MELANOTIC DIFFERENTIATION OF THE AVIAN NEURAL CREST
VOLUME 2

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MICROANATOMY OF THE ADULT BROWN LEGHORN WITH REFERENCE TO MELANOCYTE DISTRIBUTION

Fig. 1

Anterior-Posterior dissection of the eye with the palpebrae removed.

Fig. 2

Anterior-Posterior dissection of the eye with the palpebrae intact.

Fig. 3

Anterior view of the eye with the palpebrae removed to expose the nictitating membrane and corneal limbus.

Abbreviations

C	=	Cornea
C.B.	=	Ciliary Body
Ch	=	Choroid
I	=	Iris
L	=	Lens
Lg	=	Ligumenta
M.A.J.S.	=	Melanocytes of the anterior iridial stroma
M.L.	=	Melanocytes of the corneal limbus
M.L.E.	=	Melanocytes of the limbal epithelium
M.L.S.	=	Melanocytes of the limbal stroma
M.N.M.E.	=	Melanocyte of the nictitating membrane epithelium
M.N.M.S.	=	Melanocyte of the nictitating membrane stroma
N.M.	=	Nictitating Membrane
N.R.	=	Neural Retina
P.R.E.	=	Pigmented Retinal Epithelium
S.C.	=	Scleral cartilage
S.O.	=	Scleral ossicles
V.B.	=	Vitreous Body (or Choroid Body)
V.H.	=	Vitreous Humour

Fig.1

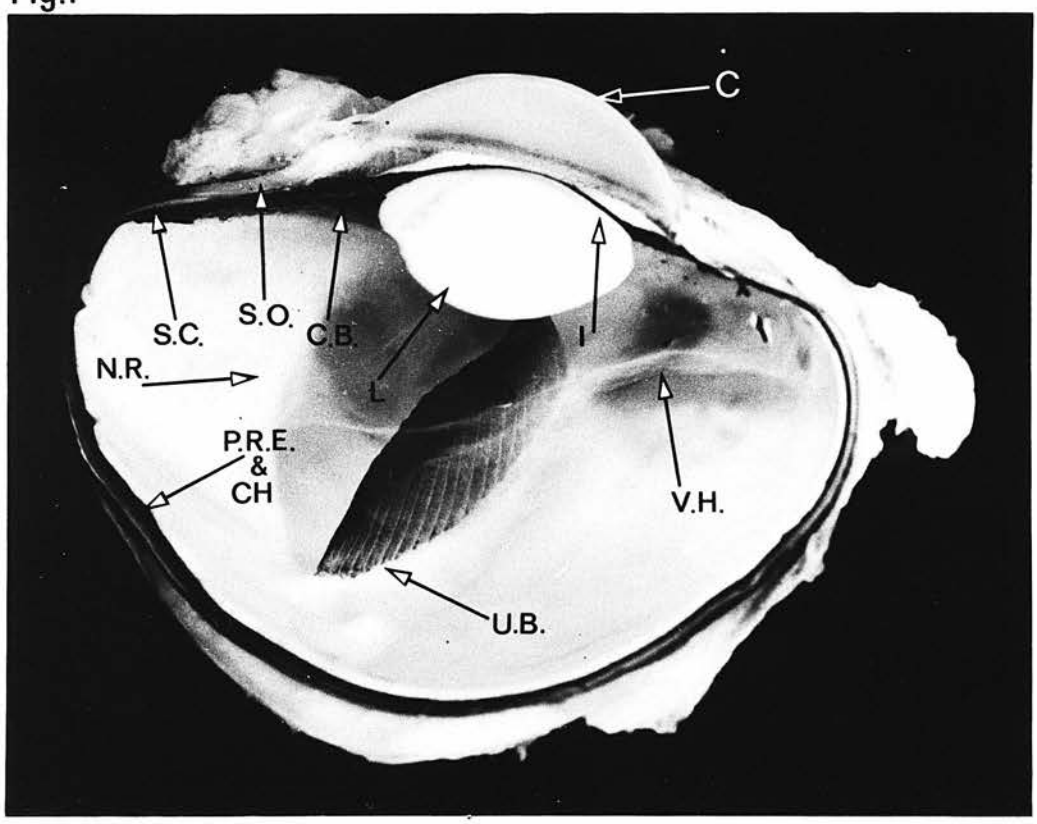


Fig.2

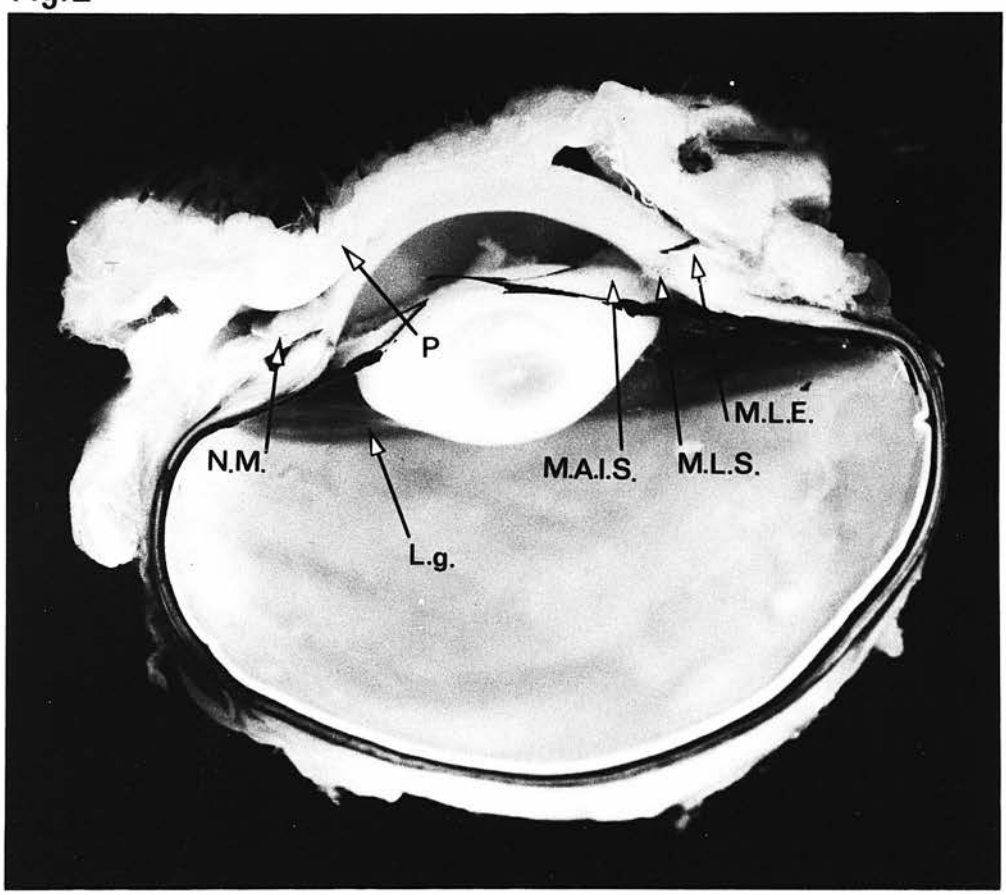


Fig.3

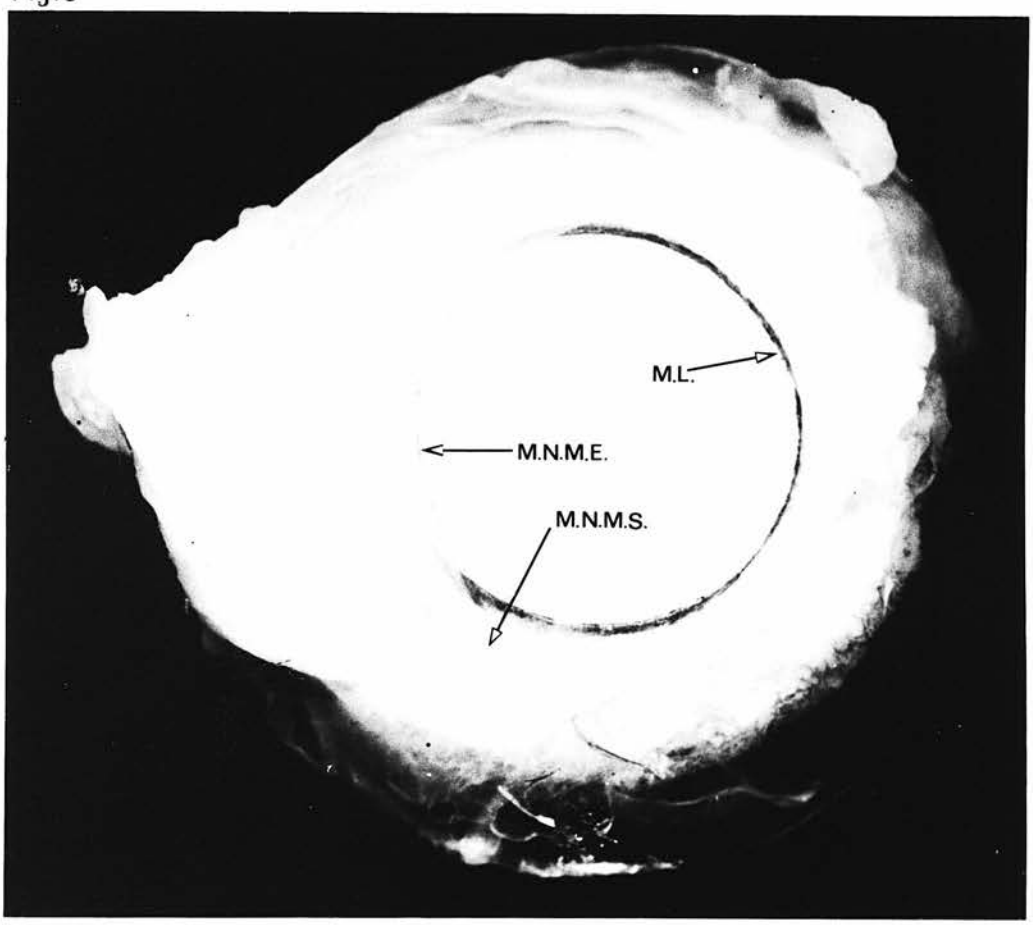


Fig. 4

Shows the ultrastructural development of pheomelanosomes and eumelanosomes. (Taken from Jimbow and Takeuchi, 1979).

Fig. 5

Shows the pathway of 'Dopa-melanin' formation which is responsible for eumelanin-type pigmentation. (Taken from Prota, 1980).

Fig. 6

Shows the relationship between the eumelanin and pheomelanin pathways, and indicates how 'intermeshing' may occur. See text for further details
(Taken from Prota, 1980).

Fig. 7

Shows the structure of some of the low molecular weight trichochrome pigments, which can be extracted from melanosomes (Taken from Prota, 1980).

