A TIMED SERIES OF EMBRYONIC DEVELOPMENTAL STAGES OF THE ADÉLIE PENGUIN (*Pygoscelis adeliae*) FROM SIGNY ISLAND, SOUTH ORKNEY ISLANDS

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ABSTRACT. An arbitrarily staged developmental series of Adélie penguin (*Pygoscelis adeliae*) embryos from Signy Island has been prepared from artificial and natural incubation. The external morphology of these embryos is described and compared with the other two species of *Pygoscelis*, the gentoo penguin (*P. papua*) and chinstrap penguin (*P. antarctica*), and with a standard series of the domestic chick (*Gallus domesticus*).

MEMBERS of several Antarctic expeditions have collected embryos of the penguin and compared their development with that of the Chinese goose (Ewart, 1922), duck (Waterston and Geddes, 1909) and chick (Parsons, 1932, 1934; Glenister, 1954; O'Gorman, 1964).

The first attempt to obtain a developmental series of embryos of known age was made by Waterston and Geddes (1909) from embryos collected during the Scottish National Antarctic Expedition, 1902–04. 30 embryos of the gentoo penguin (*Pygoscelis papua*) and the Adélie penguin (*P. adeliae*) comprised a representative series from the primitive streak stage to the pre-hatching stage. Up to 11 days of age the structure of the penguin embryo is equivalent to that of the duck, though with a slower rate of development. Later on, however, there are differences in the development of the fore-limbs, feet and plumage. Waterston and Geddes concluded that penguins originated, together with modern birds, from a common flying ancestor.

The most comprehensive descriptions of the development of *Pygoscelis* are by Parsons (1932, 1934), who described 13 *P. adeliae* and three emperor penguin (*Aptenodytes forsteri*) embryos originally collected by Wilson (1907). A comparison was made with the chick *Gallus domesticus* (Keibel and Abraham, 1900), and Parsons noted the similarity between embryos of *P. adeliae* and those of the other two pygoscelid penguins collected during the *Discovery* Investigations (1928–29, 1930–31) which he had earlier described. Various embryos (stages with raised neural folds to pre-hatching) were examined from serial sections and special consideration was given to the development of the air sacs and lungs, heart, brain, cartilaginous skeleton and feathers. The rapid elongation of the fore-limb and the dense covering of feather papillae were noted. Rudiments of dorsal air sacs on the lungs were considered to be a primitive characteristic.

More recently, Glenister (1954) investigated a series of 16 embryos of *A. forsteri*. Nine of the youngest were sectioned serially and a number of features which he considered primitive were listed. Glenister concluded that penguins are the most primitive birds, and that *A. forsteri* is the most primitive penguin. Comparisons were also made with the normal stages of development of *G. domesticus* (Hamburger and Hamilton, 1951).

The most recent description of embryos of the emperor penguin was by O'Gorman (1964) on embryos collected during the Royal Society International Geophysical Year Antarctic Expedition, 1955–59. He used the unpublished notes of J. Norman to calculate the ages of these embryos. Norman's timed series of embryos, although in poor condition and small in number, enabled O'Gorman to adopt a multiplication factor of 2·9, to calculate the ages of the penguin embryos from the ages of equivalent chick stages described by Hamburger and Hamilton (1951).

Both Glenister and O'Gorman expressed the need for an accurately timed series of developmental stages of a representative penguin. To fulfil this need a timed series of embryonic stages of *P. adeliae*, *P. antarctica* and *P. papua* was collected at Signy Island, South Orkney Islands, during the period January 1965 to February 1966. The external morphology of these embryos is described in comparison with embryos of the chick.

MATERIAL AND METHODS

The populations of *P. adeliae* and *P. antarctica*, which nest in large colonies on most of the rocky headlands of the south and west coasts of Signy Island, each comprise about 25,000

birds. There is a smaller number of *P. papua*, about 1,000 individuals in small colonies of 10–20 birds, whose nesting sites are spread over the hillside at the northern end of the island.

P. adeliae and *P. papua* adults arrive at the rookeries in the latter part of September (*P. antarctica* a month later) and immediately they commence nest-building. Within a month the first eggs are laid. Incubation takes from 35 to 38 days, the shortest period being in *P. adeliae* and the longest in *P. papua*. After hatching, the young develop rapidly and leave the rookeries 9 weeks later.

Collection of eggs

Eggs of *P. adeliae* were collected from a large colony at Pantomime Point on Gourlay Peninsula (colony III of Sladen (1958); Fig. 1) and from small colonies nearby, including Sladen's colony XI. *P. antarctica* eggs were collected from three colonies on Pageant and Gourlay Points, Gourlay Peninsula.

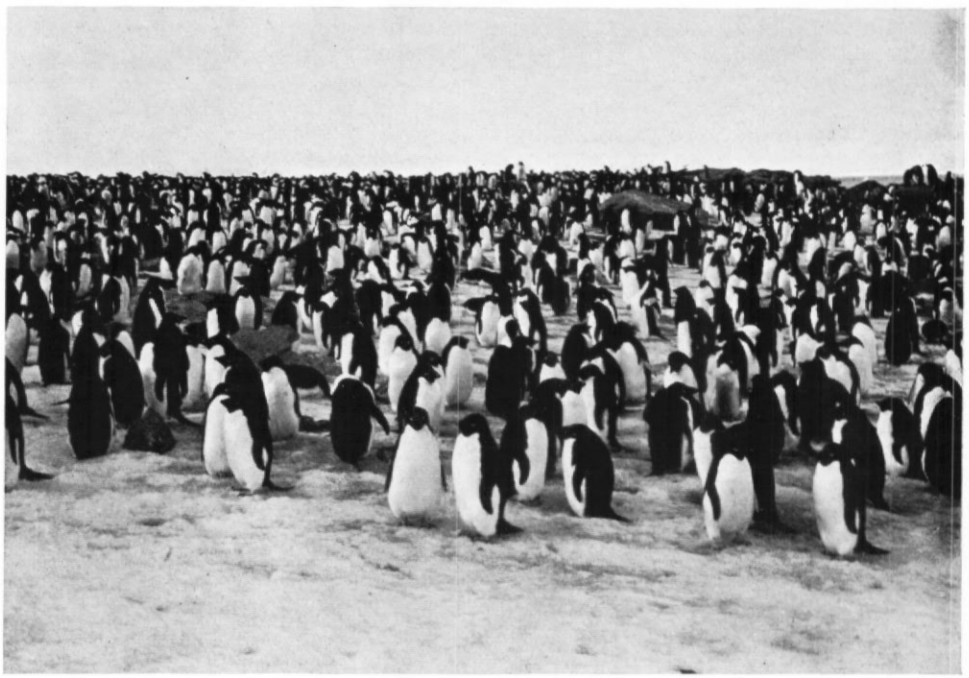


Fig. 1. *P. adeliae* colony III at Signy Island early in the breeding season before nest-building had started (7 October 1965).

Pygoscelid penguins usually lay two eggs (although *P. adeliae* (Fig. 2) and *P. antarctica* may lay three and *P. papua* four) separated by a period of 3 or 4 days. Throughout a colony, egg-laying reaches a peak (when most of the nests have a single egg) about a week after the first eggs are laid. When about 75 per cent of the nests of *P. adeliae* and *P. antarctica* each contained a single egg, a large colony and a series of smaller ones were cleared of all eggs, about 700 being removed initially from each species. 24 hr. later the nests of the same colonies were again cleared of eggs (which were thus almost certainly 0–24 hr. old), these eggs being removed to an incubator in a laboratory at the scientific station. The procedure was repeated on successive days, until over 600 eggs were in artificial incubation. Nevertheless, occasionally some embryos, when removed from the incubator, were found to be in advance of the general day's sample. Presumably these were from eggs inadvertently overlooked during the first clearing.

Transportation of *P. adeliae* eggs to the laboratory by motorized sledge took about 20 min.,

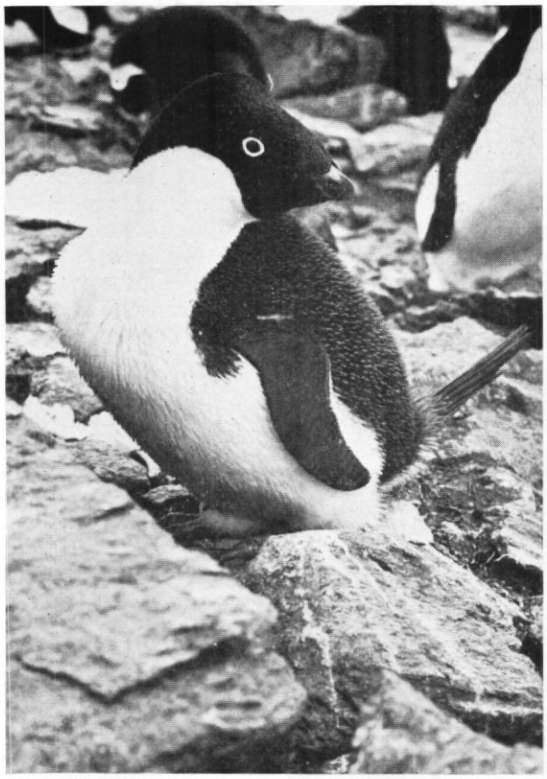


Fig. 2. *P. adeliae* on the nest at Signy Island, incubating a single egg (November 1965).

about 40 min. elapsing between removal from the parent and their introduction into the incubator. Though the eggs were in insulated containers, the ambient temperature of −4·1°C and a wind speed of 19 kt. (9·8 m./sec.) caused them to be cold to the touch on arrival at the laboratory.

P. antarctica nests on steep-sloping sites, which made egg-collecting difficult. As the sea ice had gone out, eggs were taken to the laboratory by boat, a journey lasting more than an hour. Unavoidably these eggs were more roughly handled and chilled than those of *P. adeliae*.

P. papua is extremely timid. Early on in the breeding season it deserts the nest at the first sign of human approach. Such behaviour permits skuas (*Catharacta skua maccormicki*) and sheathbills (*Chionis alba*) free access to the eggs during human intervention, with consequent loss of many of them. Access to the colony by sea was precluded by pack ice and eggs were carried back to the laboratory on foot. In order to avoid denuding the *P. papua* breeding colony, only 100 eggs were removed for laboratory incubation.

Underparent incubated eggs were also collected from all three species.

Method of embryo removal

Blastoderms up to 4 days old, complete with vitelline membrane, were excised with fine scissors and they were removed together with an underlying part of yolk on a spatula or, in the case of older more robust embryos, in a spoon. The vitelline membrane was removed with fine forceps and a fine jet of warm saline, which also facilitated the removal of surplus yolk granules.

