolk stalk passes about 8.5 mm rostrally in the yolk-sac splanchnopleure, giving off large branches near its origin, and these subdivide to supply the yolk-sac splanchnopleure and eventually extend into the peripheral yolk-sac omphalopleure. The posterior part of the yolk-sac splanchnopleure is supplied by a right and a left lateral branch of the vitelline artery. These continue onto the superficial yolk-sac omphalopleure where they take a parallel course and provide lateral fields of vascularization of the yolk-sac omphalopleure.

The venous return from the yolk-sac omphalopleure is divided into bilateral components with the main venous return via the yolk-sac splanchnopleure with venous fields more-or-less parallel to the arterial outflow. The venous drainage eventually enters two main bilateral trunks on each side of the vitelline arterial branches. The two major venous trunks eventually fuse into a single vitelline vein before entering the navel.

This mid-incubation platypus stage is marginally more developed than the mid-incubation platypus stage described by Hughes (1993).

(d) *Subterminal pre-hatching*

This specimen is from the Hill collection, Hubrecht International Embryological Laboratory, Utrecht.

Greatest length, 9 mm.

Collected by G. Inlson, 31 August 1898.

Diameter of shell of laid egg, 15 mm.

The eyes exhibited an oval peripheral rim. Eyelids were as yet undeveloped. The retina was unpigmented. The external auditory meatus was open (figure 6). The absence of epitrichial covering of the eye and the plugging of the external auditory meatus was consistent with the designation of a subterminal hatchling status.

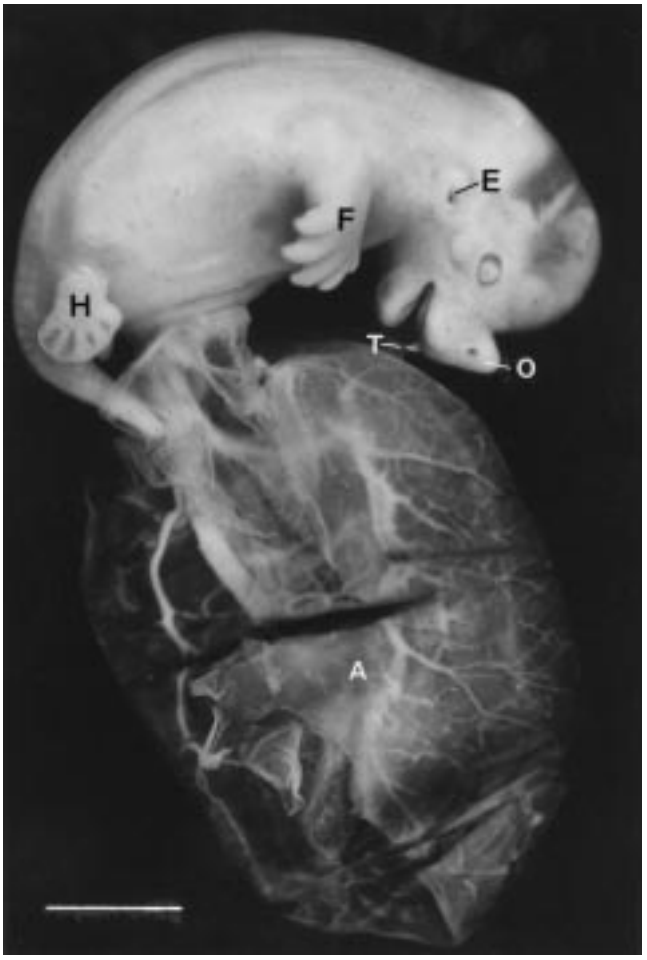

Figure 6. Photograph of subterminal pre-hatching *O. anatinus* with extra-embryonic membranes from the Hill collection. Note separated digits on forelimb (F), and touched-up shading of digital rays on hindlimb (H). External auditory meatus (E), oscaruncle (O), egg tooth (T), and allantois (A). Scale bar, 2 mm.