Fig.4

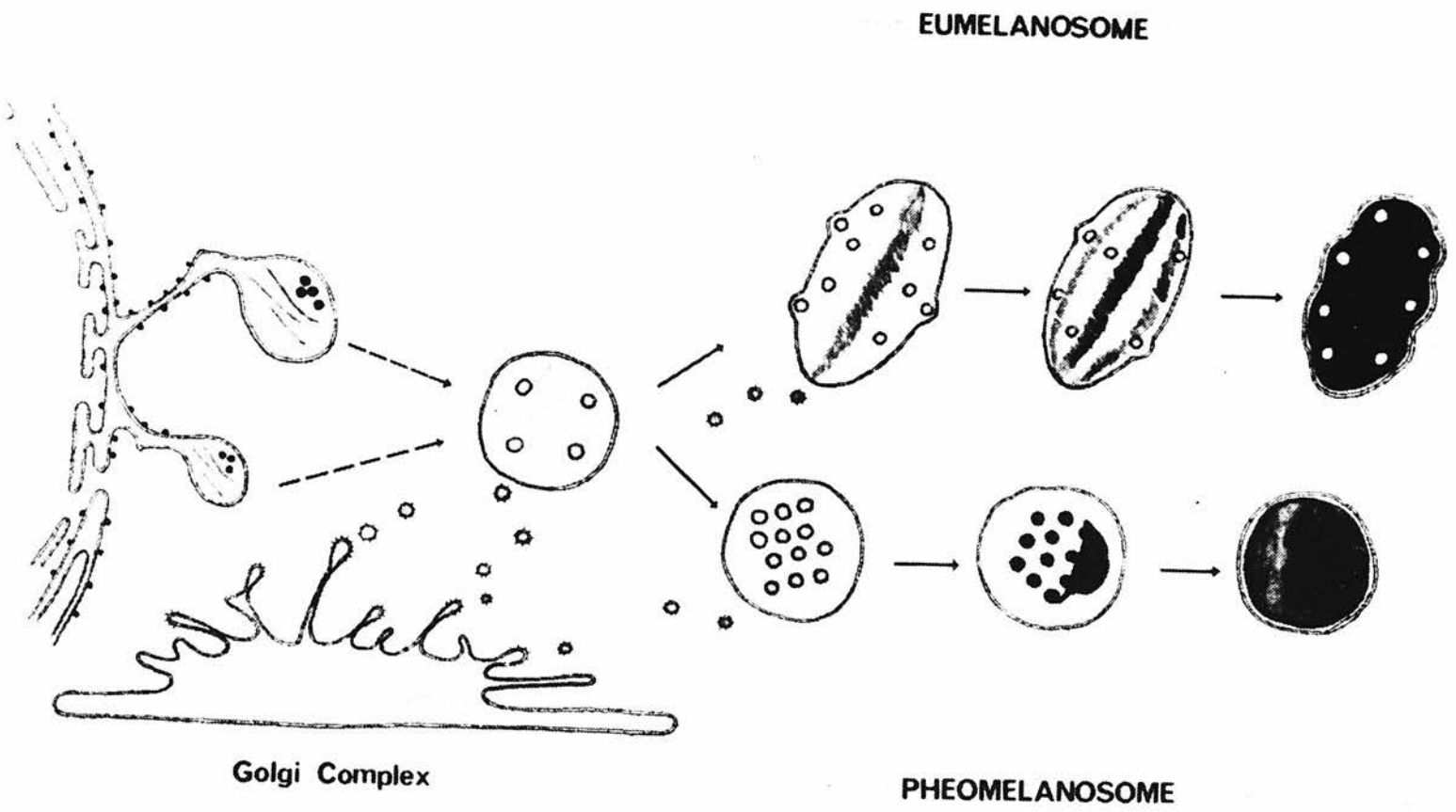


Fig.5

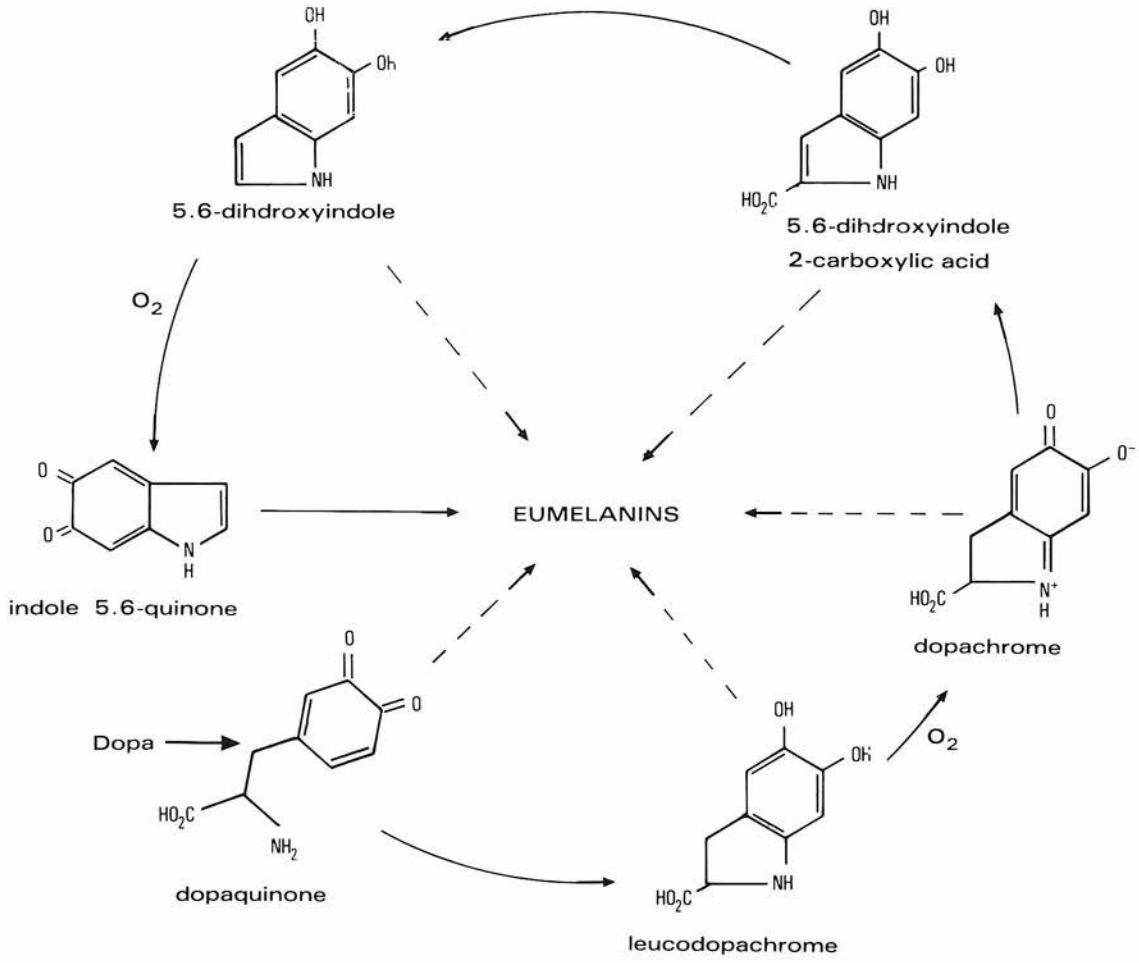

OH
OH
NH
5.6-dihdroxyindole
OH
OH
HO2C
NH
5.6-dihdroxyindole
2-carboxylic acid
O2
O
O
N
H
indole 5.6-quinone
EUMELANINS
O
O-
HO2C
N+
H
dopachrome
O
O
Dopa
HO2C
NH2
dopaquinone
OH
OH
HO2C
NH
leucodopachrome
O2

Fig.6

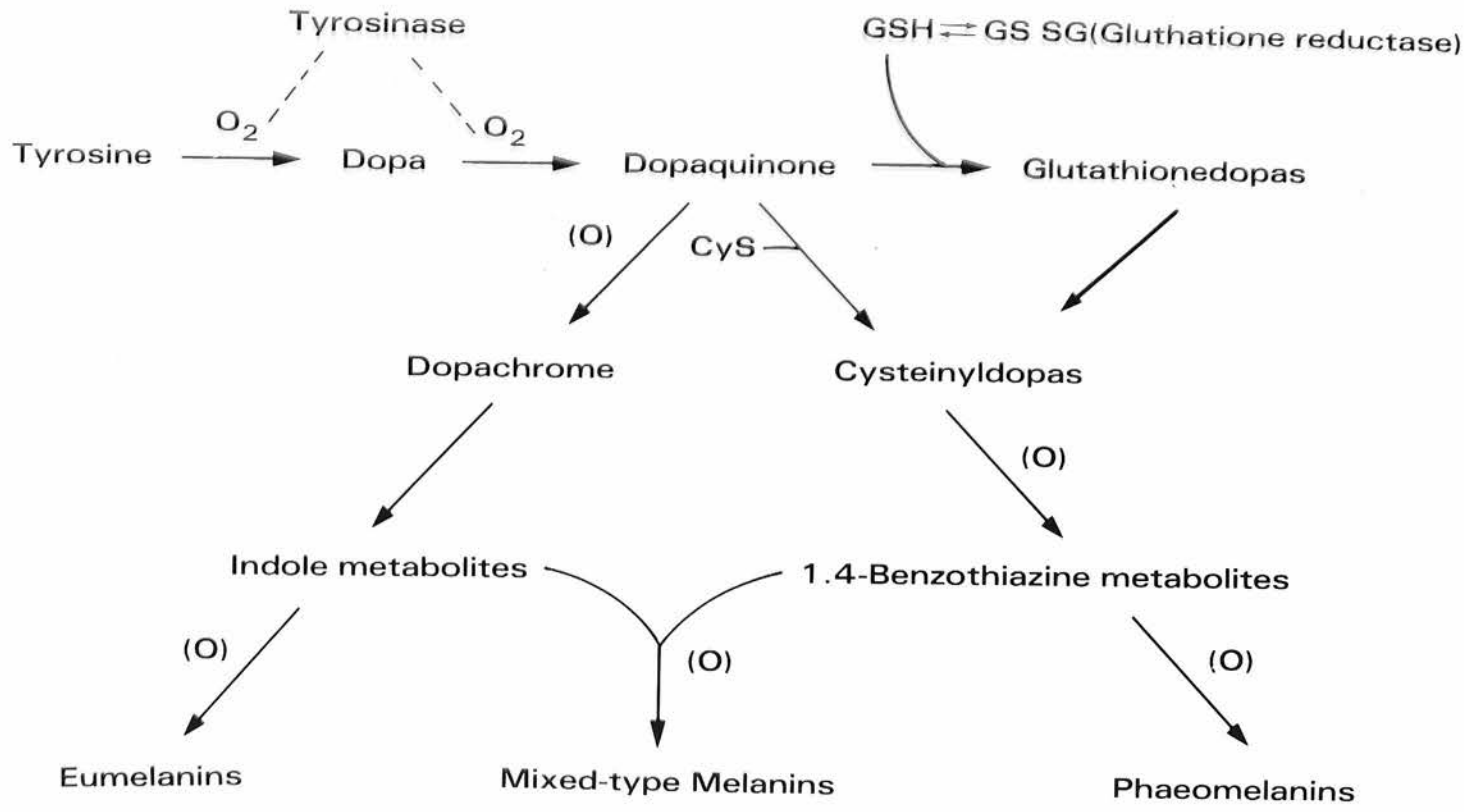
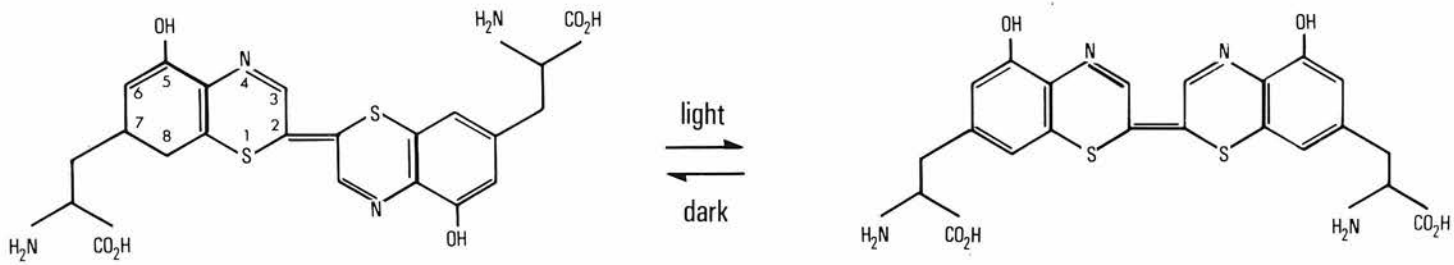
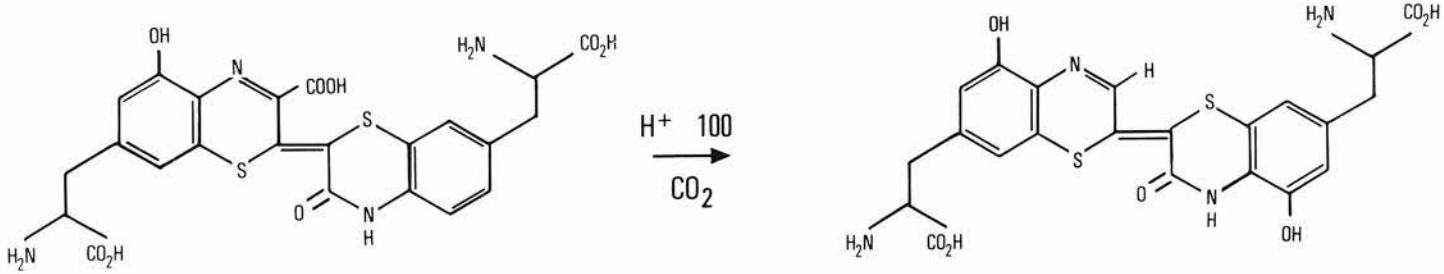

Tyrosinase
GSH ⇌ GS SG(Gluthatione reductase)
O_2
O_2
Tyrosine
Dopa
Dopaquinone
Glutathionedopas
(O)
CyS
Dopachrome
Cysteinyldopas
(O)
Indole metabolites
1.4-Benzothiazine metabolites
(O)
(O)
(O)
Eumelanins
Mixed-type Melanins
Phaeomelanins

Fig.7



trans–form　　**cis**–form

Trichochrome–F



Trichochrome–C　　**Decarboxytrichochrome–C**

FIGURES ACCOMPANYING CHAPTER 2

Figs 1-4 Microdissection of Embryonic Anterior Eye Segments

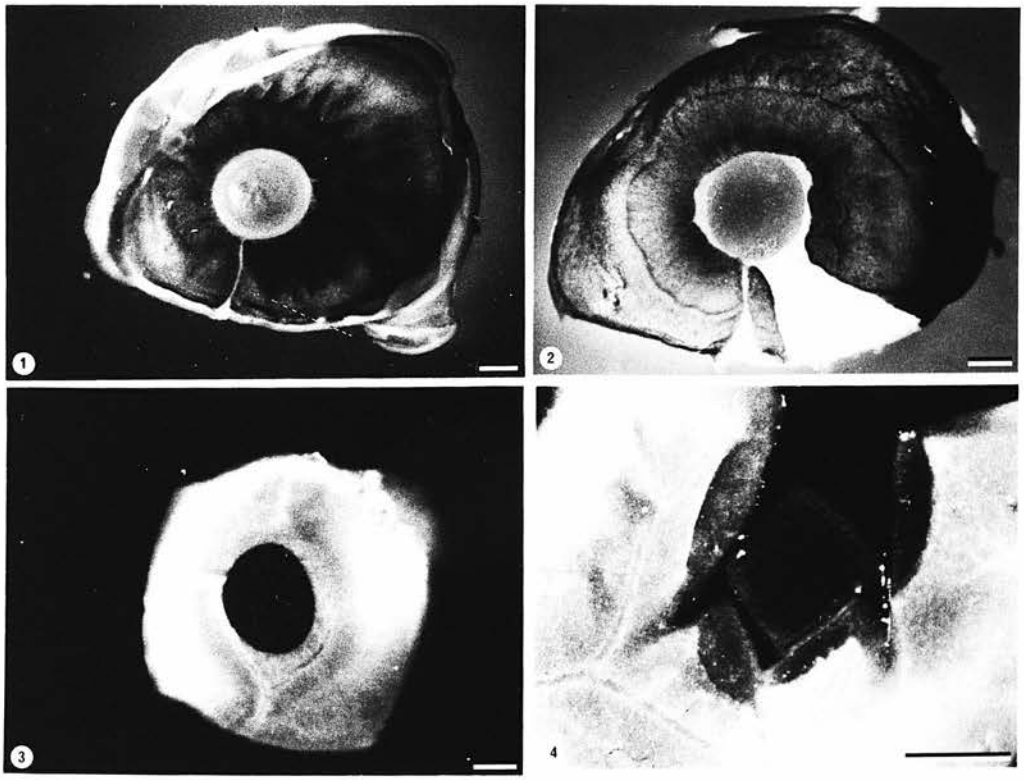
Fig 1 The whole eye segment

Fig 2 The segment after removal of lens and vitreous

Fig 3 The segment after further removal of the P.R.E. and superficial epithelium

Fig 4 Dissection of the corneal square

(Scale bar = 0.5 mm)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2
3
4

FIGURES ACCOMPANYING CHAPTER 3

Figs 1-6

Whole Mounted Pigmented Tissue

Fig 1

The blood vessels of the 18 day nictitating membrane are surrounded by melanocyte which make dendritic contact with each other (bf. scale bar = 50μm).