Blastoderms and embryos up to 20 days old were killed in Smith's fixative, and older specimens in formal-saline (0·7 per cent sodium chloride in 4 per cent formaldehyde solution) or 70 per cent alcohol. A number of blastoderms up to 20 days old (stage 19) was stained in anthracene blue (Mahoney, 1963), dehydrated, cleared and mounted in balsam (Figs. 3–19).

Incubation

Eggs were incubated in a Hearson's Anhydric S2116 incubator, modified to give a slight fresh-air feed into the air-circulating system by incorporating a 0·5 in. (12·5 mm.) inlet into the input side of the Leach blower. Two large water trays were fitted to the floor to provide a moist atmosphere, but free evaporation of water was insufficient to maintain a relative humidity of 60–80 per cent and therefore the eggs were sprayed with water every 12 hr. Eggs were turned by hand every 12 hr. and shelves were moved to different positions in the incubator in order to reduce any effects of temperature gradient during incubation. Temperatures ranged from 33·5° to 34°C as was recorded in the field by Eklund and Charlton (1958).

Artificial incubation was found not to be completely successful, and with embryos older than 18 days the percentage mortality was particularly high. Study of the second half of the developmental period, especially after 23 days, was mainly on specimens obtained from beneath parents. Only five *P. antarctica* embryos of the original collection hatched after total artificial incubation.

Developmental Stages of *Pygoscelis adeliae*

In the following account of the stages of *P. adeliae* during its development, the nearest equivalent stages in the chick (Hamburger and Hamilton, 1951), the ages of the embryos and the number of embryos measured for each stage are given in parentheses. Means and standard deviations of various embryonic components are included.

In order to facilitate examination of the text-figures, these have been given the same numbers as those used in the text for the embryonic stages of *P. adeliae*.

Stage 1 (H.1; 1–3 days; 6 embryos)

The blastoderm is undifferentiated. When alive it is recognized as a circular white area, approximately 2·5–3·0 mm. in diameter, but in fixed material the blastoderm is difficult to measure accurately owing to the vague outline of the peripheral region.

Stage 2 (H.2; 3 days; 4 embryos)

The small circular blastoderm is opaque. There is a clear central area pellucida 1·5±0·22 mm. long and 1·1±0·21 mm. wide.

Stage 3 (H.3; 4 days; 4 embryos)

The circular blastoderm includes a clear, central pear-shaped area pellucida 1·8 mm. long and 1·4±0·1 mm. wide. At the mid-posterior end there is an opaque condensation of cells (the primitive streak) 0·5±0·19 mm. long.

Stage 4 (H.3+; 4 days (approx.); 6 embryos)

The blastoderm is larger than in stage 3 but the area pellucida is still pear-shaped, measuring 2·1±0·2 mm. and 1·5±0·26 mm. in length and width, respectively. Cells of the primitive streak have accumulated on either side of the well-developed primitive groove (1·1±0·49 mm. long), which extends antero-posteriorly for up to two-thirds of the distance along the area pellucida.

Stage 5 (H.4; 4–4½ days; 4 embryos)

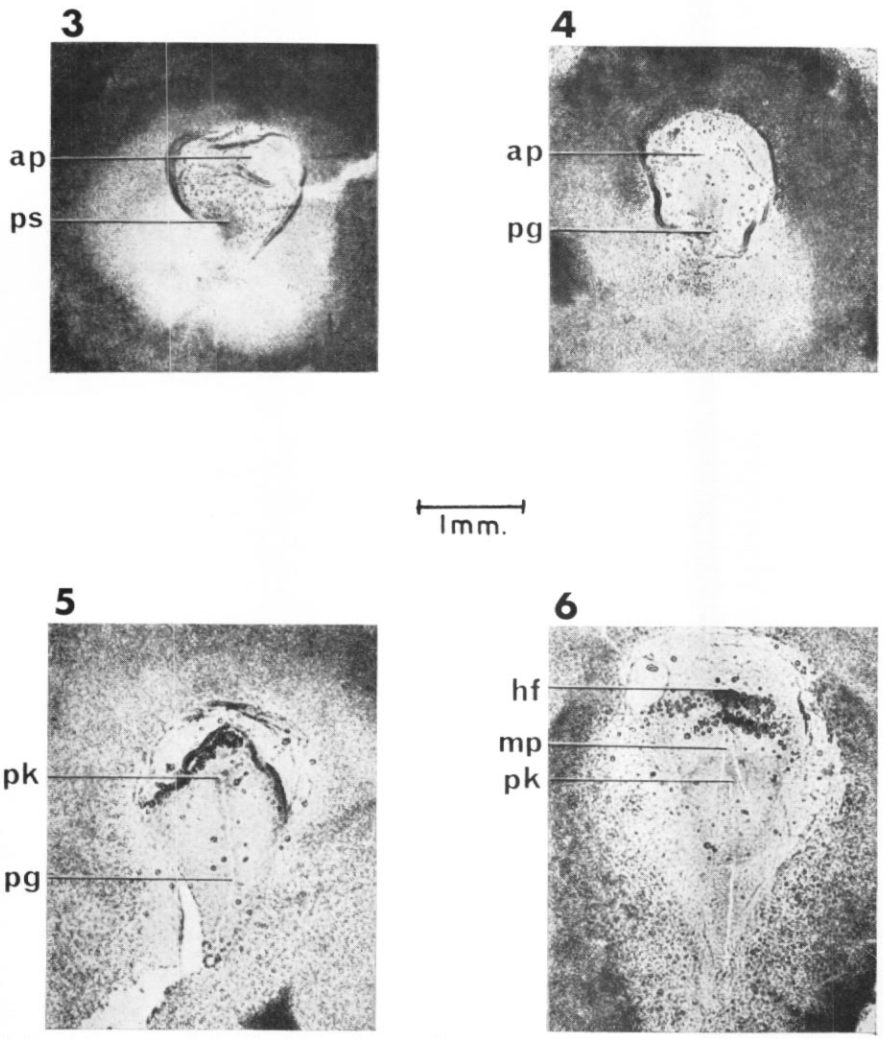
The primitive groove is extended along the entire length of the pear-shaped area pellucida, except for a small anterior raised node of cells around the primitive pit. Lengths of the area pellucida and primitive streak are 2·1±0·29 and 1·8±0·13 mm., respectively. The width of the area pellucida is 1·7±0·29 mm.

Stage 6 (H.6; 4½–5 days; 7 embryos)

The area pellucida is more elongated than in the previous stage, though still wider than in front. There is a slightly transparent anterior margin (the incipient head fold) and a grooved head process is recognizable. The primitive knot is now located further back. Lengths of the area pellucida, head process and primitive streak are 2·6±0·08, 0·9±0·19 and 1·6±0·13 mm., respectively.

Stage 7 (H.7; 5–5½ days; 7 embryos)

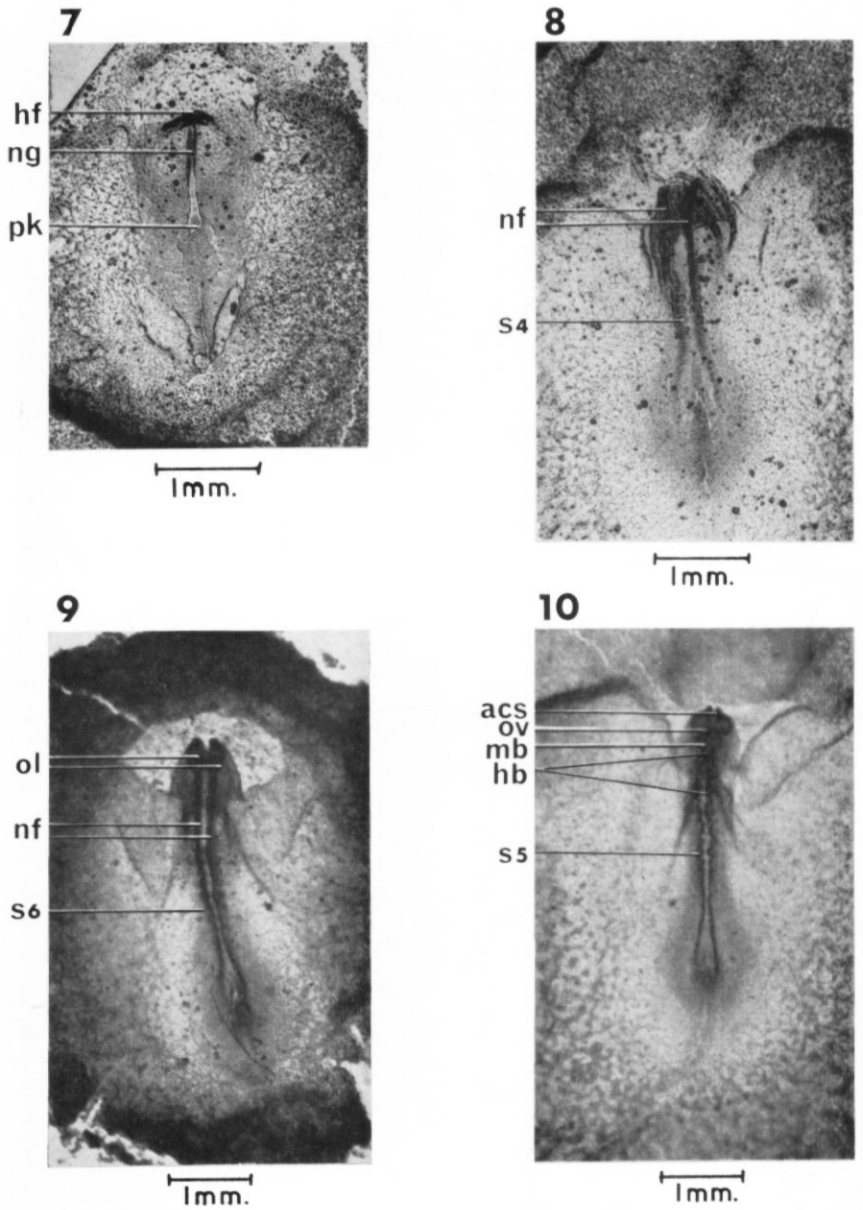
The head fold is more pronounced and the thickened medullary plate has a central neural groove. The primitive knot is situated more than one-third of the distance from the anterior margin of the area pellucida. The first two somites are recognizable as cellular condensations on either side of the primitive knot. Lengths of the primitive streak and head process are 1·2±0·27 and 1·5±0·17 mm., respectively.



Figs. 3–6. *P. adeliae* stages 3–6 stained in anthracene blue, cleared and mounted in balsam.
ap area pellucida; *hf* head fold; *mp* medullary plate; *pg* primitive groove; *pk* primitive knot; *ps* primitive streak.