The manus plates of the forelimbs were almost completely pronated with separated digits that exhibited early epitrichial claw primordia. The manus plate is equivalent to human stage CS = 23. The hindlimbs were paddles with pes plates with a smooth margin. Digit rays were faintly visible within the pes plate but in figure 6 these can be seen to be artistically enhanced. The pes plate is equivalent to human CS = 19.

The tail was not markedly recurved but was clasped by the plantar surfaces of the pes plates.

Coils of the gut were herniated on the left side of the umbilicus. Portions of the highly vascularized extra-embryonic membranes were attached to the umbilical region. On the left side of the umbilicus a flattened vesicular allantois of 10 mm × 6.5 mm exhibited a distal region fused with the chorion. In human development the allantois remains small and does not form a vesicle that fuses with the chorion. However, the blood vessels of the human allantoic stalk vascularize the discordal placenta and traverse the umbilical cord.

The upturned snout constitutes the developing oscaruncle. A sharp recurved egg tooth 0.29 mm in length, situated in the median portion of the upper jaw, is further

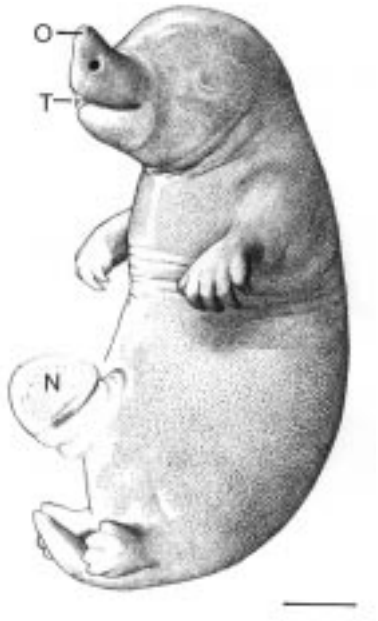

Figure 7. Drawing of a newly hatched *O. anatinus*, (specimen WW), from the Hill collection. Eyelids now cover the eyes, digits have appeared on the hindfeet, there is a prominent yolk navel (N), oscaruncle (O), and egg tooth (T). Scale bar, 2 mm.

described by Hill & De Beer (1949). These features have no equivalent in human embryonic development.

4. NEWLY HATCHED YOUNG

This specimen is from the Hill collection, Hubrecht International Embryological Laboratory, Utrecht.

Specimen 'WW'. Greatest length, 16.75 mm; head length, 6.0 mm.

Collected by J. P. Kenny; first week of August 1899.

Platypus WW was one of two nestlings taken along with their mother and egg shells. The estimated age was presumed to be not more than two days. The body dimensions and external morphology, together with the presence of a yolk-navel, were very similar to those reported as newly hatched echidna by Griffiths *et al.* (1969).

A drawing of platypus WW is shown in figure 7. The fused eyelids, as well as the primordia of the external auditory meatus, were covered by epitrichium. The mobile forelimbs (slightly less massive than the newly hatched echidna) exhibited five separated digits bearing blunt recurved epitrichial claws. The manus plate is pronated for digito-palmar prehension. In relation to human development the final stages of pronation of the manus plate are nearing completion at the end of the human embryonic period CS = 23 at about 56 post-ovulatory days. In platypus WW the immobile hindlimbs were paddles at a much earlier stage (CS = 20, approximately 50–51 post-ovulatory days in humans) of modelling. The pes digit rays were sculptured on the external surface of the pes plate and are distally notched. The medial surface of the unrotated pes plate clasps the recurved tail (figures 6 and 7). In transverse sections the tail was rounded. The prominent genital tubercle adjacent to the inner surface of the tail curvature was slightly bilobed with a median ridge extending over its summit and extending down its rostral surface.