Fig 2

The epithelium of the nictitating membrane is made up of very small epithelial cells, amongst which are interspersed very small melanocytes. The cell indicated (arrow) extends over 30μm (approx) (dic, scale bar = 30μm).

Fig 3

The basal cells of the 18 day dorsal skin epithelium are much larger than those found within the nictitating membrane. The melanocytes which are interspersed amongst the keratinocytes are larger than those seen in fig 2. The cell indicated (arrow) extends over 95μm (approx) (dic, scale bar = 100μm).

Fig 4

The small epithelial melanocyte of the 18 day nictitating membrane have much narrower dendritic processes than the subadjacent stromal melanocytes (dic, scale bar = 30μm).

Fig 5

The most pigmented tissue within the Brown Leghorn embryo is the highly vascular choroid. A great majority of the melanocytes are associated with blood vessels (dic, scale bar = 50μm).

Fig 6

Melanocytes are attached to large diameter blood vessels are often flatter than the more dendritic cells apposed to capillaries (see fig 5 also) (dic, scale bar = 100μm).

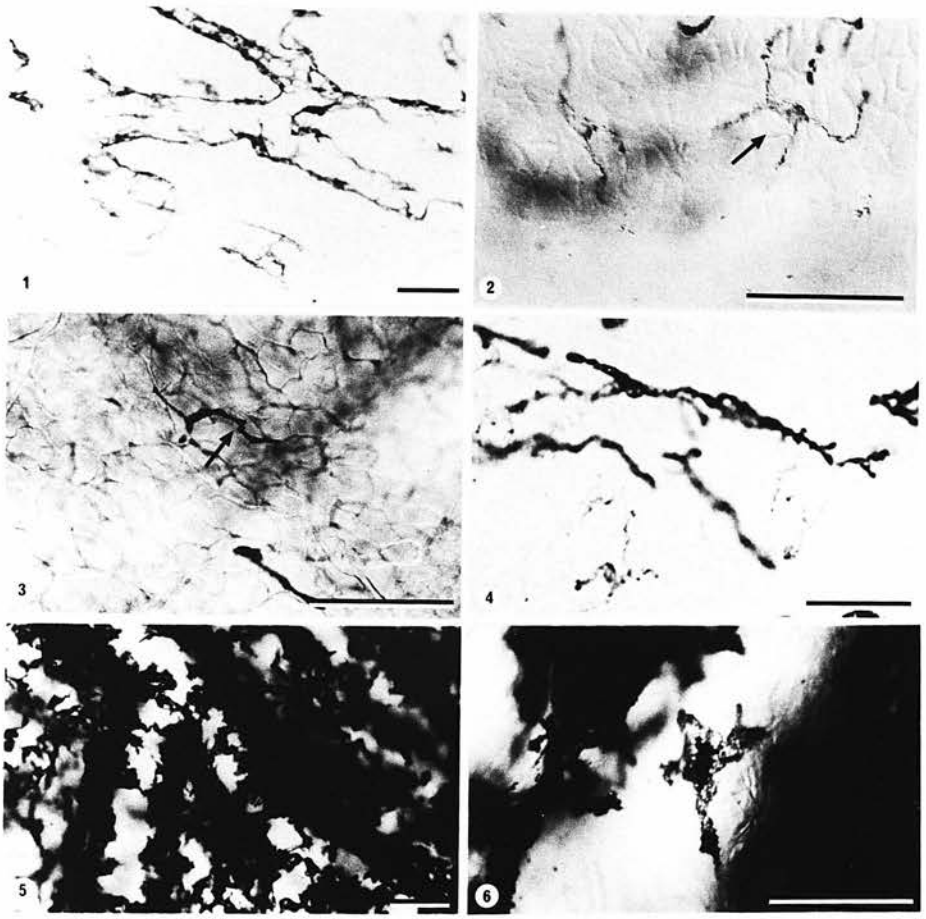
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Fig 7-8 Interaction of the Melanocytes with the Extracellular Matrix

Fig 7

The shape of stromal melanocytes is influenced by the gross architecture of the extracellular matrix. Processes of both melanocytes and fibroblasts extend around collagen bundles (arrows) (scale bar = 2μm).

Fig 8

A melanocyte lies in close apposition to the subendothelial matrix of a limbal capillary (arrow) (scale bar = 2μm).

7

8

Figs 9-11 Pinching-off of Dendrite Tips

Fig 9

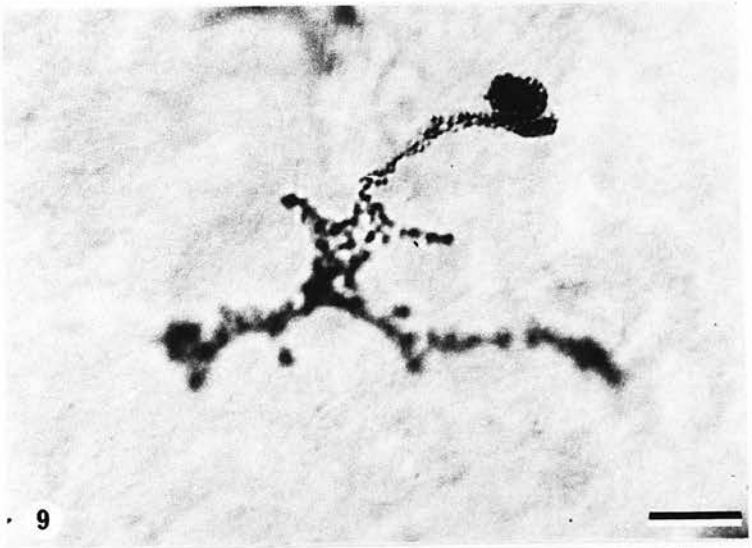
The isolated melanocyte of connective tissue overlying the 18 day skull bone is in contact with or is shedding a melanosome clump (dic, scale bar = 10μm).

Fig 10

One of the melanocytes from the same source as above has dendrites with bulbous terminations (dic, scale bar = 10μm).

Fig 11

Melanocytes from the same source as above which have bulbous endings and dendrite constrictions (dic, scale bar = 10μm)



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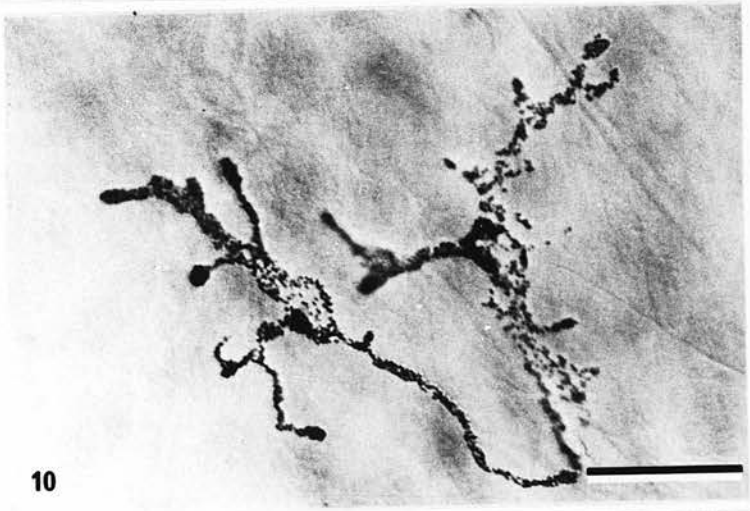

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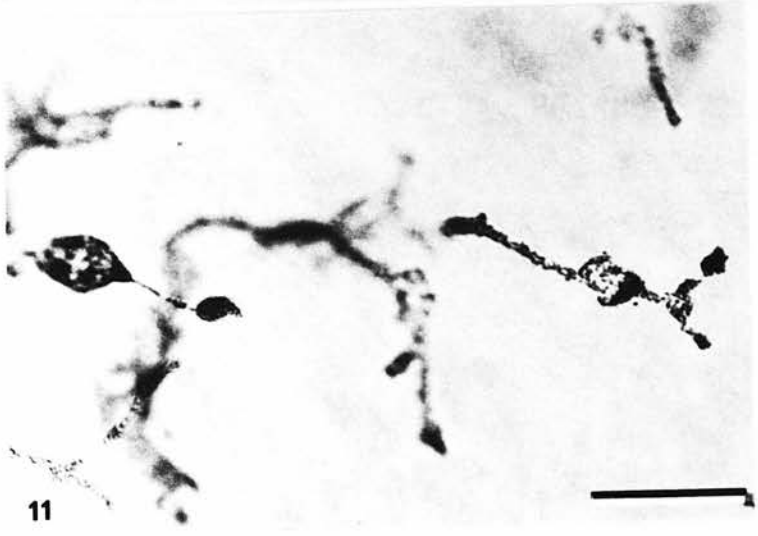

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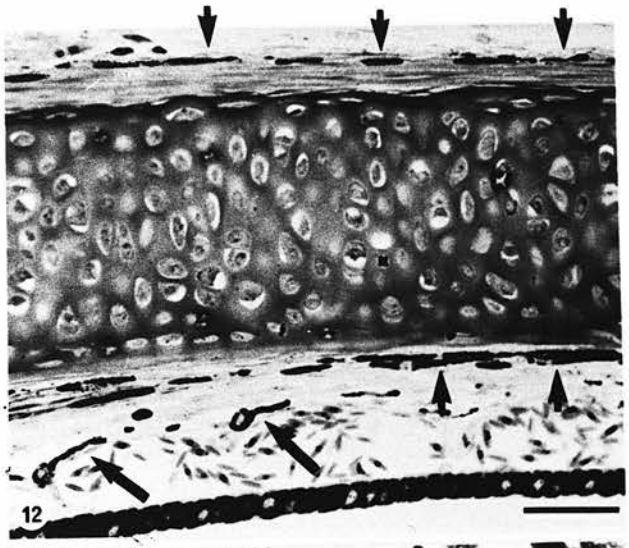
Figs 12-14 Flattened Epithelioid Melanocytes of Uvea

Fig 12

A transverse section through the P.R.E. choroid, and sclera of an 18 day eye shows the close association of melanocytes with the blood vessels (large arrows) and the fibrous sheath surrounding the scleral cartilage (small arrows). In the latter situation the melanocytes are aligned parallel to the matrix. (Tangential sections shows the sheath associated melanocytes to be epithelioid) (scale bar approx 40μm).

Fig 13-14

Epithelioid melanocyte near the surface of the scleral ossicle (whole mount, 18 day). Note the absence of melanosomes from the nuclear area (dic, scale bar = 20μm).



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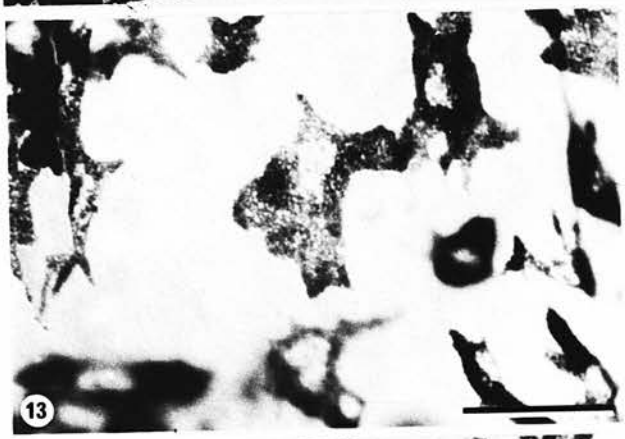

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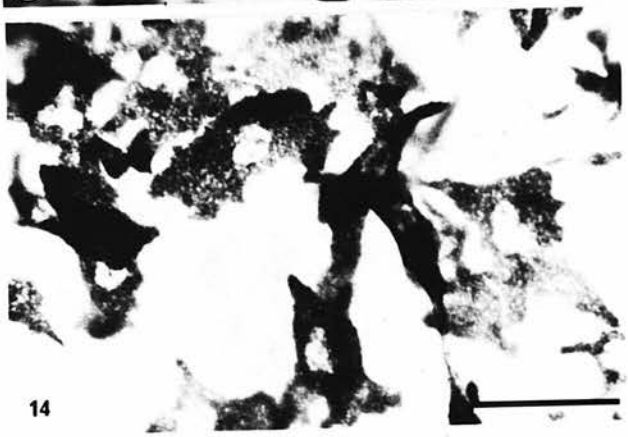

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Figs 15-17 Melanocyte Size Distribution in Three Locations

Figs 15-17

Shows the size distribution of melanocytes within three locations of the 18 day embryo. Note that the measurement is sensitive to changes of dendrite shape and distribution as well as to dendrite length.