Stage 8 (H.8; 5½–6 days; 8 embryos)

The prominent neural folds, which extend for two-thirds of the length of the embryo, are down-turned at the front, but they do not meet dorsally at any point. The well-developed head fold is 0·5±0·13 mm. long. The distance from the front of the embryo to the primitive knot is 2·6±0·39 mm. There are four or five somites.



Figs. 7–10. *P. adeliae* stages 7–10 stained in anthracene blue, cleared and mounted in balsam.
acs anterior cerebral suture; *hb* hind-brain; *hf* head fold; *mb* mid-brain; *nf* neural fold; *ng* neural groove; *ol* optic lobe; *ov* optic vesicle; *pk* primitive knot; *s*4, 5, 6 fourth, fifth and sixth somites.

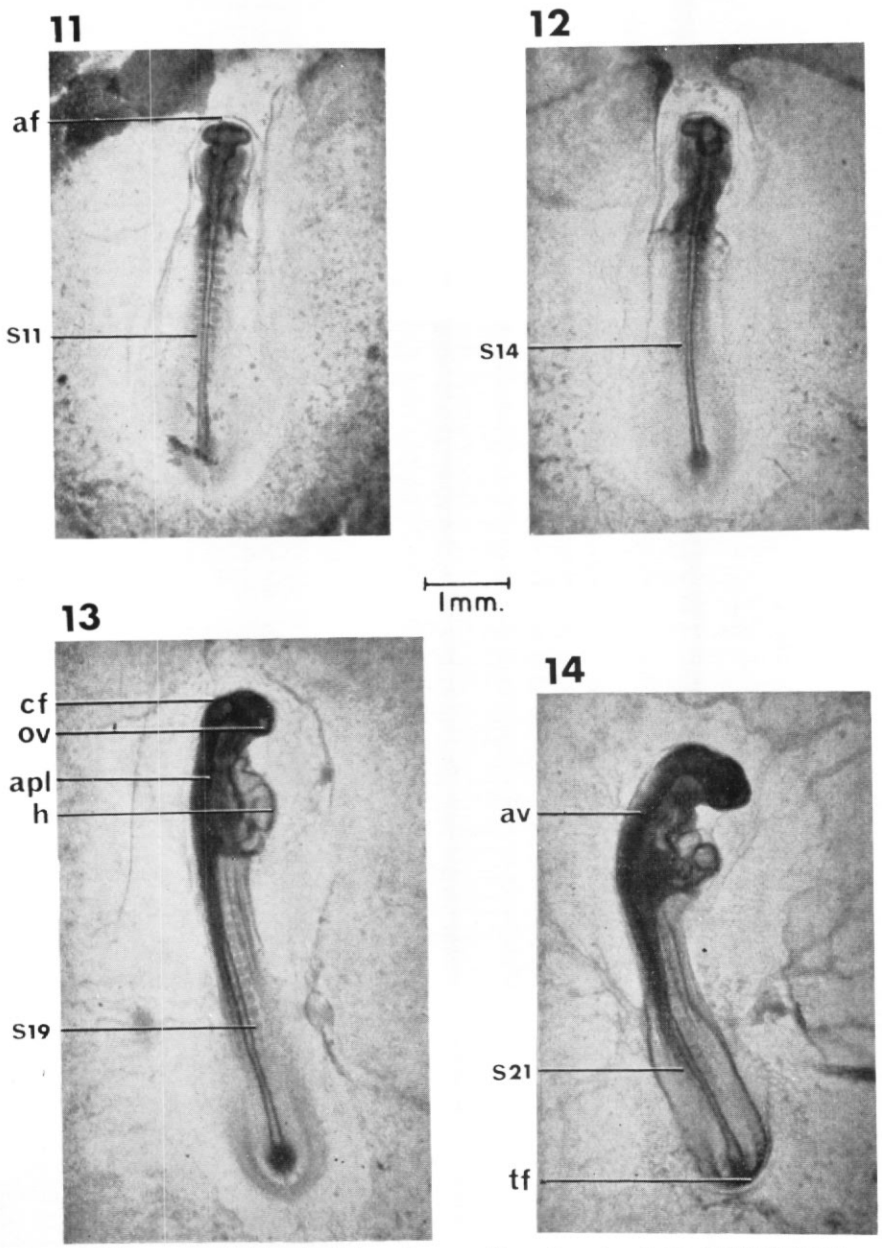
Stage 9 (H.9; 6 days; 2 embryos)
The neural folds still do not fuse at the front but they meet in the region of the hind-brain. Optic lobes are present. Measurements are about the same as in stage 8. There are seven somites.

Stage 10 (H.11; 6–6½ days; 6 embryos)
The three primary brain vesicles and the optic lobes are well developed. The neural folds

have fused in the region of the hind-brain but they have not yet done so in the fore-brain region. Length of the head fold is $0{\cdot}6 \pm 0{\cdot}17$ mm. Distance from the front of the embryo to the primitive knot is $3{\cdot}1 \pm 0{\cdot}19$ mm. There are nine somites.

Stage 11 (H.12; 6–7½ days; 12 embryos)

The optic lobes are vesicular. The neural tube has fused dorsally along its whole length. The



Figs. 11–14. *P. adeliae* stages 11–14 stained in anthracene blue, cleared and mounted in balsam.
af amniotic fold; *apl* auditory placode; *av* auditory vesicle; *cf* cranial flexure; *h* heart; *ov* optic vesicle; *s*11,14,19,21 11th, 14th, 19th and 21st somites; *tf* tail fold.

tissues in front of the head are more transparent than in the previous stage and a raised amniotic fold is recognizable. The mean lengths of the embryos and their head-folds are $4{\cdot}4 \pm 0{\cdot}34$ and $1{\cdot}1 \pm 0{\cdot}24$ mm., respectively. There are 10 to 12 somites.

Stage 12 (H.13; $6\frac{1}{2}$–$7\frac{1}{2}$ days; 7 embryos)

The first indications of cranial flexure are apparent. The fore-brain is enclosed by an amniotic fold. The neural tube terminates at the hind end of the embryo in a dense flat mass of cells. An incipient auditory placode is recognizable. Lengths of the embryo and amnion are $5{\cdot}0 \pm 0{\cdot}37$ and $0{\cdot}6 \pm 0{\cdot}22$ mm., respectively. There are 15 somites.

Stage 13 (H.13+; $6\frac{1}{2}$–$7\frac{1}{2}$ days; 12 embryos)

The head is inclined to the right with the right eye uppermost. The body is partially turned to the right. Cranial flexure is confined to the fore-brain. The elongated auditory placode is U-shaped. The amnion now extends in a posterior direction for one-third of the distance along the embryo. Lengths of the embryo and amnion are $5{\cdot}0 \pm 0{\cdot}45$ and $1{\cdot}4 \pm 0{\cdot}29$ mm., respectively. There are about 19 somites.

Stage 14 (H.14; $7\frac{1}{2}$–$8\frac{1}{2}$ days; 16 embryos)

The amnion has extended from one-half to two-thirds of the distance along the embryo. The entire front half of the embryo is rotated to the right and it lies on its left side. The angle of cranial flexure between the fore- and hind-brains is 90°. The auditory placode is circular. Lengths of the embryo and amnion are $5{\cdot}5 \pm 0{\cdot}66$ and $3{\cdot}5 \pm 0{\cdot}92$ mm., respectively. There are 23 to 24 somites.

Stage 15 (H.15; $8\frac{1}{2}$–10 days; 14 embryos)

The amnion has almost completely covered the embryo, except for an oval aperture (amniotic umbilicus) situated just dorso-anterior to or over the tail bud. The cranial flexure between fore- and hind-brains is now at an acute angle. The dorsal contour of the body is convex. The rotation of the body has extended to the junction of the omphalo-mesenteric arteries. Lengths of the body and amnion are $8{\cdot}7 \pm 0{\cdot}94$ and $6{\cdot}4 \pm 0{\cdot}43$ mm., respectively. 27 to 29 somites are present.

The somites become increasingly difficult to determine accurately partly as a result of the dispersal of the most anterior somites and, in later stages, to the curvature of the tail. The somite counts described hereafter are therefore not reliable enough for use as a stage characteristic.

Stage 16 (H.17; 9–10 days; 9 embryos)

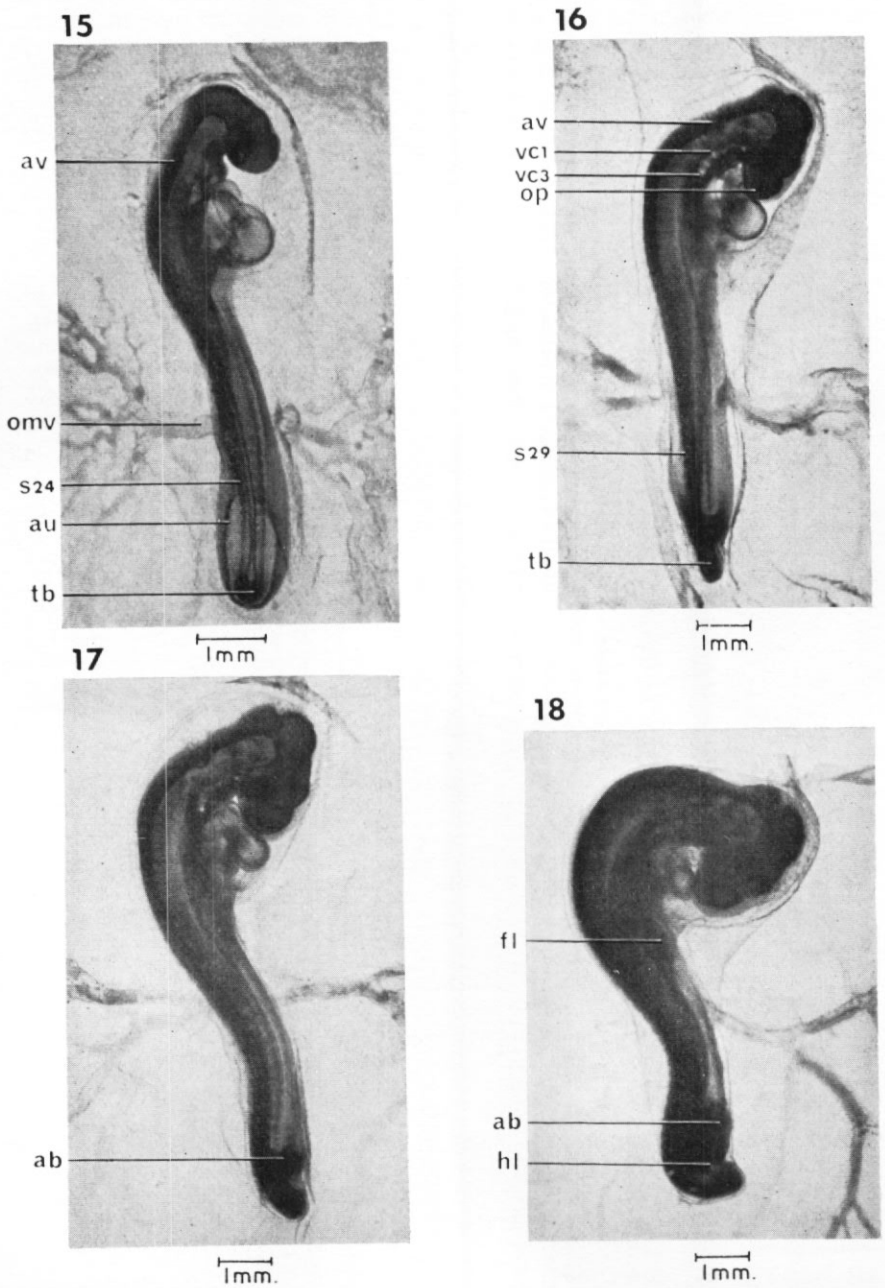
The amniotic umbilicus is vestigial. The cervical flexure is greater than 90°. In the posterior region there is a slight dorsal convexity and the tail bud is inclined underneath the hind end of the body. The lateral body folds have extended entirely round the body. When the overlying membranes are removed, three visceral arches and associated visceral clefts 1, 2 and 3 are recognized. The pear-shaped auditory vesicle still retains a single opening from the upper narrow region. Length of the body and width of the amniotic umbilicus are $9{\cdot}9 \pm 0{\cdot}5$ and $1{\cdot}1 \pm 0{\cdot}57$ mm., respectively. About 36 somites are recognizable.