A prominent yolk navel was present on the median surface of the abdomen (figure 7). This is a remnant of the extra-embryonic yolk-sac of the earlier incubation period. The yolk navel projected ventrally from the abdominal cavity for 2.8 mm. It had a rostrocaudal axis of 2.5 mm. According to Griffiths *et al.* (1969), the yolk navel of the echidna disappeared 6 h after hatching. The presence of a yolk navel in platypus WW is interpreted as consistent with its designation as an early hatchling.

A prominent feature of platypus hatchling WW is the presence of a bluntly conical oscaruncle. This upturned prerostral structure measured 1.7 mm from the margin of the upper lip to the tip of the caruncle. Internally the osseous skeletal foundation of the caruncle is derived from the fusion of two upturned inferior lamellae of the premaxillae, to form a nodule of bone that is capped externally by a cornified epidermis; further details have been reported by Hill & De Beer (1949). In addition to the oscaruncle, an egg tooth is also present and consists of a thick basal portion of 2.4 mm in width, which provides a remarkably strong support. Distally the egg tooth tapers and bends slightly inwards as a curved lancet-like structure of about 0.35 mm in length. The egg tooth exhibits a translucent horn-like appearance and, according to Hill & De Beer (1949), consists of a pulp region, vascularized by small capillaries, successively inverted by two inner layers of dentine and an outer film-like layer of presumptive enamel. The oscaruncle and the egg tooth assist the hatchling to extricate itself from the egg shell at the conclusion of the incubation period. These structures are also well developed at the pre-hatchling stage (figure 6).

It is of interest to note that the newly hatched nestling of the platypus has an external form reminiscent of a newborn marsupial (Griffiths *et al.* 1969; Hughes 1984, 1993). The only precursor of the adult distinctive surface anatomy is a presumptive webbing ridge at the margins of the palmar surface of the mannus plate as previously described.

5. DISCUSSION

The intrauterine embryonic development of the platypus exhibits a dual spectrum of features.

First, the axial post-primitive streak expression of the central nervous system closely followed by paraxial somitogenesis forms a core of primary bases for the expression of the underlying pattern of the definitive body. In the platypus the rate of somitogenesis is faster in relation to CNS morphogenesis than that of the human. The size of the early definitive embryonal area is massive in relation to that of the human. The differences in the rate of morphogenesis between the CNS and somites between the platypus and humans give rise to embryos with divergent organogenetic patterns, so that comparative staging between human and platypus embryos as a whole becomes progressively inappropriate. Other fundamental patterns of organogenetic divergence include cardiovascular development and, at a later stage, the development of the limbs, respiratory system and excretory systems.

Secondary patterns of organogenesis are superimposed on the fundamental primary patterns and these are dictated by extra-embryonic parameters pertinent to both pre-hatching and post-hatching survival. The earliest of

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these secondary patterns include the morphogenesis of the extra-embryonic membranes with a unique morphology and function that defies comparative staging with eutherian mammalian taxa. At no stage in extra-embryonic membrane development in monotremes is comparative human Carnegie Staging appropriate.

Intra-embryonic structures adapted for altricial survival of the hatchling require the acquisition of function competence at an earlier stage of organogenesis than in eutherians. The establishment in the platypus of a functional respiratory membrane at the terminal sac stage of lung development is an example of early out-of-phase vascularization in relation to the development of the lung of eutherians. Out-of-phase early myogenesis in the preumbilical region of the body results in the generation of striated embryonic myotubules for breach of the egg shell at hatching, together with post-hatching locomotion and sucking and swallowing. The newly hatched platypus is served by a mesonephric-based excretory system. These special adaptations that permit survival with otherwise incomplete morphogenesis of organ systems confer a unique morphology at near-terminal stages of pre-hatching development; they are not amenable to comparative staging of the pre-hatchling *in toto* with human development by assigning equivalent Carnegie Staging. The pattern of pre-hatchling development is sufficiently parallel to marsupial patterns for equivalent staging with this taxon (Hughes & Hall 1988; Hughes 1993).