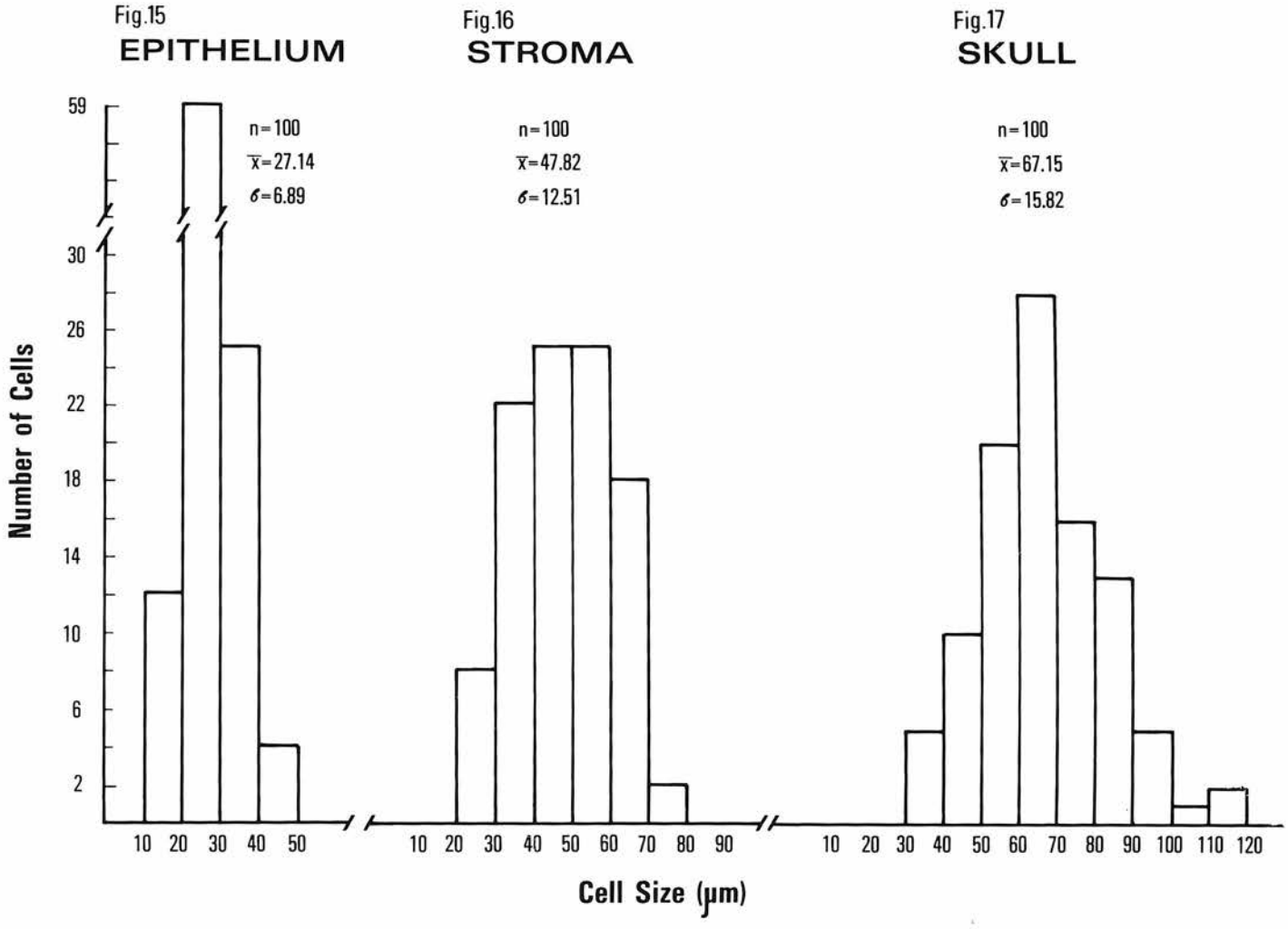


Fig.15
EPITHELIUM
n=100
x̄=27.14
σ=6.89
Fig.16
STROMA
n=100
x̄=47.82
σ=12.51
Fig.17
SKULL
n=100
x̄=67.15
σ=15.82
Number of Cells
59
30
26
22
18
14
10
6
2
10 20 30 40 50
10 20 30 40 50 60 70 80 90
10 20 30 40 50 60 70 80 90 100 110 120
Cell Size (μm)

Fig 18-23 Differentation of Premigratory Crest Cells In Neural Tube Cultures

Fig 18

A neural tube explant has bent back on itself and produced a circumferential outgrowth of neural crest cells during the first 1.5 days of culture (10% F.C.S. + 2% C.E.E. ph, scale approx 120μm).

Fig 19

An isolated outgrowth of stellate crest cells after 2.5 days in culture (10% F.C.S. + 2% C.E.E., ph, scale bar approx 100μm).

Fig 20

The periphery of a highly pigmented outgrowth after 10 days of culture shows the very light (brown) colour of the pigment (15% F.C.S. + 2% C.E.E., si, Scale bar = 60μm).

Fig 21

Phase illumination of the same field shows the stellate morphology of the cells at high density. Note the existence of short blunt ended processes (ph, scale bar = 60μm).

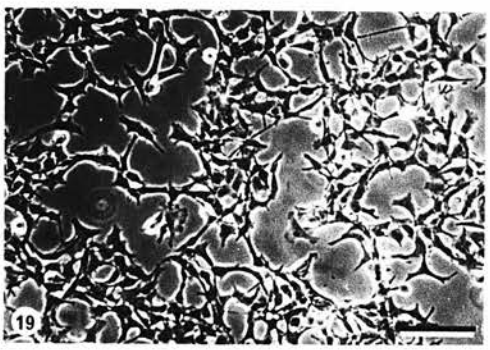
Fig 22

This was the most pure melanocyte culture produced by neural tube explantation, after 13 days incubation. The broken line indicates where the edge of the neural tube had been (5% H.S. + 2% C.E.E., bf, scale bar = 100μm

Fig 23

Phase illumination of the same field shows the stellate morphology of the cells (Note the large clumps of melanocytes which formed after the onset of pigmentation) (ph, scale bar = 100 m).


18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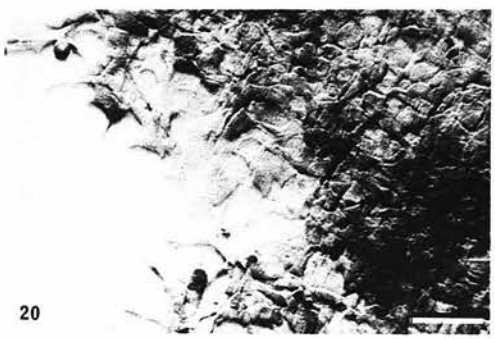
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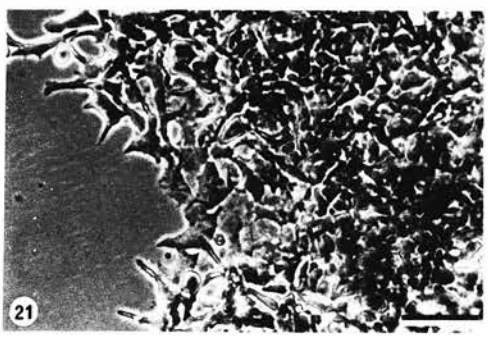
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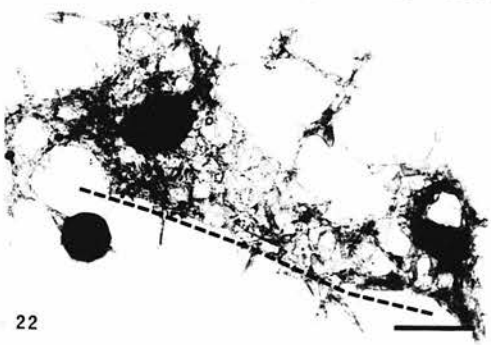
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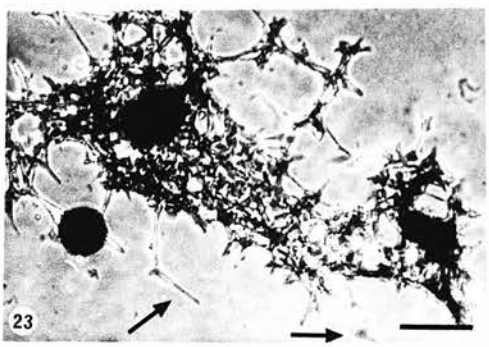
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Fig 24-25

Large fibroblastic cells are present in low frequency at the periphery of outgrowths (5.5 days, 10% F.C.S. and 5% C.E.D., dic, scale bar = 100μm).

Fig 26

Large epithelioid pitches were observed on rare occasions within neural tube outgrowths (ph, scale bar = 100μm).

Fig 27

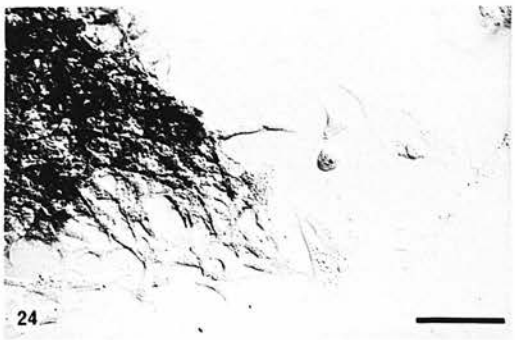
Explants of notochord dissociated to form sheets of epithelial cells which became vacular within 1 week and then died shortly after (ph, scale bar = 100μm).

Fig 28

When neural tube explants were left in situ neuronal processes emerged. This field shows a branched neuronal fascule after 3.5 days of culture (10% F.C.S. + 5% C.E.E., ph, scale bar = 100μm).

Fig 29

When the neural tube was left in place migrant neurones were also present within the outgrowths. Neural growth cones were adherent to the apical surface of fibroblastic cells(double arrows). A laterally blebbed neuronal process runs toward a clump of melanocytes (single arrow)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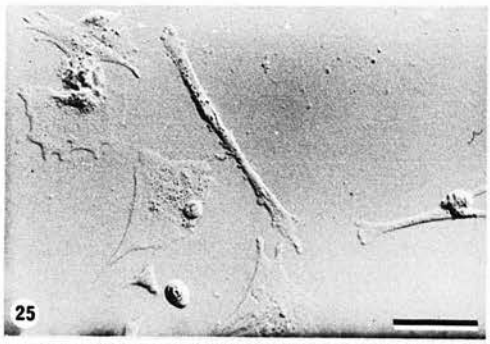
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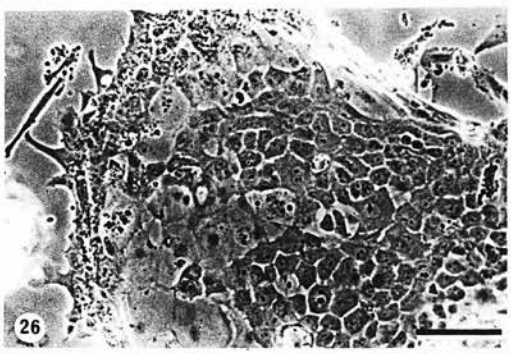
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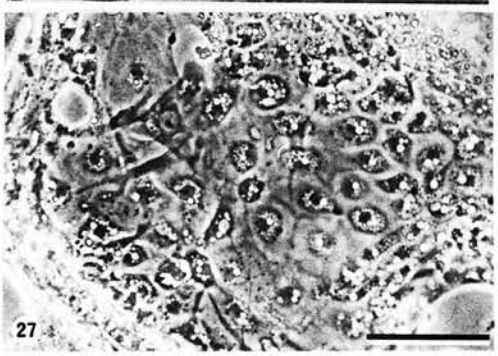
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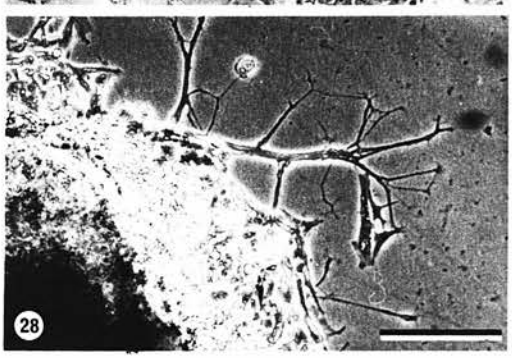
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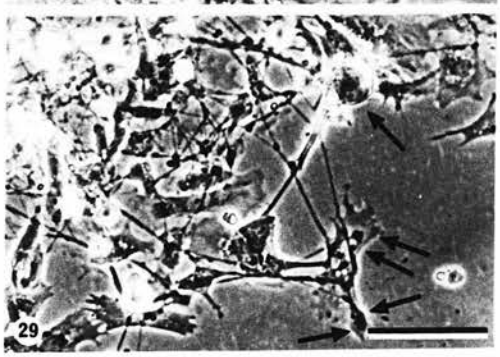
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Fig 30

Outgrowth in medium supplemented with high concentrations of serum and embryo extract clumped within 36 hours. (10% F.C.S. + 5% C.E.E., ph, scale bar = 50μm).

Fig 31

Unpigmented clumps of melanoblasts from the neural tube (free floating in the illustration). The clump illustrated (arrow) has begun to pigment after 2.5 days in culture. (ph, scale bar = 250μm).

Fig 32

A large melanocyte aggregate and several smaller clumps have formed (arrows) after 11 days in culture. (10% F.C.S. + 5% C.E.E., ph scale bar = 250μm).

Fig 33

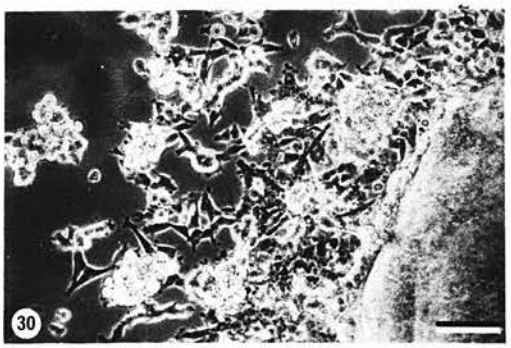
The outgrowth from the neural tube above contained large peripheral melanocyte aggregates. (culture data as above, scale bar = 250μm).

Fig 34

Two cultures had such extensive peripheral aggregates of melanocytes that a pigmented torus developed within 5 days of culture. (15% F.C.S. + 5% C.E.E., ph, scale bar = 500μm).