Stage 17 (H.18; $9\frac{1}{2}$–$10\frac{1}{2}$ days; 12 embryos)

The embryo lies on its left side and its lumbar region has developed a slight dorsal concavity. The aperture of the auditory vesicle is closed to the exterior. Nasal pits and visceral clefts 1 and 2 are prominent. The primordia of the fore- and hind-limbs are condensations of tissue elongated antero-posteriorly on the lateral body folds. Length of the embryo is $10{\cdot}8 \pm 0{\cdot}61$ mm. and the width of the fore-limb is $1{\cdot}1 \pm 0{\cdot}01$ mm. (Fig. 19b). There are about 39 somites.

Stage 18 (H.19; 10–11 days; 13 embryos)

The pronounced curvature of the head and neck causes the fore-brain to reach the region of the fore-limb. The limb rudiments are easier to recognize as they are now raised up from the lateral body folds. A septum has developed in the upper narrow region of the auditory vesicle. Length and width of the fore-limb are $0{\cdot}2 \pm 0{\cdot}02$ and $1{\cdot}2 \pm 0{\cdot}07$ mm., respectively. There are about 41 somites.



Figs. 15–18. *P. adeliae* stages 15–18 stained in anthracene blue, cleared and mounted in balsam.
ab allantoic bud; *au* amniotic umbilicus; *av* auditory vesicle; *fl* fore-limb; *hl* hind-limb; *omv* omphalomesenteric blood vessels; *op* olfactory pit; *s*24,29 24th and 29th somites; *tb* tail bud; *vc*1,3 visceral clefts 1 and 3.

Stage 19 (H.20 and 21; 11–12 days; 9 embryos)

In appearance the embryo is similar to stage 18 but the body extends in a straight line between the fore- and hind-limbs. The first visceral arch is separated into an upper maxillary and a lower mandibulary process. The second visceral arch projects backwards as the collar. The third and fourth visceral arches are just recognizable. Lengths of the fore- and hind-limbs are $0{\cdot}6 \pm 0{\cdot}15$ and $0{\cdot}4 \pm 0{\cdot}17$ mm., respectively. The widths of both fore- and hind-limbs are approximately 1·2 mm., a measurement which is relatively constant until stage 23. 45 to 50 somites are recognizable.

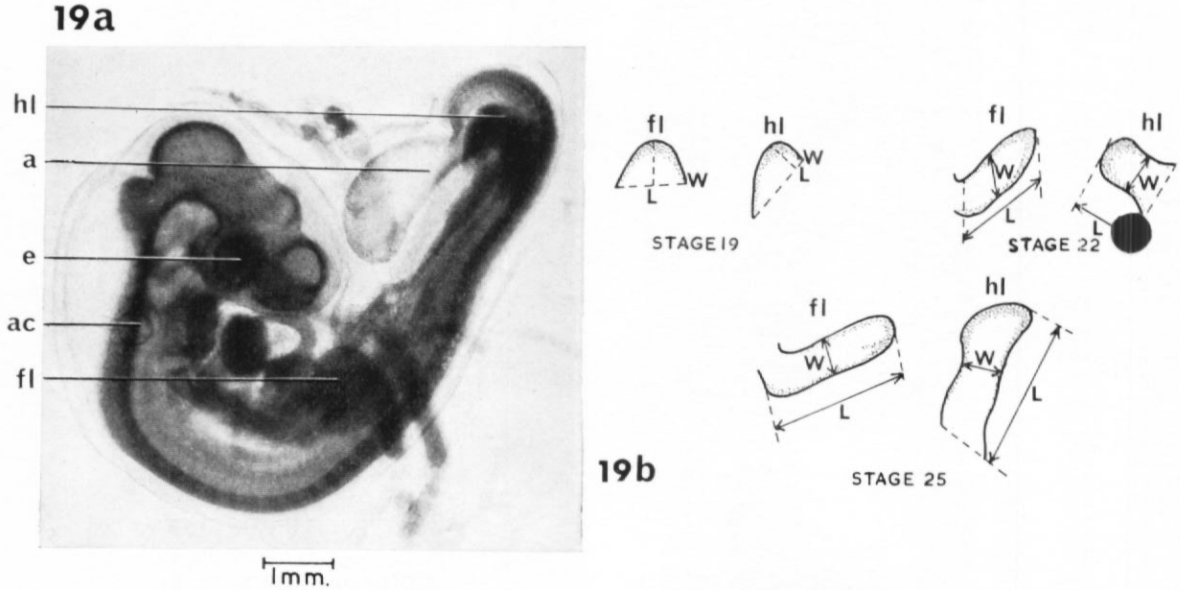


Fig. 19. a. *P. adeliae* stage 19 stained in anthracene blue, cleared and mounted in balsam. *a* allantois; *ac* auditory capsule; *e* eye; *fl* fore-limb; *hl* hind-limb.
b. Diagram of the limb measurements in early stages of embryonic development. *fl* fore-limb; *hl* hind-limb; *L* length; *W* width.

Stage 20 (H.22; 9 embryos)

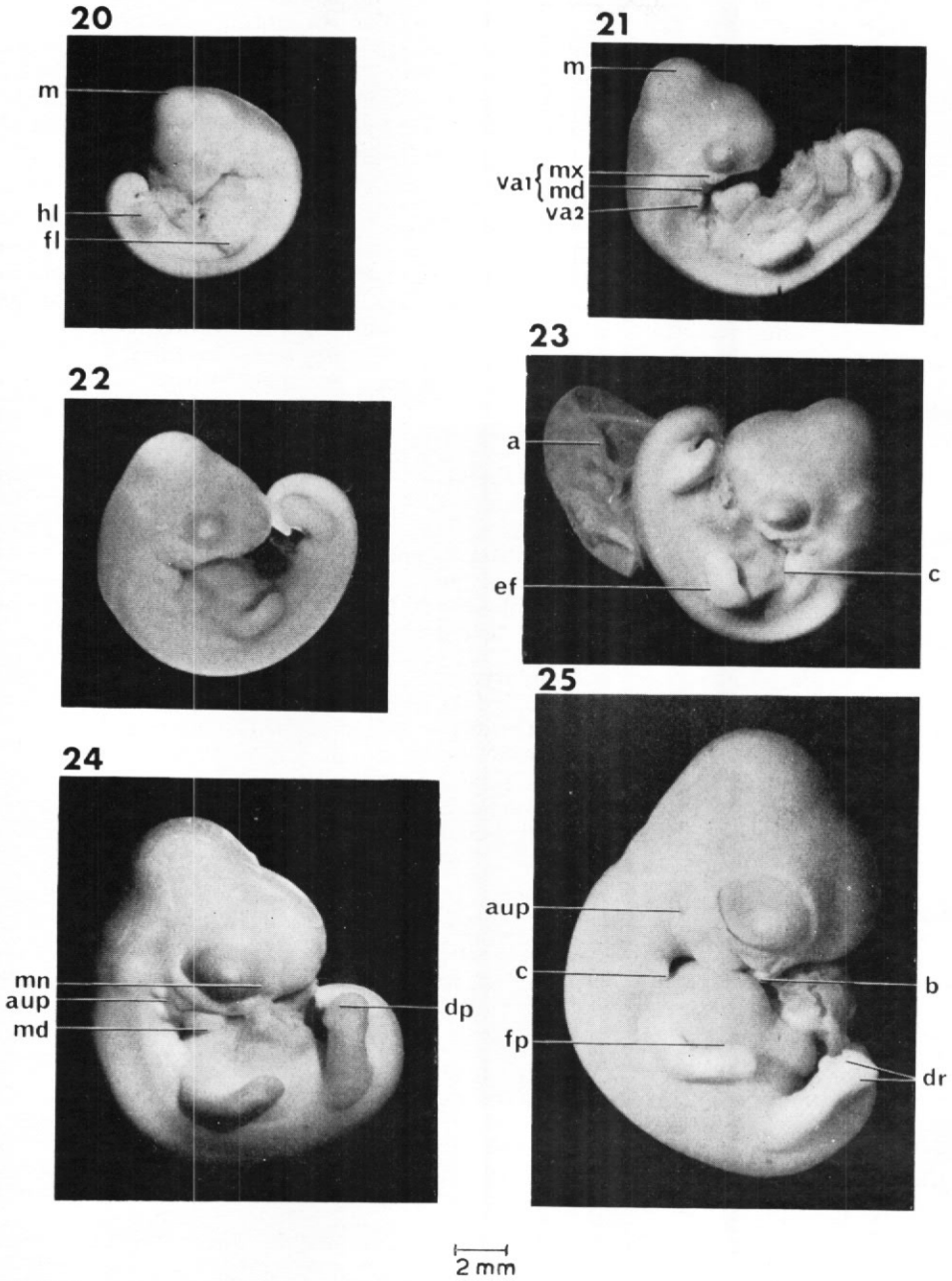
The trunk is now curved through 180° and because of this curvature the tail almost meets the fore-brain. The fore- and hind-limbs are more clearly demarcated from the body. The mesencephalon shows incipient enlargement. The lengths of the fore- and hind-limbs are $0{\cdot}9 \pm 0{\cdot}18$ and $0{\cdot}6 \pm 0{\cdot}08$ mm., respectively.