The platypus hatchling is neither an embryo nor a foetus but a specialized stage in the unique monotreme life-cycle (table 2).

The distinctive external morphology of the platypus hatchling includes a rostral caruncle and a median egg tooth to assist in emergence from the egg shell. This is also assisted by the precociously mobile forelimbs with epitrichial clawed digits capable of digito-palmar flexion. Among mammalian taxa the platypus hatchling, like that of the echidna, most closely resembles the neonatal kangaroo-like marsupials (Griffiths *et al*. 1969; Hughes 1993). These features were summarized in table 2.

At hatching the unique external features of the adult platypus body are as yet undeveloped. The distinctive aspects of the platypus external anatomy such as the bill, webbing of the limb digits, flattened tail, as well as the thick body fur have morphogenetic profiles confined exclusively to the post-hatching suckling period. The continuation of the sequence of post-hatching development of external body form will be described in the paper by Manger, Hall & Pettigrew (this issue).

(a) *Speculation concerning monotreme and marsupial patterns of organogenesis*

Griffiths *et al*. (1969) and Hughes (1993) have commented on the large suite of monotreme features at hatching that are shared with new-born marsupials and particularly with the neonatal kangaroo-like marsupials such as *Macropus eugenii* (Hughes *et al*. 1989).

The 18–20 somite stage at the conclusion of intrauterine development in the platypus as described in this paper is believed to represent a subterminal stage in the first quarter of pre-hatching organogenesis, as defined by a time interval from primitive streak to hatching.

The full-term interuterine stage of the echidna is equivalent to that of the platypus (Hughes 1984). In the echidna this represents 10–10.5 days pre-hatching (Griffiths *et al*. 1969). In the kangaroo-like marsupial *Macropus eugenii*, at the end of the first quarter of post-primitive streak/pre-parturient organogenesis the embryo is about 9 days before birth. The *Macropus eugenii* embryo is then at a 17-somite stage. At this stage of development, *Macropus eugenii* is roughly at a stage comparable to the 25-somite stage of the platypus at 1–2 days post-laying (Hughes 1993). On this basis it might be presumed that the full-term intrauterine monotreme embryo could represent a subterminal stage in the first quarter of post-primitive streak/pre-hatching organogenesis.

The pharyngeal arch stage of platypus J in this paper represents an earlier point in the profile of development with reference to the late pharyngeal arch stage at the end of the second quarter of prenatal organogenesis in *Macropus eugenii* (Hughes *et al*. 1989). However, platypus J is presumed to be later than the end of the first monotreme organogenetic quarter. This possibly places platypus J within the second organogenetic quarter of post-primitive streak/pre-hatching organogenesis.

The platypus specimen in this paper designated as mid-incubation period stage is also less developed than *Macropus eugenii* at the terminal part of the third quarter of pre-parturient organogenesis, but slightly more advanced than at the end of the second quarter. In consequence, this mid-incubation platypus stage has been presumptively designated as representative of the third pre-hatching quarter of monotreme organogenesis. The mid-incubation platypus stage described by Hughes (1993) is also considered to be characteristic of the third pre-hatching quarter of monotreme organogenesis.

In relation to the subterminal pre-hatching stage of the platypus with an overall length of 9 mm, it is clear that this subterminal stage is quite similar in development to the early platypus hatchling. It almost certainly represents the subterminal portion of the fourth quarter of post-primitive streak/pre-hatchling monotreme organogenesis.

It is of interest that despite more than 90 years having elapsed since the basic features of monotreme embryonic morphogenesis were established, the precise temporal staging of platypus organogenesis awaits experimental confirmation.

The authors thank the curators of the Hubrecht International Embryological Laboratory, Utrecht, for access and use of material housed in the J. P. Hill collection. We also thank Erica Maddock for her help in the preparation of the manuscript.

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