Fig 35

The periphery of a culture which differentiated to form melanocytes prior to cell aggregation (5% F.C.S. + 2% C.E.E., si, scale bar = 50 μm)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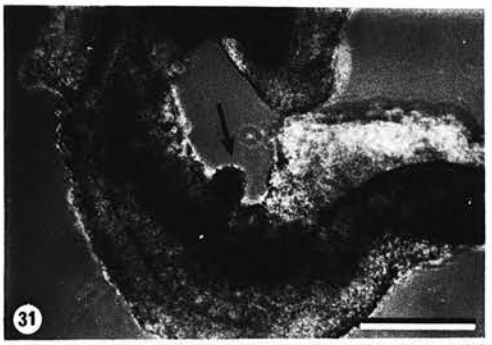
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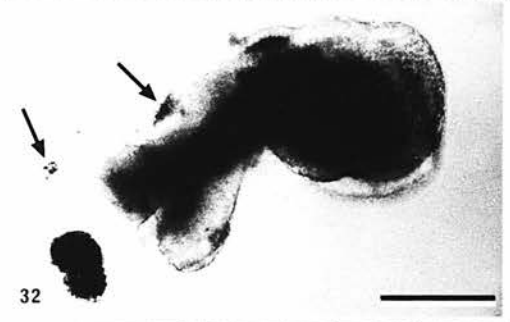
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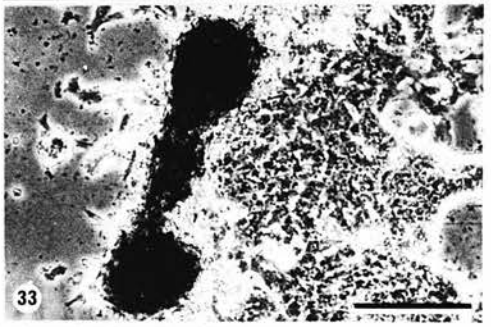
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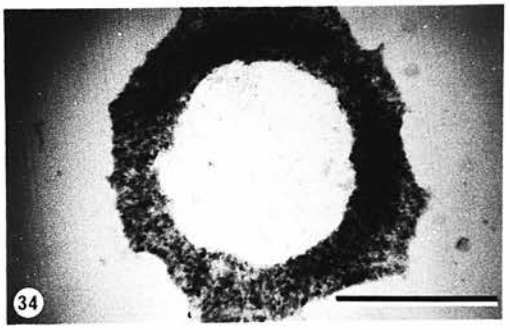
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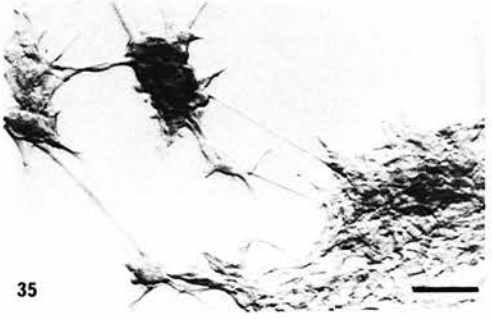
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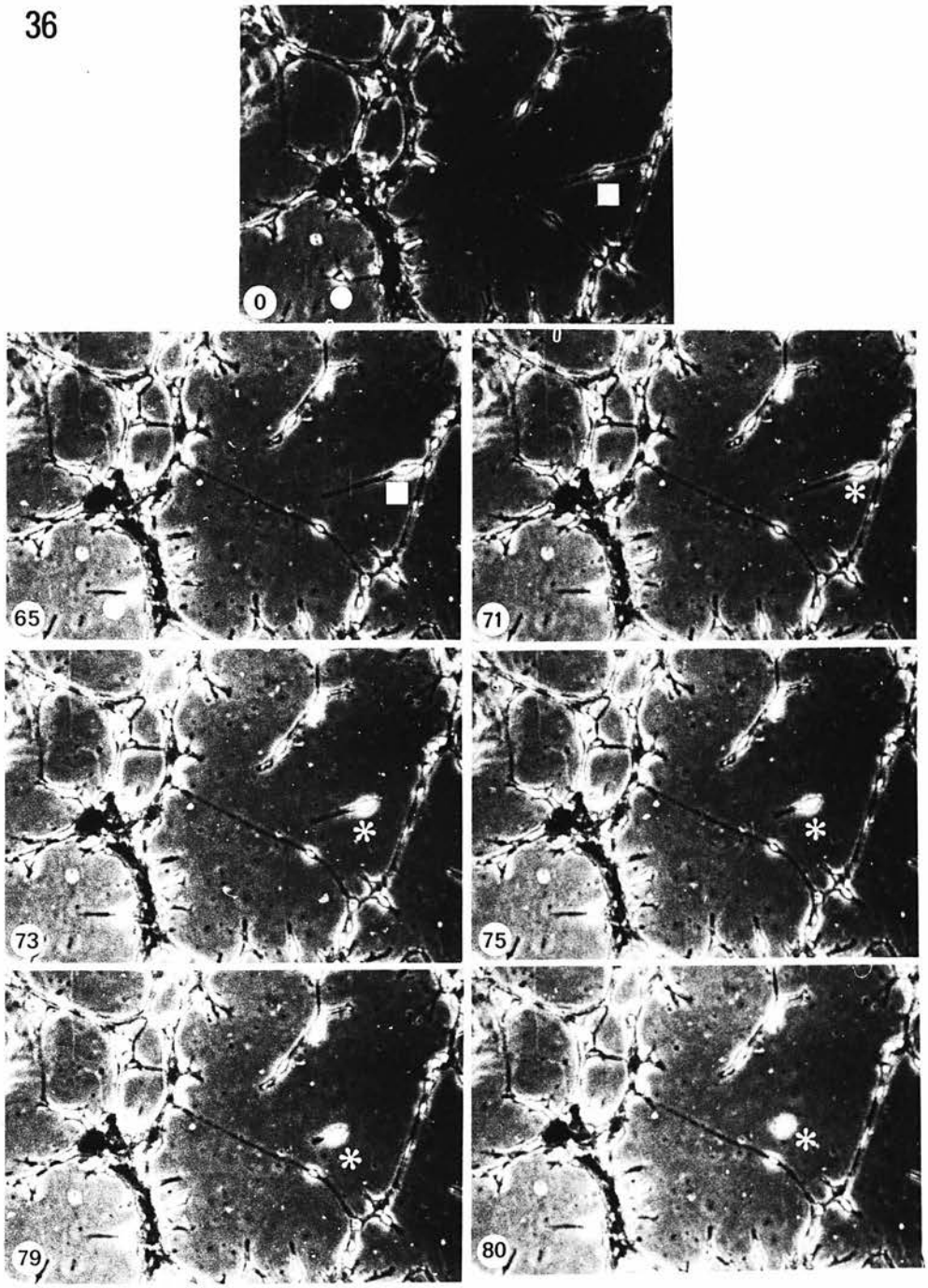
Fig 36

Cephalic melanocytes were filmed 12 days after outgrowth from a segment of mesencephalic roof (7% F.C.S. + 2% C.E.E). The frames illustrated are part of a time-lapse sequence shot at a lapse rate of 2 frames per minute. The time of each frame illustrated is given in minutes from the initial zero point. The three events described as 1, 2 and 3 in the text are labelled, o, □ , and * respectively (ph).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Figs 37-40 Dibutyryl-cyclic AMP treatment of Red Minorca Melanocytes

Fig 37

After 4 days of incubation in medium containing 1mM Dibutyryl-cyclic-AMP the Red Minorca cells pigmented and aggregated. (5% H.S. + 2% C.E.E., bf, scale bar = 100μm).

Fig 38

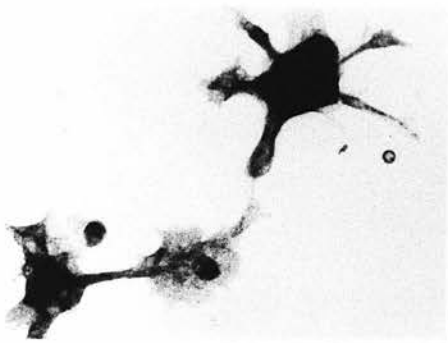
Phase illumination of the second field shows the existence of round unpigmented cells (arrows), which suggests that the untreated population were not exclusively melanoblastic. (scale bar = 100μm).

Fig 39

The 'control' culture, at the same time, showed absolutely no trace of pigmentation. (f% H.S. + 2% C.E.E., bf, scale bar = 100μm).

Fig 40

Phase illumination of the same field shows the presence of stellate and well-spread cells within the untreated culture. (ph, scale bar = 100μm).



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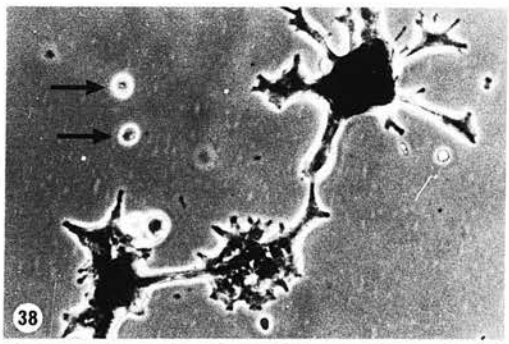

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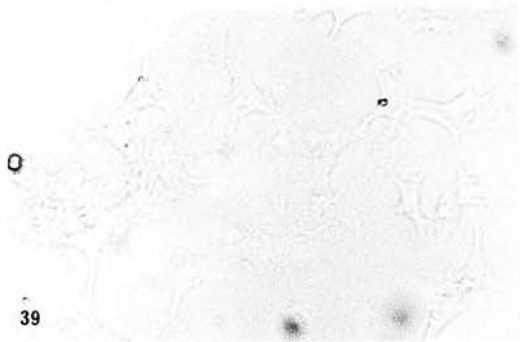

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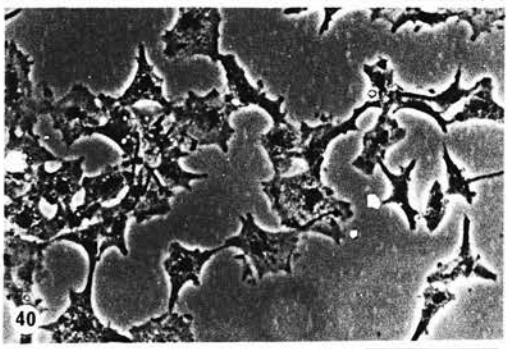

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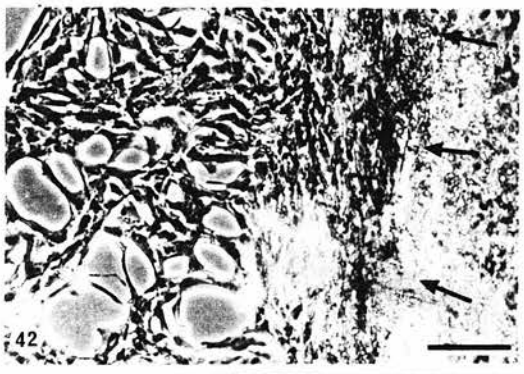
Figs 41-42 Trunk Segment Explantation

Fig 41

A whole trunk segment is shown just prior to explantation. (scale bar = 1mm).

Fig 42

The periphery of a trunk explant contains a band of brown pigmented cells (arrows) after 6 days (scale bar = 100μm).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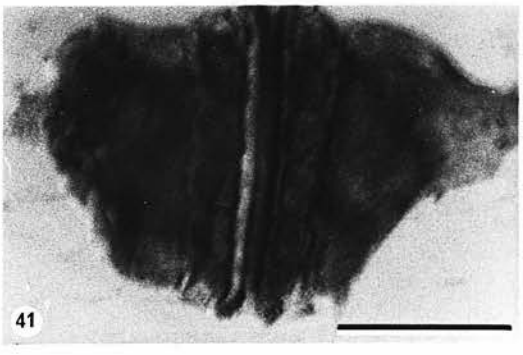
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Figs 43-48 Periorbital Mesenchyme Explantation

Fig 43

An explant of stage 29 anterior mesenchyme after 3 days of culture has become completely black with melanocytes. The outgrowth, within the proximity of the explant, consists of fibroblasts and a few dendritic black melanocytes which have migrated from the explant. (ph, scale bar = 100μm).

Fig 44

Migration of black melanocytes from the explant, during the third day, is shown more clearly by bright field illumination. Note also the presence of isolated melanosome clumps (arrows). (bf, scale bar = 100μm).

Fig 45

A section (1 m) of the mesenchyme 4 days after explantation shows the distribution of dendritic melanosomes with elongate black melanosomes some of which are approximately 1μm in length. (ph, scale bar = 10μm).

Fig 46

A toluidine blue stained section of a 12 day neural crest culture was photographed at very high magnification. The brown melanosomes are round, rather than elongate and less than 0.5 m in diameter. Note also the less dendritic morphology of aggregating cells. (bf, scale bar = 10μm).

Fig 47

A dense clump of brown melanocytes is present at the periphery of a mesenchymal outgrowth after 18 days of culture. (10% F.C.S. + 5% C.E.E., ph, scale bar = 100μm).

Fig 48

Osmophilic lipid vesicles (arrows) are present within the cytoplasm of fibroblasts, but not melanocytes, after 4 days of culture. (10% F.C.S. + 5% C.E.E., bf, scale bar = 100μm).


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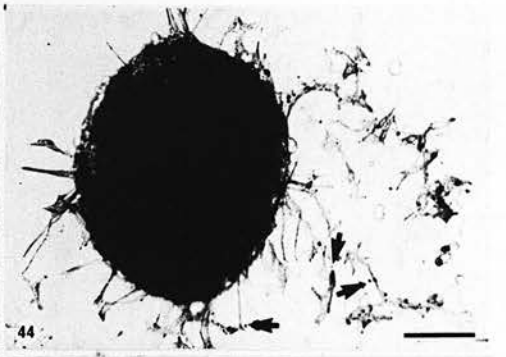
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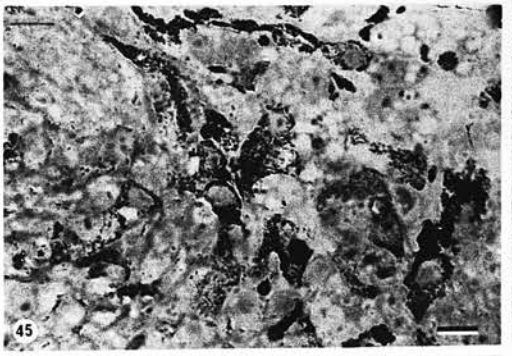
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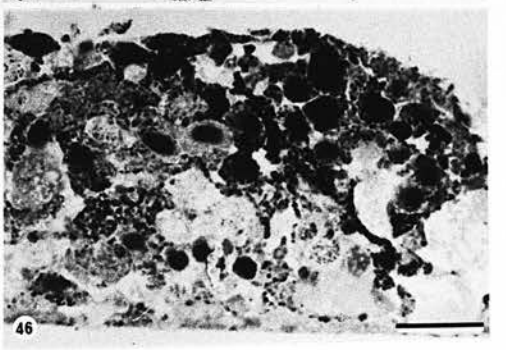
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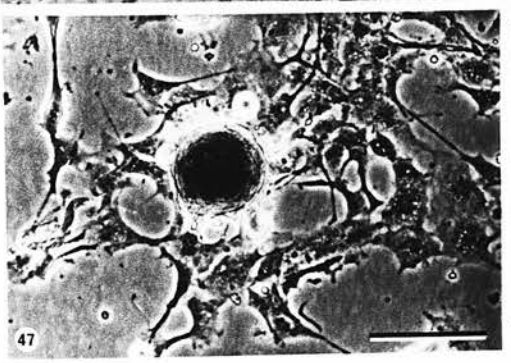
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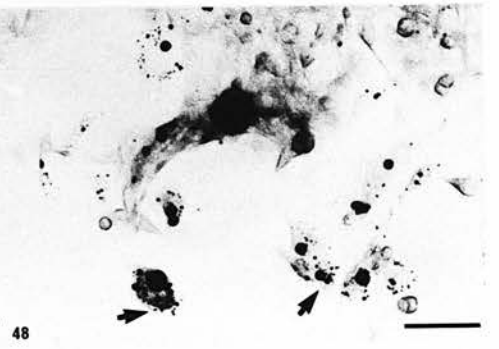
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Figs 49-53 Behavioural Studies of Post-Migratory Melanocytes

Fig 49

A stage 30 orbital mesenchyme was cultured for 3 days in 10% F.C.S. + 5% C.E.E. The outgrowth was filmed at a lapse rate of 3 frames per minute, using bright field illumination. The time of each frame illustrated is given in minutes from the initial zero point. The open arrow (frame 423) denotes the formation of a melanocyte aggregate. The closed arrow (frame 265) denotes the shedding of a melanosome clump.