Stage 21 (H.23; 12–13 days; 21 embryos)

The dorsal contour, from mesencephalon to tail, is now a smooth curve. The maxillary part of the first visceral arch has now extended forwards below the eye. The latter is pigmented and larger than in the previous stages. The fore-limb is now inclined posteriorly. The lengths of the fore- and hind-limbs are $1{\cdot}4 \pm 0{\cdot}16$ and $1{\cdot}1 \pm 0{\cdot}15$ mm., respectively.

Stage 22 (H.24; 12–14 days; 16 embryos)

The mandibular arch has paired protuberances on its posterior margin, as has the second visceral arch on its anterior surface. The maxillary part of the first visceral arch has extended forwards below and in front of the central region of the eye. The fore-limb is flattened; the hind-limb, situated alongside of the tail, is wider distally. Lengths of the fore- and hind-limbs are $1{\cdot}9 \pm 0{\cdot}25$ and $1{\cdot}8 \pm 0{\cdot}28$ mm., respectively.



Figs. 20–25 *P. adeliae* stages 20–25; opaque fixed embryos.
a allantois; *aup* auditory pit; *b* beak; *c* collar; *dp* digital plate; *dr* digital ridge; *ef* elbow flexion; *fl* fore-limb; *fp* fore-limb protuberance; *hl* hind-limb; *m* mesencephalon; *md* mandibulary part of first visceral arch; *mx* maxillary part of first visceral arch; *nm* nictitating membrane; *va*1,2 visceral arches 1 and 2.

Stage 23 (H.26; 13–15 days; 19 embryos)

A superficial auditory pit can be recognized between the mandibular process and the second visceral arch. The collar is well formed and the maxillary primordia have extended further forwards below the eyes. The fore-limb shows the first signs of elbow flexion. A raised ridge (incipient eyelids) encircles the eye. The mesencephalon is much enlarged. Lengths of the fore- and hind-limbs are $2{\cdot}2 \pm 0{\cdot}24$ and $2{\cdot}2 \pm 0{\cdot}17$ mm., respectively. Width of the fore-limb is still about 1·2 mm. but that of the hind-limb is about 1·2 mm. in the proximal part and about 1·4 mm. across the digital plate.

Stage 24 (H.27 and 28; 15–16 days; 16 embryos)

The auditory pit, deeper than in the previous stage, is situated between the two posterior protuberances of the mandibular arch. The neck has commenced to elongate. The maxillary part of the first arch has reached the lachrymal region of the eye. The olecranon joint of the flattened fore-limb is bent at an angle of 120–130° and is slightly grooved. The hind-limb is spatulate distally and there are signs of digital condensations. A nictitating membrane is just visible at the anterior margin of the eye. Lengths of the fore- and hind-limbs are $2{\cdot}2 \pm 0{\cdot}18$ and $3{\cdot}1 \pm 0{\cdot}29$ mm., respectively. The widths of the fore- and hind-limbs are about the same (1·2–1·3 mm.), except for the foot which is now 1·8 mm. wide.

Stage 25 (H.29; 15–16 days; 9 embryos)

The deep auditory pit is situated independently of the mandibular process. The fore-limb has a protuberance on its leading edge, and a delicate ridge extends for one-third of the distance from the tip. The hind-limb is longer than in the previous stage; distally there are three clearly defined digital ridges. The nictitating membrane is further developed. The beak is knob-like. Primordia of the rectrices are recognizable on either side of the tail. Lengths of the fore- and hind-limbs are $2{\cdot}8 \pm 0{\cdot}18$ and $3{\cdot}9 \pm 0{\cdot}25$ mm., respectively. The widths of the fore-limb, hind-limb and digital plate are 1·4, 1·4 and 2·1 mm., respectively.

Stage 26 (H.30 and 31; 16–17 days; 10 embryos)

The raised primordia of the eyelids are further developed, and two pairs of posterior and anterior scleral papillae encircle the iris. The elbow flexion of the fore-limb is almost 90° and its protuberance is very pronounced. Distally, the hind-limb has an angular outline with the first digit represented as a small plate of tissue. The neck is considerably lengthened. The collar is no longer clearly defined, and the mandible terminates just behind the tip of the beak. The primordia of the rectrices are elongated ridges. Lengths of the fore- and hind-limbs are $4{\cdot}6 \pm 0{\cdot}39$ and $5{\cdot}6 \pm 0{\cdot}32$ mm., respectively.

Stage 27 (H.32 and 33; 17–18 days; 5 embryos)

Nine to 12 scleral papillae encircle the iris. The length of the mandible equals that of the beak. The fore-limb has two well-defined ridges (incipient radius and ulna). The foot comprises a separate first digit and three other toes each joined by a concave webbing. Lengths of the fore- and hind-limbs are $5{\cdot}3 \pm 0{\cdot}15$ and $6{\cdot}2 \pm 0{\cdot}24$ mm., respectively.

Stage 28 (H.34; 18–20 days; 6 embryos)

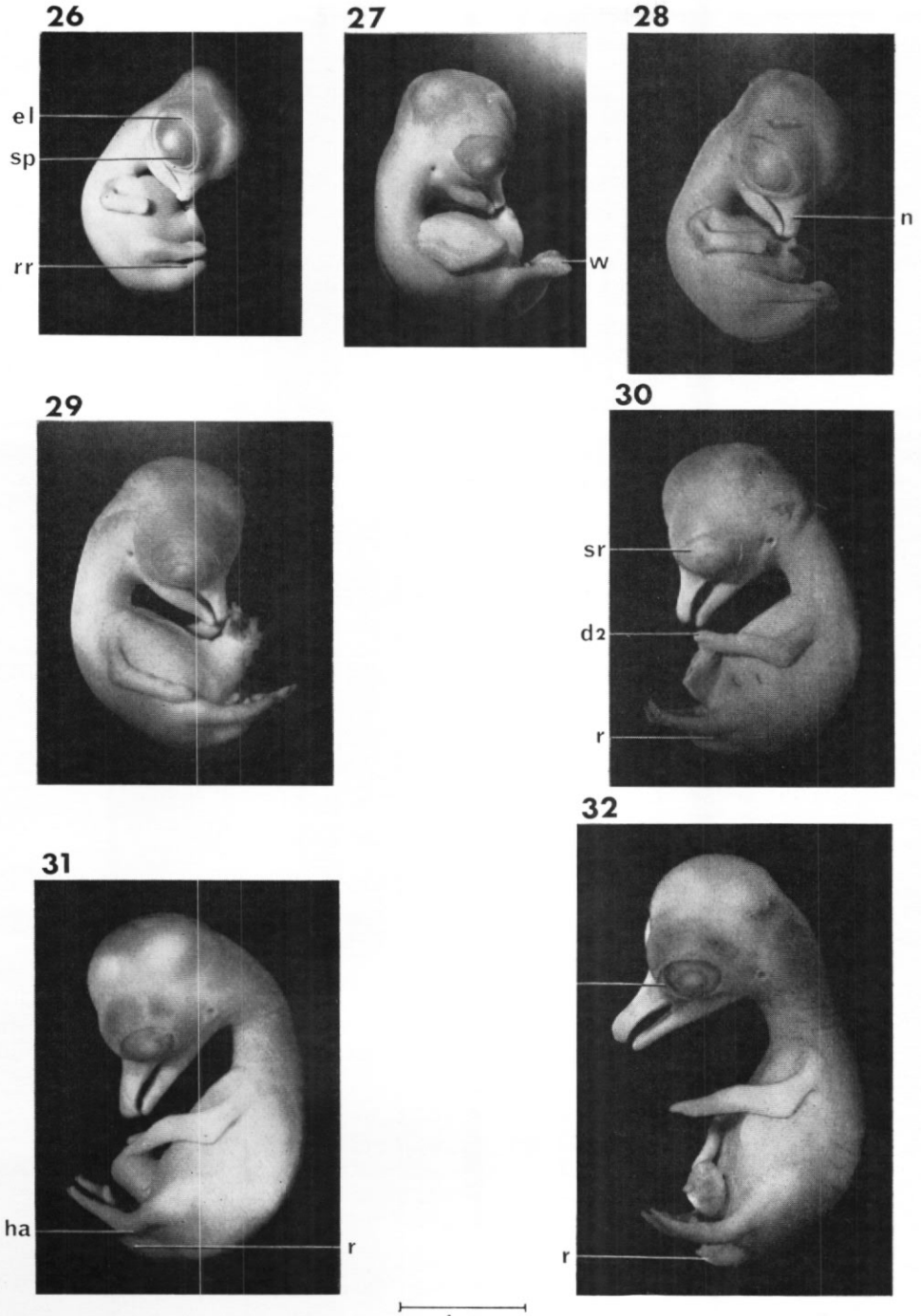
The fore- and hind-limbs are larger than in the previous stage. The protuberance on the leading edge of the fore-limb is elongated. The nictitating membrane extends from the eye-rim for less than one-half of the distance to the scleral papillae. Nostrils are now small raised areas situated about half-way along the beak. Incipient rectrices each comprise separate papillae. Lengths of the fore- and hind-limbs are $5{\cdot}6 \pm 0{\cdot}15$ and $6{\cdot}7 \pm 0{\cdot}23$ mm., respectively.

Stage 29 (H.34; 20–21 days; 3 embryos)

The fore-limb is more pointed distally than in the previous stage. The hind-limb is orientated at right-angles to the tail. Digits are more pronounced. A uropygial gland is just visible on the dorsal region of the tail. Lengths of the fore- and hind-limbs are 6·6 and 7·1 mm., respectively.

Stage 30 (H.35; 21 days (approx.); 2 embryos)

The pointed fore-limb is slightly curved. An incipient carpo-metacarpal articulation is recognized half-way along its length. The webbing of the hind-limbs is at an acute angle between the toes and there is a slight development of pads on the soles of the feet. The



Figs. 26–32. *P. adeliae* stages 26–32; opaque fixed embryos.
d2 second digit; *el* eyelids; *ha* heel articulation; *n* nostril; *nm* nictitating membrane; *r* rectrices; *rr* rectrical ridge; *sp* scleral papillae; *sr* scleral ring; *w* web between digits.

nictitating membrane reaches half-way to the scleral ring (fused scleral papillae). There are six or seven small individual conical rectrices on either side of the tail. The uropygial gland is now a heart-shaped depression. A few white spots (incipient feather germs) are recognizable on the head above the eyes. Lengths of the fore- and hind-limbs are 8·4 and 8·1 mm., respectively.

Stage 31 (H.36; 21–22 days; 8 embryos)
The fore-limb is more wedge-shaped and its protuberance has almost disappeared. The webbing on the foot joins the second, third and fourth toes; the first toe is completely separate from the others. The hind-limb is bent at the "heel". The beak has a keratinized tip. The rectrices are elongated cones. The nictitating membrane has now reached the scleral ring and the eyelids are ellipsoidal. Lengths of the fore- and hind-limbs are $9 \cdot 5 \pm 0 \cdot 54$ and $9 \cdot 9 \pm 0 \cdot 55$ mm., respectively.

Stage 32 (21–22 days; 9 embryos)
The embryo is larger than in the previous stage; likewise the fore-limb is more flipper-like and the beak more keratinized. The rectrices are long tapered filaments. Other lines of feather germs (pterylae) are situated above them on the tail. Additional pterylae cause the lumbar region to have a stippled appearance. The nictitating membrane covers the anterior part of the scleral ring. The upper eyelids are more highly developed than the lower ones. Lengths of the fore- and hind-limbs are $10 \cdot 4 \pm 0 \cdot 21$ and $11 \cdot 6 \pm 0 \cdot 49$ mm., respectively. The length of the beak, from the posterior border of the nostril to the tip, is $3 \cdot 2 \pm 0 \cdot 19$ mm.

Stage 33 (22–23 days; 10 embryos)
A faint line of feather germs is present along the trailing edge of the flipper. Pterylae cover the whole body, except for the edge of the eyelids, upper and lower jaws and the feet. The eyelids are separated by a biconvex aperture. The incipient claws of the toes are not yet keratinized. Lengths of the fore-limbs, hind-limbs and beak are $11 \cdot 2 \pm 0 \cdot 44$, $12 \cdot 2 \pm 0 \cdot 64$ and $3 \cdot 4 \pm 0 \cdot 12$ mm., respectively.

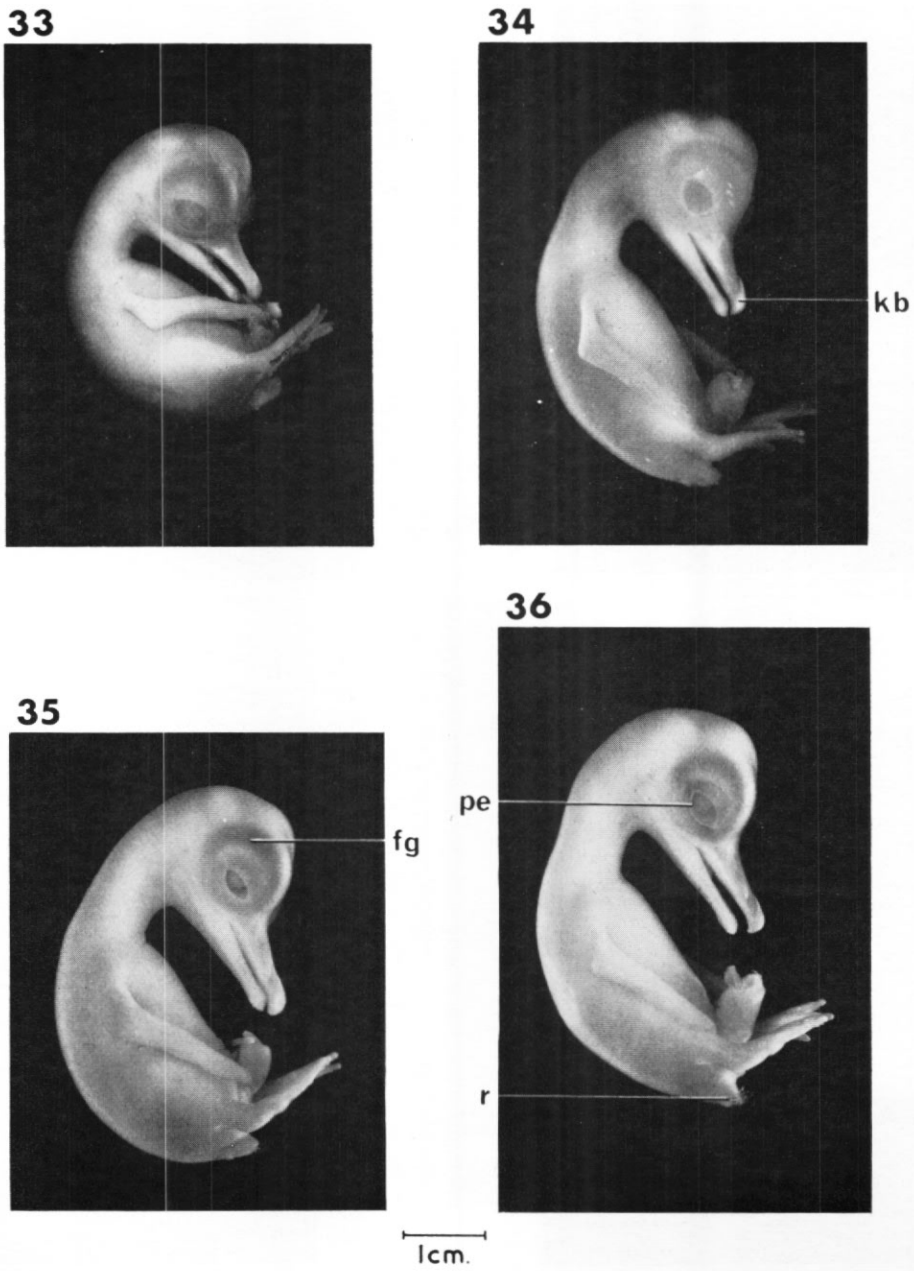
Stage 34 (23 days (approx.); 13 embryos)
The lower jaw is keratinized at the tip. The long smooth flipper is wedge-shaped. The lengths of the fore-limb, hind-limb and beak are $12 \cdot 1 \pm 0 \cdot 52$, $13 \cdot 4 \pm 0 \cdot 41$ and $3 \cdot 7 \pm 0 \cdot 30$ mm., respectively.

Stage 35 (24 days (approx.); 6 embryos)
Raised feather germs cover the whole body. The upper eyelid extends over almost half the pupil area. The iris is slightly pigmented. Lengths of the fore-limb, hind-limb and beak are $13 \cdot 0 \pm 0 \cdot 24$, $14 \cdot 8 \pm 0 \cdot 36$ and $3 \cdot 9 \pm 0 \cdot 36$ mm., respectively.

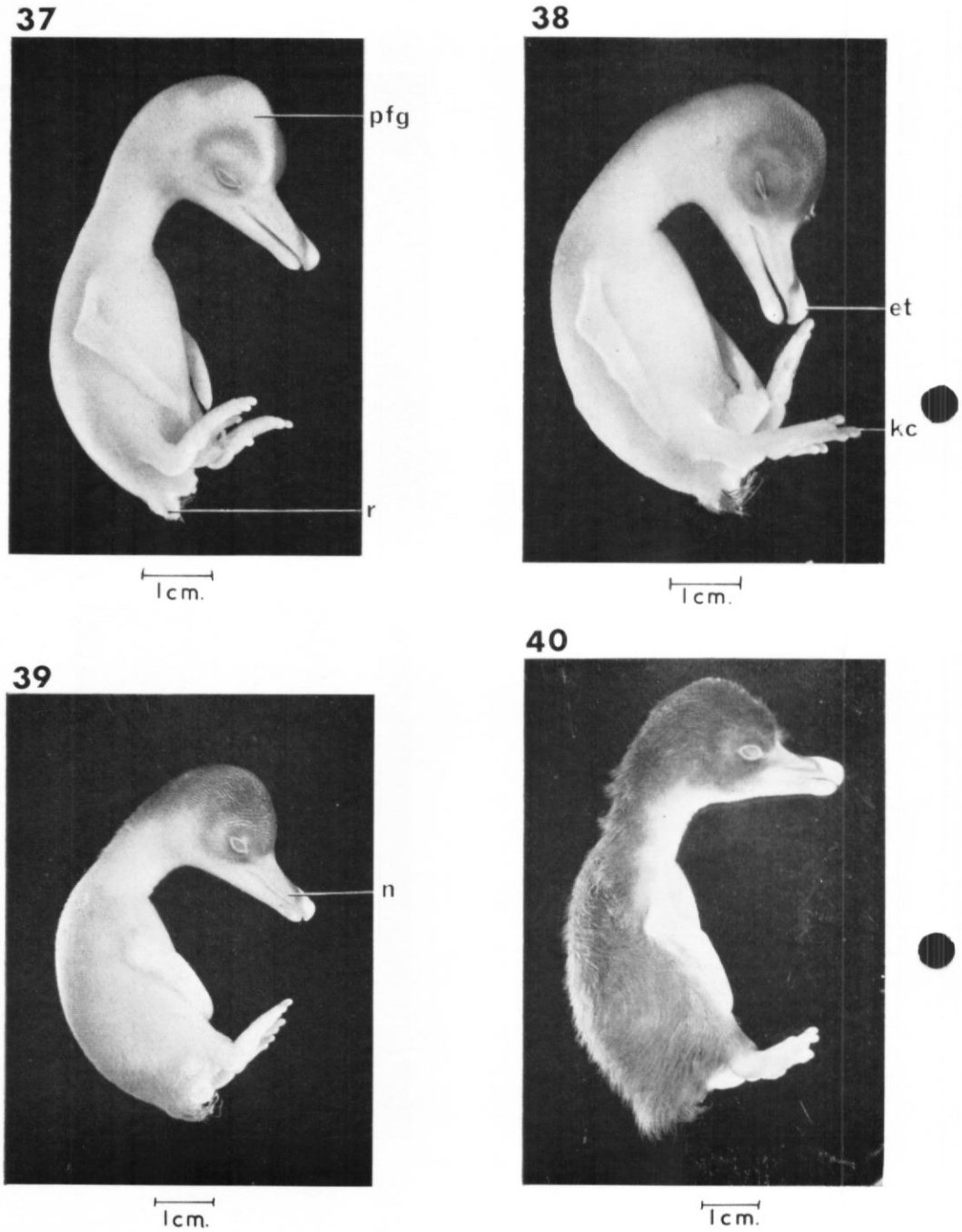
Stage 36 (24–25 days; 3 embryos)
Feather germs cover the beak. The eyelids, iris, rectrices and the tip of the beak all have faint black pigmentation. Lengths of the fore-limb, beak and third toe (measured ventrally from the tip of the claw to the front of the large tarsular pad) are 13·8, 4·3 and 6·5 mm., respectively.