Fig 50

Detailed analysis of the sequence above over a short time interval demonstrated rapid change of cell shape as described in the text. The time of each frame illustrated is given in minutes from the initial zero point. Shading denotes regions of high melanosomal density (scale bar = 50μm).

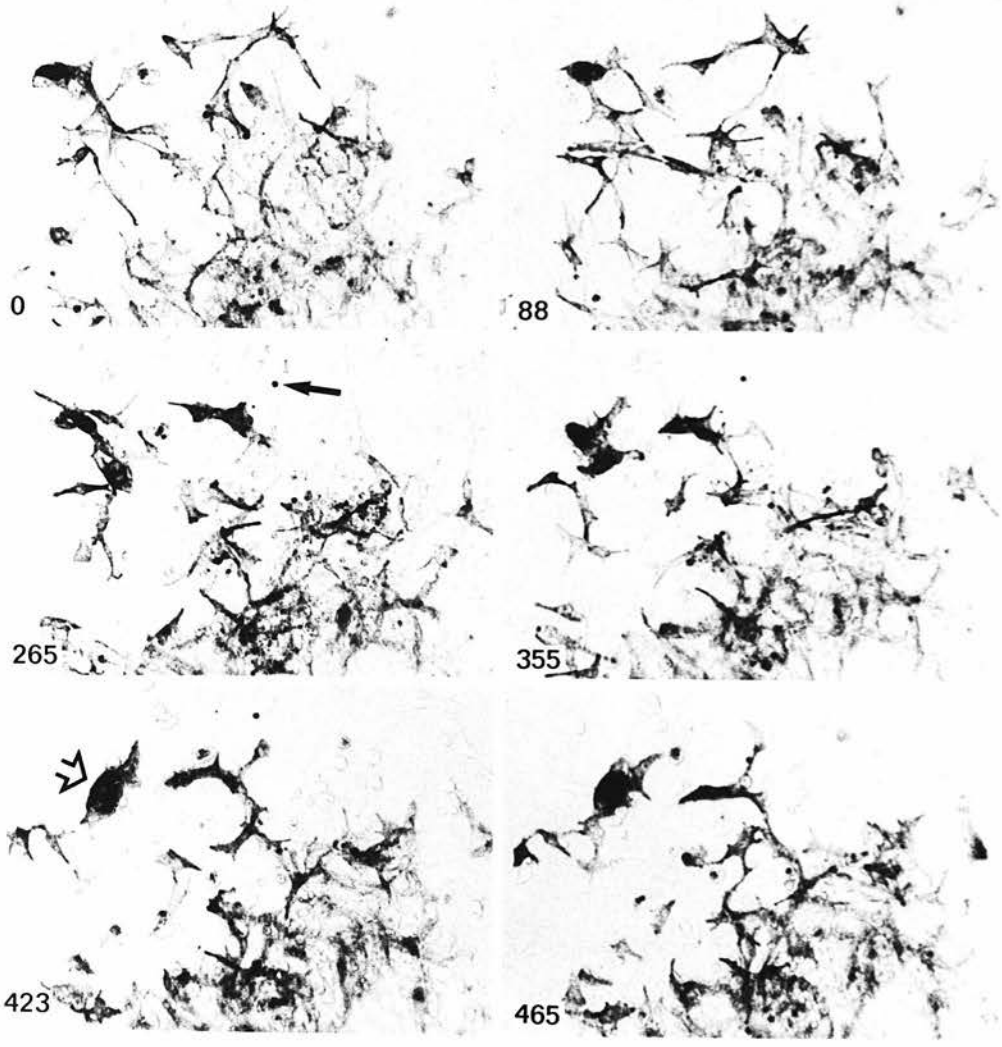
Fig 51

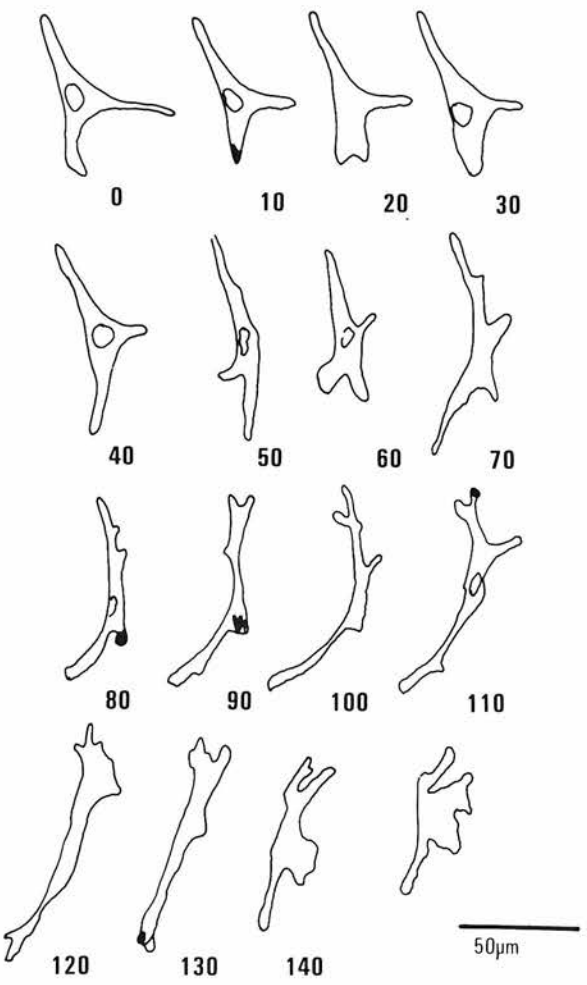
A similar sequence to that above, filmed after 5 days of culture, shows rapid construction and pinching-off at the dendrite tip (arrow), as well as dendritic extension and retraction. (data as above).

Figs 52-53

Epithelioid melanocytes within mesenchymal explants undergo shape changes over large areas of their periphery (data as above).

49

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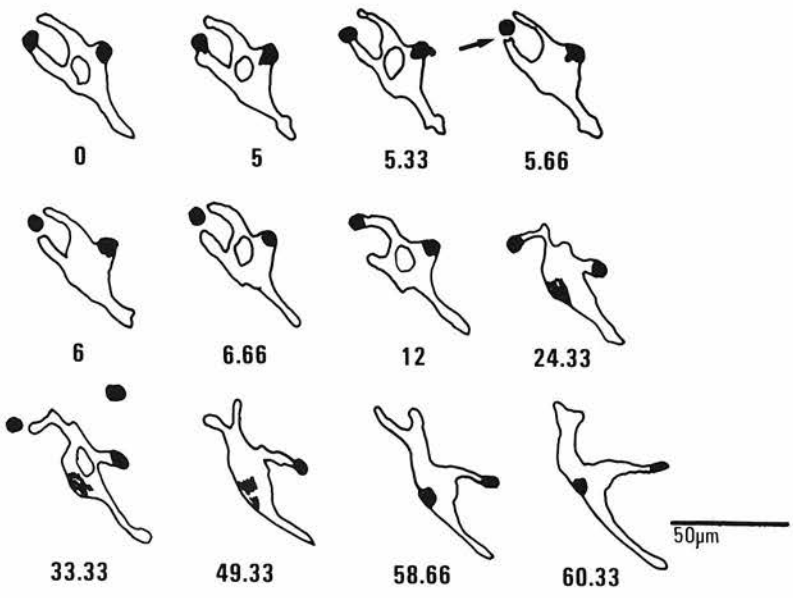

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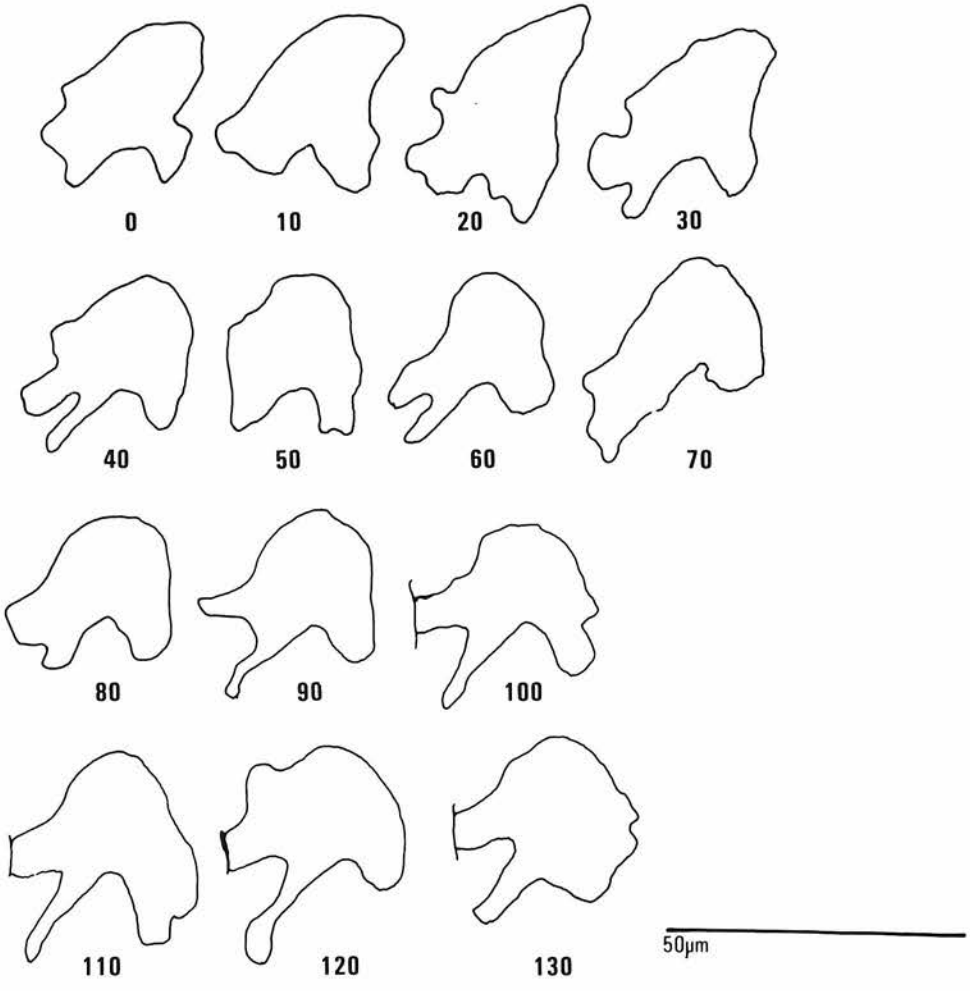

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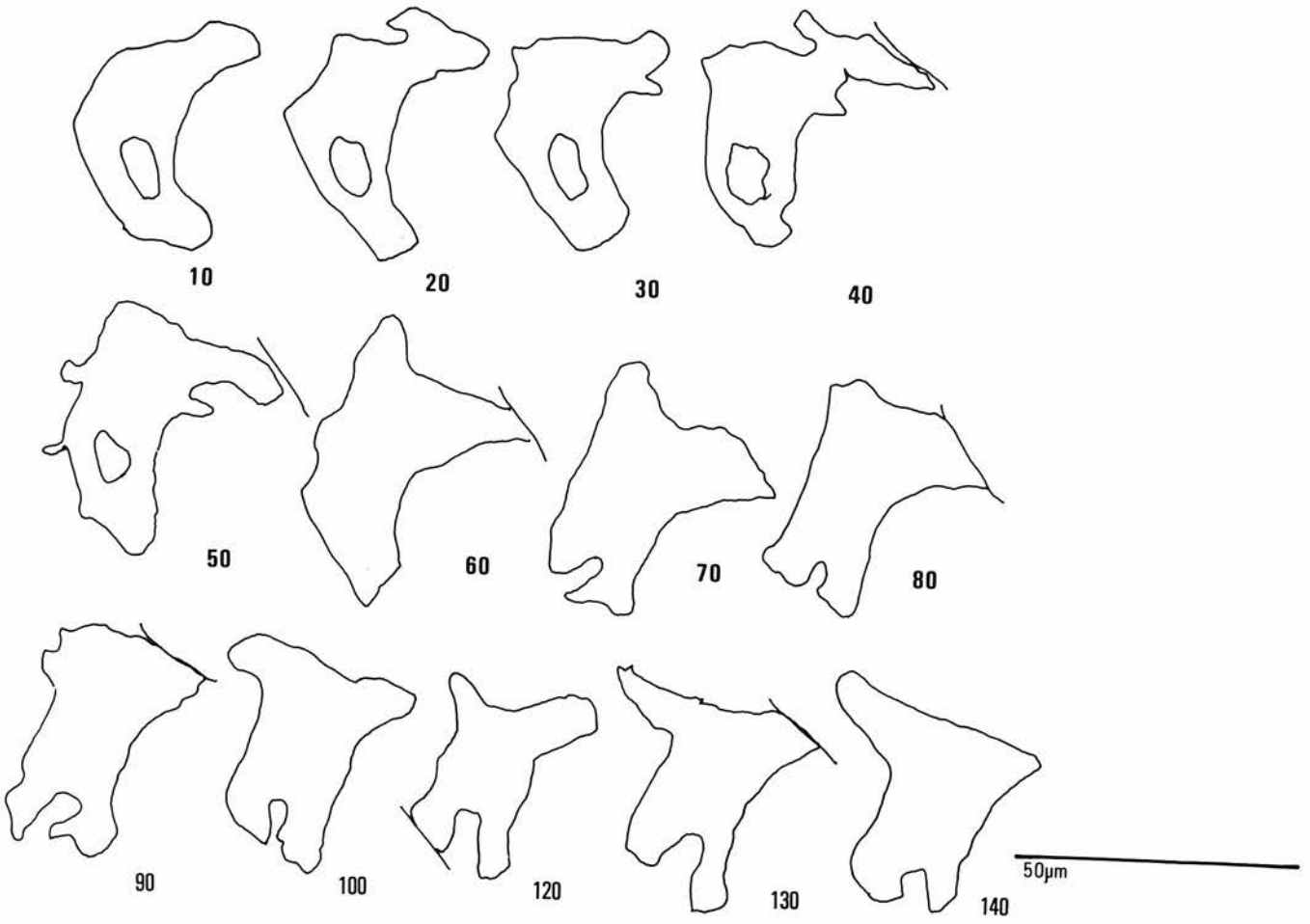


Figs 54-59 Pigmented Retinal Epithelium and Mesenchyme after 1 week in culture

Fig 54

The explant has produced a dense epithelial outgrowth which became depigmented toward its periphery (arrow). (10% F.C.S + 5% C.E.E., bf, scale bar = 100μm).

Fig 55

Fibroblasts and clumped brown melanocytes are present at the edge of the outgrowth. (ph, scale bar = 100μm).

Fig 56

After 1 week in culture, dendritic black melanocytes are present, even although their counterparts in mesenchymal cultures had retracted their processes. The are outlined is shown in fig 57. (si, scale bar = 100μm).

Fig 57

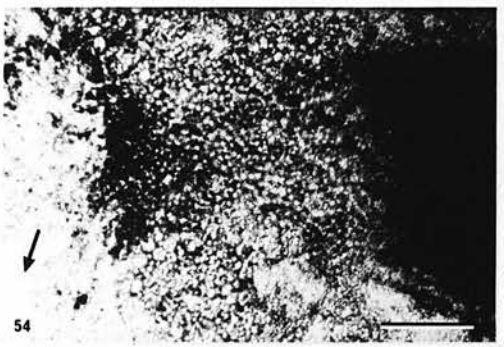
Phase illumination of the area outlined in fig 47 (scale bar = 100μm).

Fig 58

A dendritic melanocyte, in the centre of the field, has bulbous dendrite termini from which melanosomes are being shed (si, scale bar = 100μm).

Fig 59

Phase illumination of the same field. (ph, scale bar = 100μm).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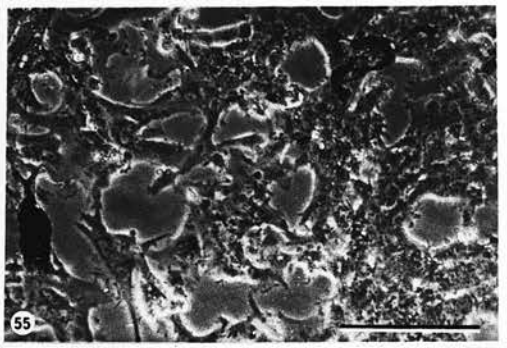
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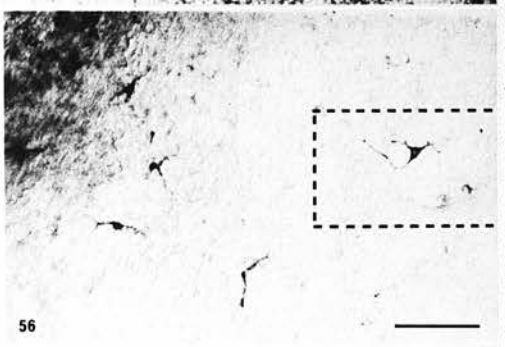
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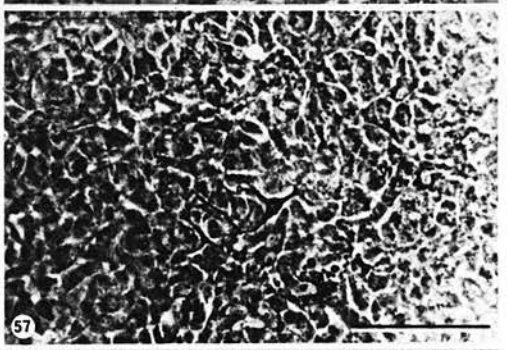
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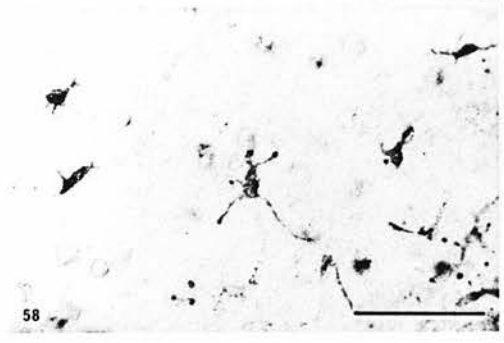
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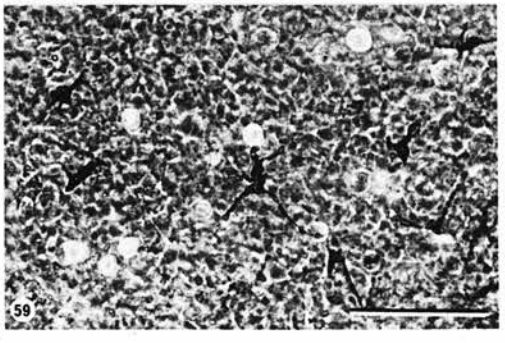
59

Figs 1-4 Figures Accompanying Chapter 4
Ocular Melanocytes In Vivo

Fig 1

Melanosomes in a 14 day Ciliary Body Melanocyte. (scale bar = 0.2μm).

Fig 2

Melanosomes in an 18 day Iridial Melanocyte. (scale bar = 0.2μm).

Fig 3

Transfered melanosome within 14 day epithelial cells of the corneal limbus. (scale bar= 0.4μm).

Fig 4

A Basal Melanocyte within the 18 day corneal limbal epithelium. (scale bar = 1μm).

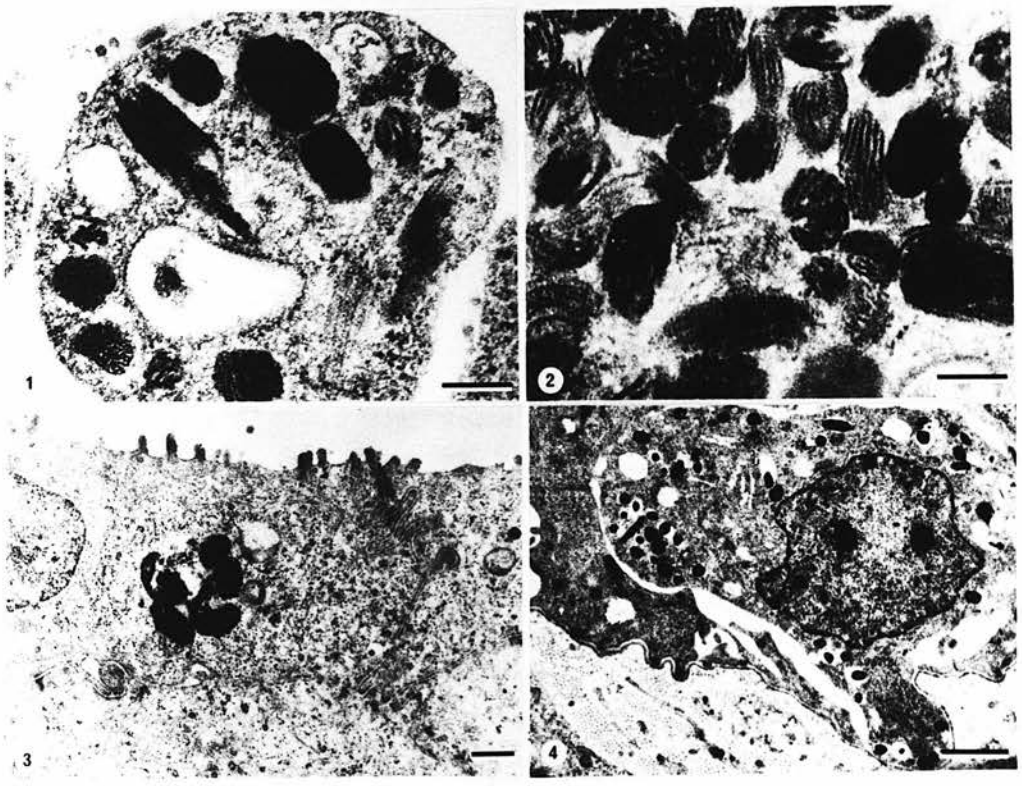
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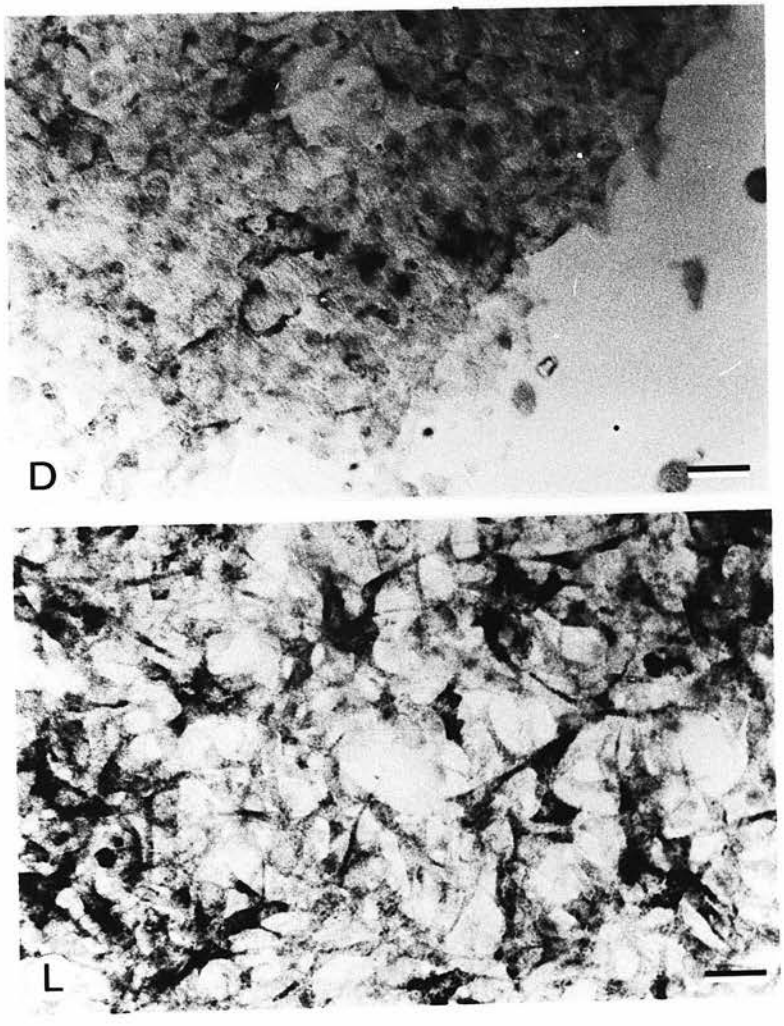
Fig 5 Dopa Staining of Premigratory Cultures Grown in medium supplemented with 10% serum

Fig 5

L, staining with L-Dopa, identifies many tyrosinase positive cells in largely unpigmented cultures. (scale bar = 20μm).

D, control stain with D-Dopa, shows no elevation of pigmentation above that already present. (scale bar = 20μm).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L

Fig 6

Early melanosomal stage containing a few vesicles and fine filamentous material. Taken from a melanocyte clump on the neural tube. (scale bar = 0.2μm).

Fig 7

Early melanosomal stages illustrating vesicle continuity with the melanosomal membrane arrow. (scale bar = 0.1μm).

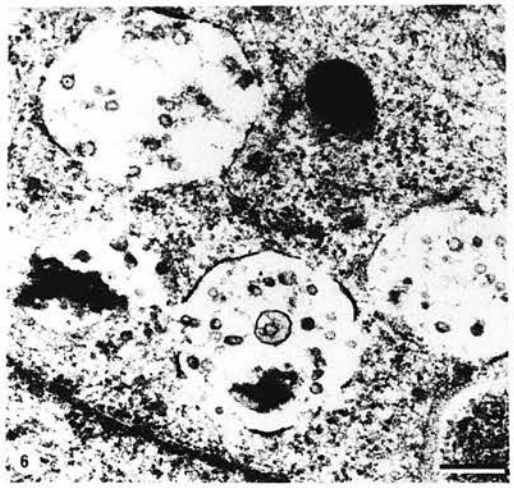
Fig 8

Golgi cisternae and coated vesicles (arrowed) in the vicinity of immature melanosomes. Taken from a 5 day culture. (scale bar = 0.2μm).

Fig 9

Melanosome showing preservation of the mitochondrial matrix. Taken from a 3 day culture. (scale bar = 0.1μm).