Stage 37 (26 days (approx.); 6 embryos)
The head feather germs comprise slightly pigmented raised cones. The feet have scale germs on their upper surfaces. Claws are slightly keratinized. Rectrices are longer and curlier than in the previous stage. The uropygial gland is visible as an external pore. Lengths of the fore-limb, beak and third toe are $14 \cdot 7 \pm 0 \cdot 54$, $4 \cdot 9 \pm 0 \cdot 23$ and $7 \cdot 0 \pm 0 \cdot 34$ mm., respectively.

Stage 38 (26–27 days; 3 embryos)
All the feather germs are elongated, tapered pigmented cones, which cover the body with a soft "stubble"; the tail feathers are almost "down-like". A dull area (the incipient egg tooth) is recognized at the tip of a well-keratinized beak. A line of demarcation, between the scales and feathers is recognized on the heel, and the claws are pigmented. Lengths of the fore-limb, beak and third toe are 16, 5·4 and 7·8 mm., respectively.



Figs. 33–36. *P. adeliae* stages 33–36; opaque fixed embryos.
fg feather germs; *kb* keratinized beak; *pe* pigmented eyelids; *r* rectrices.



Figs. 37–40. *P. adeliae* stages 37–40; opaque fixed embryos.
et egg tooth; *kc* keratinized claws; *n* nostril; *pfg* pigmented feather germs; *r* rectrices.

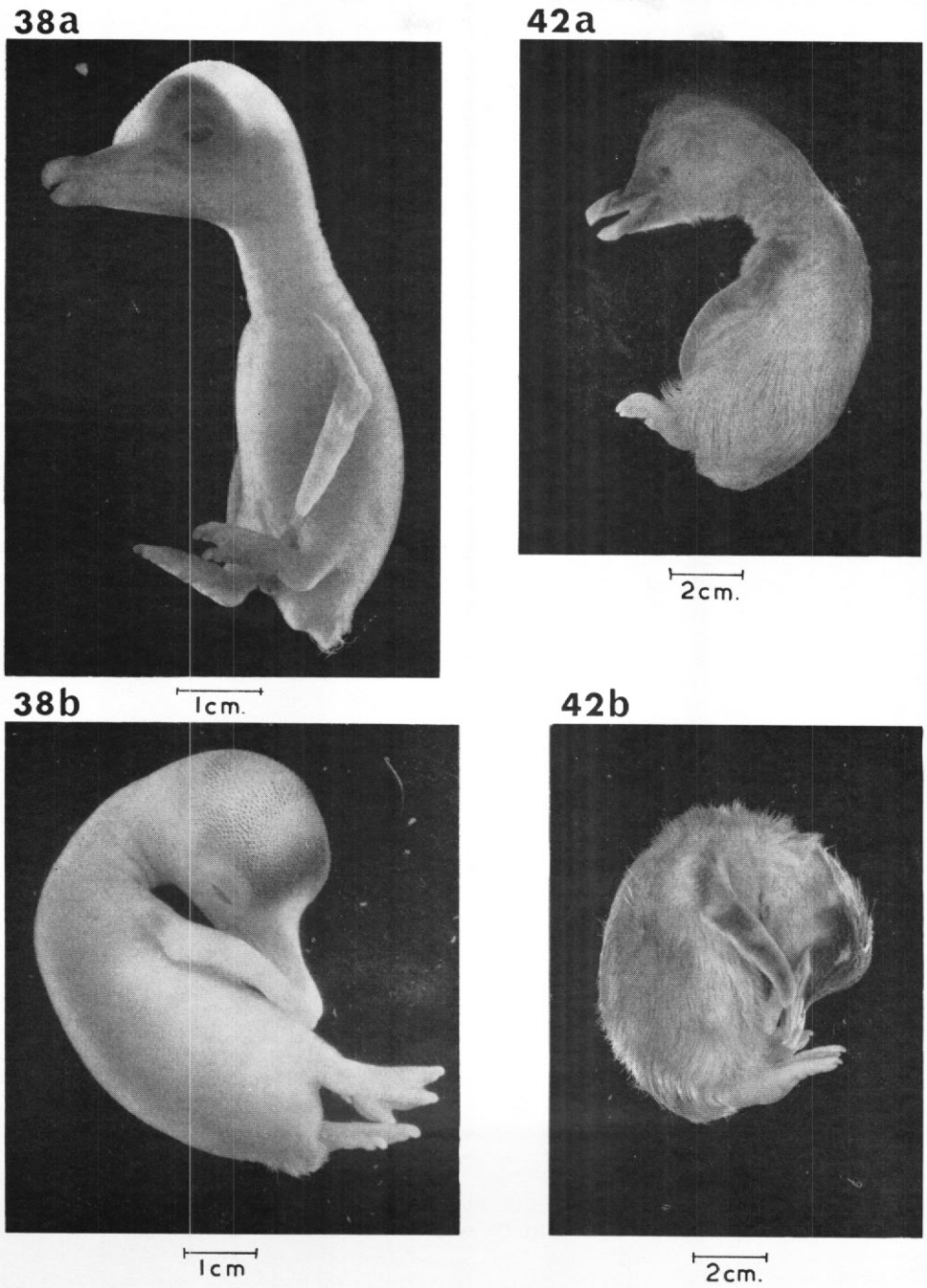


Fig. 38. a. *P. antarctica* stage 38; opaque fixed embryo.
b. *P. papua* stage 38; opaque fixed embryo.
Fig. 42. a. *P. antarctica* stage 42; opaque fixed embryo.
b. *P. papua* stage 42; opaque fixed embryo.

Stage 39 (27–28 days; 12 embryos)
The whole body is covered with soft down. The nostrils are circular white pits. The lengths of the fore-limb, beak and third toe are $17{\cdot}3 \pm 0{\cdot}88$, $5{\cdot}7 \pm 0{\cdot}22$ and $8{\cdot}7 \pm 0{\cdot}41$ mm., respectively.

Stage 40 (28–29 days; 11 embryos)
The head and hind edge of the flipper are covered with long black down feathers, which are still in the feather sheaths and hence their long tapered appearance. The rest of the flipper is covered with small triangular feathers. The soles of the feet are highly scaled. The lengths of the fore-limb, beak and third toe are $18{\cdot}6 \pm 0{\cdot}98$, $6{\cdot}2 \pm 0{\cdot}33$ and $9{\cdot}5 \pm 0{\cdot}69$ mm., respectively.

Stage 41 (29–31 days; 15 embryos)
All the claws and body feathers are pigmented, except for those feathers on the leading edge of the flippers. The lengths of the fore-limb, beak and third toe are $20{\cdot}0 \pm 0{\cdot}78$, $7{\cdot}0 \pm 0{\cdot}42$ and $10{\cdot}8 \pm 0{\cdot}8$ mm., respectively.

Stage 42 (31–33 days; 15 embryos)
The body feathers are now almost 1 cm. long. Only those on the leading edge of the outermost part of the flipper are still free from pigment. The egg tooth is more conical. The lengths of the fore-limb, beak and third toe are $21{\cdot}5 \pm 0{\cdot}77$, $7{\cdot}7 \pm 0{\cdot}29$ and $11{\cdot}5 \pm 0{\cdot}53$ mm., respectively.

Stage 43 (32–34 days; 7 embryos)
The fore-limbs are completely pigmented. The feet are thickly padded and grey in colour. The yolk sac is small and bilobed. The lengths of the fore-limb, beak and third toe are $22{\cdot}4 \pm 0{\cdot}82$, $8{\cdot}5 \pm 0{\cdot}42$ and $12{\cdot}6 \pm 0{\cdot}43$ mm., respectively.

Stage 44 (33–36 days; 5 embryos)
The yolk sac is practically withdrawn. The nostrils are almost perforate. The allantois and amnion are of a "sticky" consistency. The inner membrane of the air space may be punctured. The lengths of the fore-limb, beak and third toe are $23{\cdot}8 \pm 1{\cdot}46$, $8{\cdot}7 \pm 0{\cdot}51$ and $14{\cdot}3 \pm 0{\cdot}36$ mm., respectively.

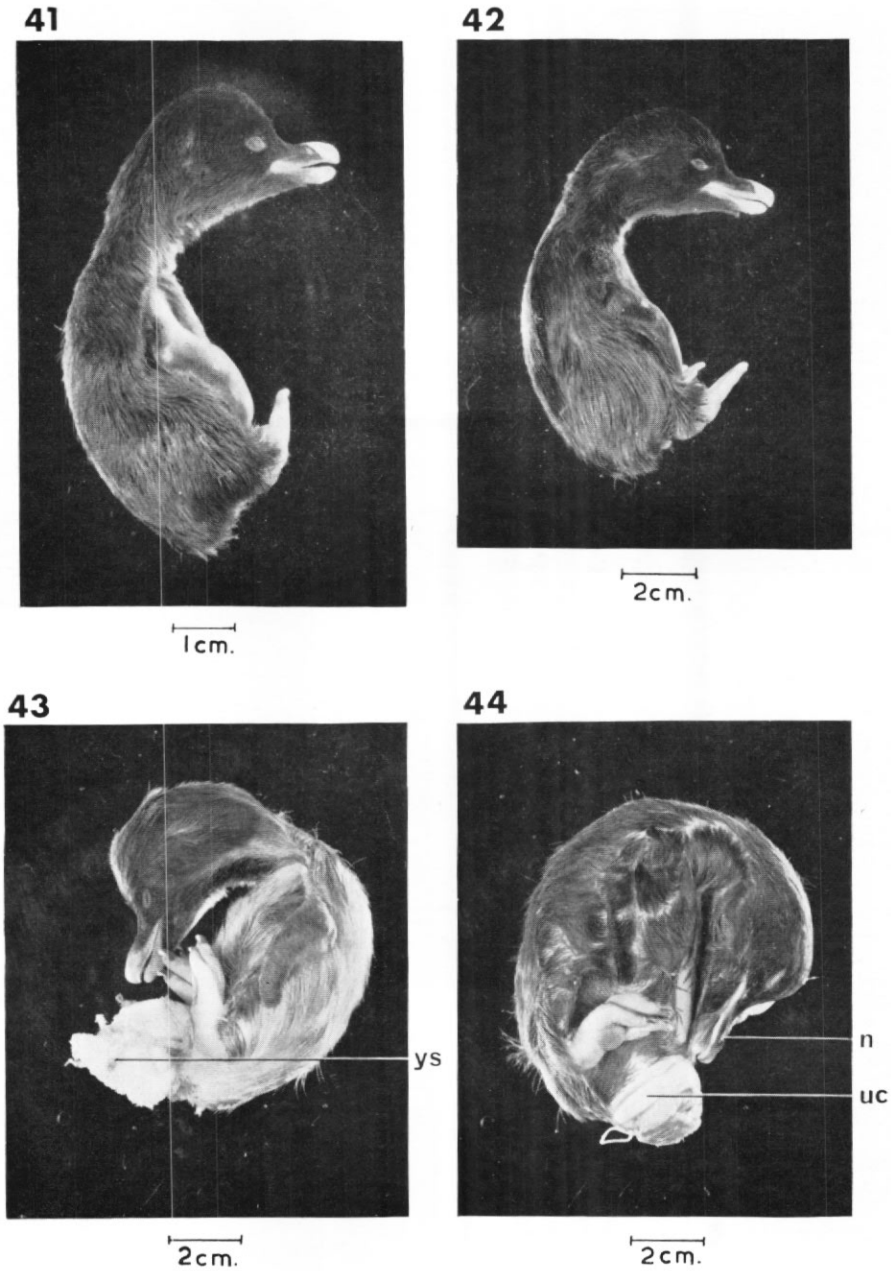
Stage 45 (Hatching)
The yolk sac is now completely absorbed and the ventral abdominal aperture sealed, although a few dried blood vessels may be visible externally. At this stage the young penguin begins to chip a hole in the shell and often emits high-pitched peeping sounds. Hatching takes from 15 to 36 hr.

Comparison of Embryos of the Three Species of Pygoscelid Penguin

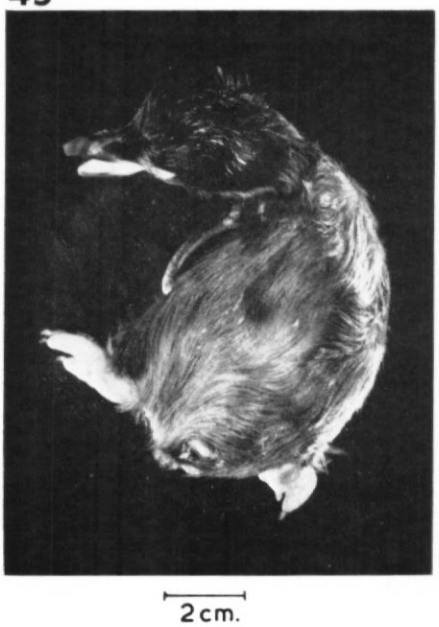
Embryos of *P. adeliae* and *P. antarctica* are approximately the same size at all stages; *P. papua*, however, is somewhat larger during the last six stages (stages 40–45). *P. adeliae* hatches after an incubation period of from 34 to 36 days, which confirms the results of Reid (1965). *P. antarctica* and *P. papua* hatch when a little older, after 36 days incubation, which agrees with the information given by Bagshawe (1938).

Differences in the development of the three species appear from stage 36. Pigmentation, which is recognizable in the beak, eyelids and rectrices of *P. adeliae* at this stage, is not yet found in *P. antarctica* and *P. papua*. In *P. adeliae* and *P. papua*, at stage 38, the head is covered with pigmented pterylae; at a similar stage the feather germs of *P. antarctica* are not pigmented.

At stage 42, when the embryo is completely covered with down feathers, *P. adeliae* is either very dark grey or black and *P. antarctica* is coloured a light silvery grey. *P. papua*, however, has a black head merging to a dark grey-coloured back and flippers; the front is white and there is a small white flash through the eye. At this stage the feet and beak of *P. adeliae* and *P. antarctica* are grey, while those of *P. papua* are a flesh pink colour.



Figs. 41–44. *P. adeliae* stages 41–44; opaque fixed embryos.
n nostril; *uc* umbilicus closing; *ys* yolk sac.



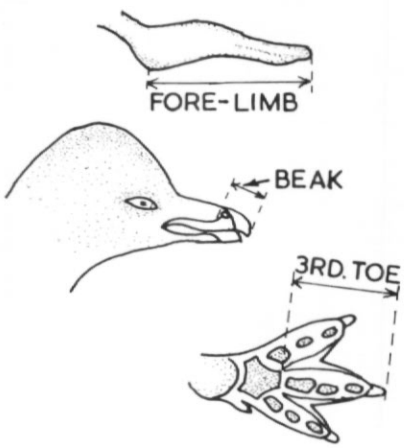


Fig. 45. *P. adeliae* stage 45; opaque fixed embryo.
45. a. *P. antarctica* stage 45; opaque fixed embryo.
45. b. *P. papua* stage 45; opaque fixed embryo.
Fig. 46. Diagrams of the fore-limb, beak and third toe measurements in later stages.

At hatching, *P. papua* is slightly larger than the other two species, and when its down feathers are fluffed out it has a black back and a white front. The feathers are a uniform black in *P. adeliae* and silvery grey in *P. antarctica*. Also, in *P. papua* the underside of the feet and the premaxilla of the beak have a slight amount of dark grey pigmentation.

Comparison of the Development of *Pygoscelis adeliae* and *Gallus domesticus* (Table I)

The incubation period (laying to hatching) of *P. adeliae* is from 34 to 36 days compared with 20 to 21 days of the chick. Thus at equivalent stages of development a 5½ to 6 day embryo penguin (stage 9) is equivalent to a 26 to 29 hr. embryo chick (stage 8; Hamburger and Hamilton, 1951). When 15 to 16 days old (stages 24–25), *P. adeliae* closely resembles a chick 6

Table I. Comparison of the development of *Pygoscelis adeliae* and *Gallus domesticus*

Pygoscelis adeliae (Adélie penguin)		*Gallus domesticus* (domestic chick)	
Stage	*Age* (days)	*Stage* (Hamburger and Hamilton, 1951)	*Age*
1	1–3	1	
2	3	2	6–7 hr.
3	4	3	12–13
4	4	3+	13–18
5	4–4½	4	18–19
6	4½–5	5–6	23–25
7	5–5½	7	23–26
8	5½–6	8	26–29
9	6	9	29–33
10	6–6½	11	40–45
11	6–7½	12	45–49
12	6½–7½	13	48–52
13	6½–7½	13+	
14	7½–8½	14	50–53
15	8½–10	15	ca. 50–55
16	9–10	17	ca. 52–64
17	9½–10½	18	3 days
18	10–11	19	3–3½
19	11–12	20–21	3–3½
20	11–12	22	3½–4
21	12–13	23	4
22	12–14	24	4½
23	13–15	26	5
24	15–16	27–28	5–6
25	15–16	29	6–6½
26	16–17	30–31	6½–7
27	17–18	32–33	7½–8
28	18–20	34	8
29	20–21	34	8
30	21	35	8½–9
31	21–22	36	10
32	21–22		
33	22–23	Thereafter, because of the specific anatomical specializations in the penguin, comparisons with the chick are not strictly valid.	
34	23		
35	24		
36	24–25		
37	26		
38	26–27		
39	27–28		
40	28–29		
41	29–31		
42	31–33		
43	32–34		
44	33–36		
45	Hatching		

to 6½ days old (H.27–29). The oldest comparable stage of the penguin is at stage 31 (21 to 22 days old), when it is equivalent to a chick of stage H.36 (10 days old). Up to stage H.36, embryos of both genera are similar in form and only differ in rate of development. Thereafter there are pronounced differences in the general body size and shape, limbs and feathers.

Differences in the rate of development are especially noticeable in the differentiation of the limbs. At stage 24 (15 to 16 days) the fore-limb of the penguin has no recognizable first digit, whereas the fore-limb of the equivalent stage of chick (H.27–28; 5 to 6 days old) includes a pronounced pollex. In later stages of the penguin (up to stage 29), a first digit is formed though it does not form a separate structure, the bastard wing, as in the case of the chick. Furthermore, the penguin fore-limb elongates considerably but its distal end remains blunter than in the case of the chick, until stage 29; thereafter the second digit elongates, giving rise to a pointed limb. From stage 31 onwards the fore-limb of the penguin has no protruding first digit and it develops as a long smooth wedge-shaped flipper with merely a suggestion of an articulation at the wrist.

The hind-limb of the penguin also shows considerable differences in shape compared with that of the chick. The tarso-metatarsal region of the Adélie penguin is much shorter and is aligned with the toes, giving rise to a plantigrade foot. Flexure occurs only at the heel and knee joints, the former being more noticeable at a somewhat later stage than in the chick. Webbing occurs in a similar fashion in both penguins (stages 26–27; 16 to 18 days old) and the chick (stage H.29; 6 to 6½ days old) between the second, third and fourth toes, but it is retained only in the former genus.

Again, in the penguin the scales of the foot originate considerably later in development and are not represented in the stage equivalent to that of the chick. Only a few overlapping scales are found near the bases of the claws; other penguin scales are the polygonal papillae, which are extremely similar to those of the ventral surface of the chick foot. In contrast to that of the chick, the first digit of *P. adeliae* is always vestigeal.

Dorsal body pterylae are already recognizable in the chick at stages H.30–31, equivalent to stage 26 in the penguin. Rectrices appear at stages H.31–32. In the penguin, however, these are apparent relatively earlier (stage 25). Body pterylae of the penguin, which are more numerous than in the chick, are somewhat retarded and they do not appear until stage 31 (equivalent to stage H.36 in the chick). Flight feathers of the chick appear at stage H.34 (equivalent to stage 29 of *P. adeliae*), but in the penguin they do not originate until stage 33 (equivalent to a chick embryo in advance of stage H.36). In the penguin, after stage 33, flipper feathers become more numerous until, by stage 40, the flippers are completely covered. In number these feathers are probably far in excess of those found on the wings of the chick.

The beak of the penguin is larger and its egg tooth originates relatively later than in the chick of comparable stage. The nostrils of the chick start as a groove and narrow to a slit by stage H.36. In contrast, the small round nostrils of the Adélie penguin are situated further forward and do not perforate until a few days before hatching (stage 44).

ACKNOWLEDGEMENTS

I wish to thank Dr. H. Fox for his encouragement and advice during the preparation of this paper. I also wish to thank all of my colleagues at Signy Island during the season 1965–66, for their invaluable assistance during the collection of the eggs; Dr. M. W. Holdgate, who originally suggested the topic; Professor M. Abercrombie, for the generous facilities in the Department of Zoology, University College, London; Dr. Ruth Bellairs, who kindly read the manuscript, and Mr. K. Hunt, who prepared the photographs.

MS. received 6 June 1967

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