(Cells were cultured in medium supplemented by 10% F.C.S. + 5% C.E.E. for 3 or 5 days).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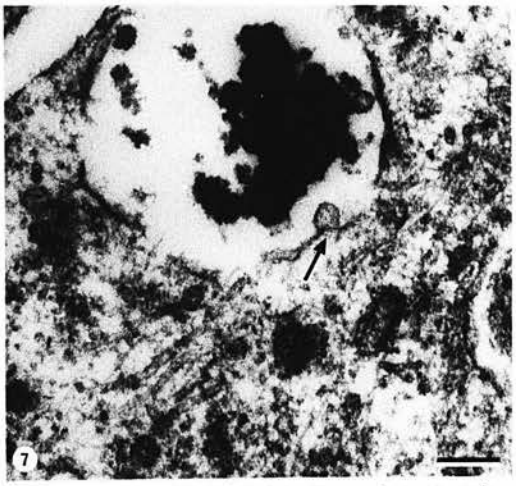
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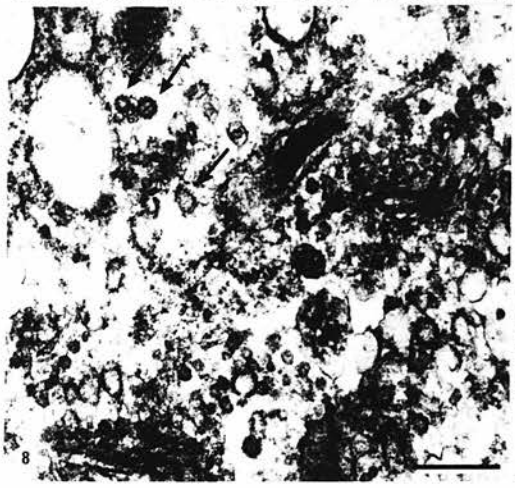
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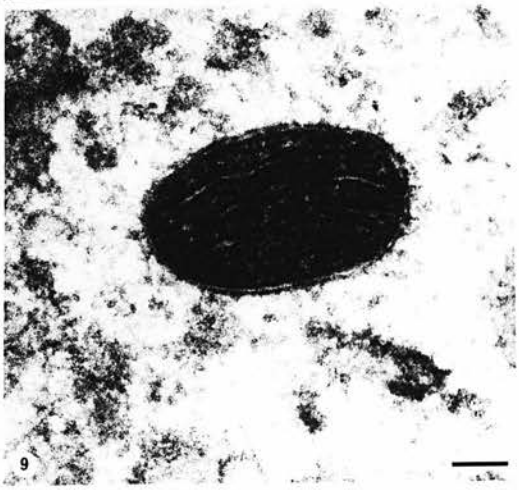
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Figs 10-14 Later Melanosomal Stages in Premigratory Crest Cultures

Fig 10

Immature melanosomes and more melanised granular melanosomes taken from a 3.5 day culture. (scale bar = 0.4μm).

Fig 11

A variety of melanosomal stages are present, most are granular and a minority are filamentous (5.5 days, scale bar = 0.5μm).

Fig 12

A variety of melanosomal stages are present, most melanosomes contain filaments, some of which are melanised. The filament distribution is disorganized and melanisation irregular. (3.5 days, scale bar = 0.7μm).

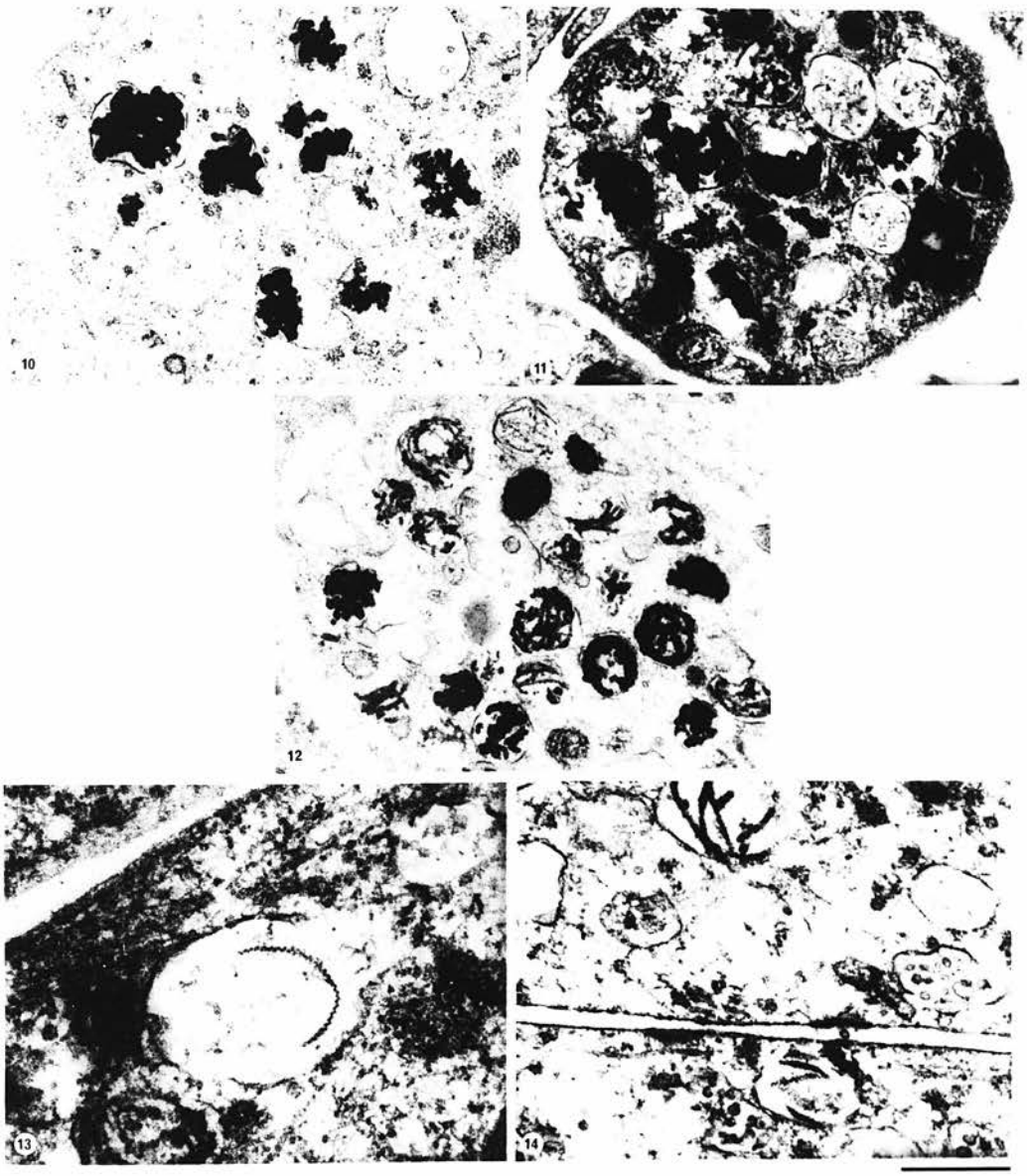
Fig 13

An unmelanised melanosomal filament has a spiral or repeat pattern around a central core. (3.5 days, scale bar = 0.3μm).

Fig 14

Melanosomes are seen here within 2 cells. The pattern within the filament is becoming obscured by melanisation. (scale bar = 0.35μm).

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.).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Figs 15-15 More Melanised Melanosomes after 35 days in culture

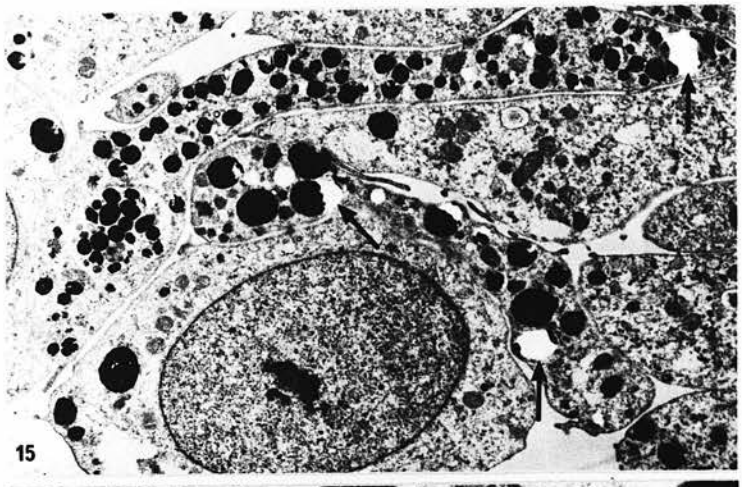
Fig 15

More dense round melanosomes are present. The holes within the section (arrowed) are a shearing artefect produced by cutting well-melanised organelles. (scale bar = 16μm).

Fig 16

A mixture of granular, and more melanised organelles with small holes (arrowed) are present. (scale bar = 0.22μm).

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.).



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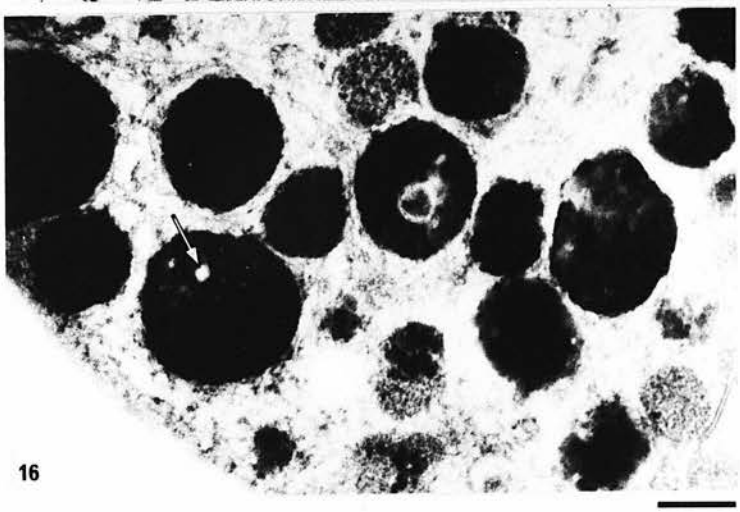

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Figs 17-21 Melanosomal Membrane Continuity and Melanosome Aggregation

Fig 17

Early melanosomal stages in close proximity to centrioles and golgi cisternae. A tubule-like extension of one melanosome is indicated (arrow). (3 day, scale bar = 1μm).

Fig 18

The melanosomal membrane sometimes appears continuous between melanosomes (arrows). (3.5 day, scale bar = 1μm).

Fig 19

An extremely large vesicle containing granular melanosomes appears to be in intimate contact with other melanosomes. (5.5 day, scale bar = 20μm).

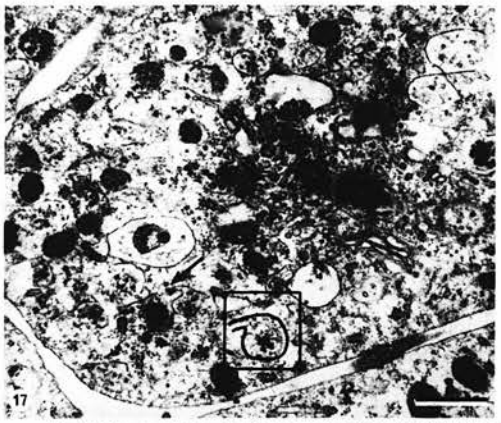
Fig 20

A bizarre collection of fused early melanosomal stages existing within a vesicle. The melanosomal mass appears to be membrane bound (arrows). (5.5 days, scale bar = 1μm).

Fig 21

A giant melanosome with some substructure. (scale bar = 1μm).

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.)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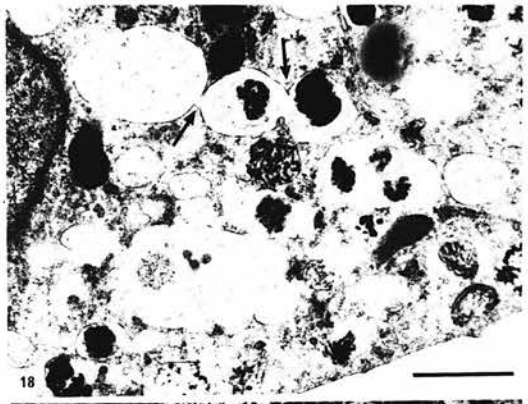
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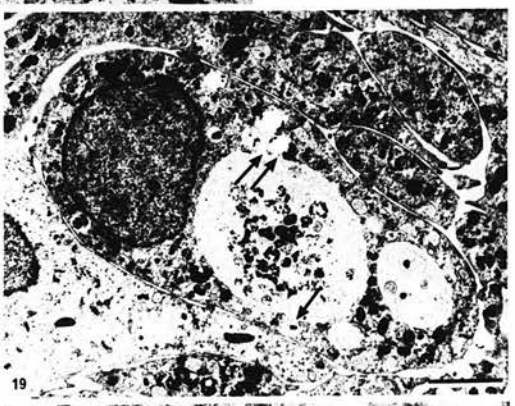
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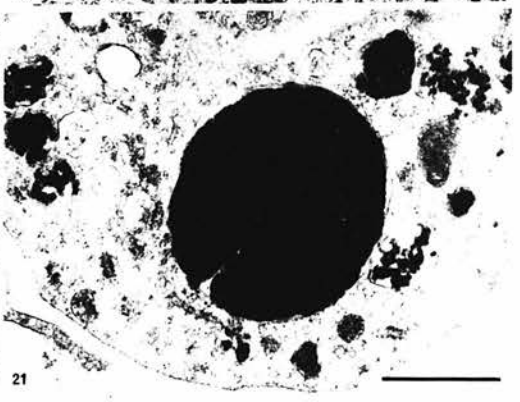
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Figs 22-25 Normality of the Golgi Apparatus and Tyrosinase Distribution

Fig 22

A centriole and golgi cisternae with vesicles budding from the 'trans' face (arrowed) in close proximity to an early melanosomal stage. (3.5 days, scale bar = 0.5μm).

Fig 23

A cilium recessed into the cell in the vicinity of the golgi apparatus. (3.5 days, scale bar = 0.2μm).

Fig 24

L-Dopa staining showing the presence of tyrosinase within golgi associated cisternae and within vesicles. (3.5 days, scale bar = 1.0μm).

Fig 25

L-Dopa staining of melanosome produced a dense reaction demonstrating similar levels of tyrosinase found in golgi associated cisternae. (3.5 days, scale bar = 0.5μm).

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.).


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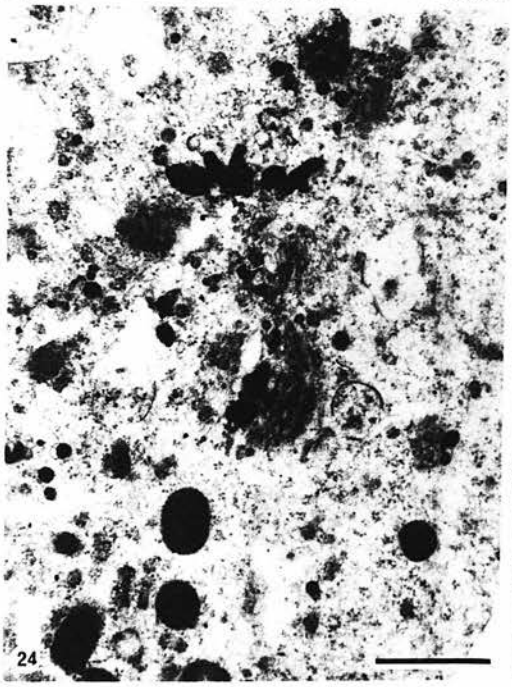
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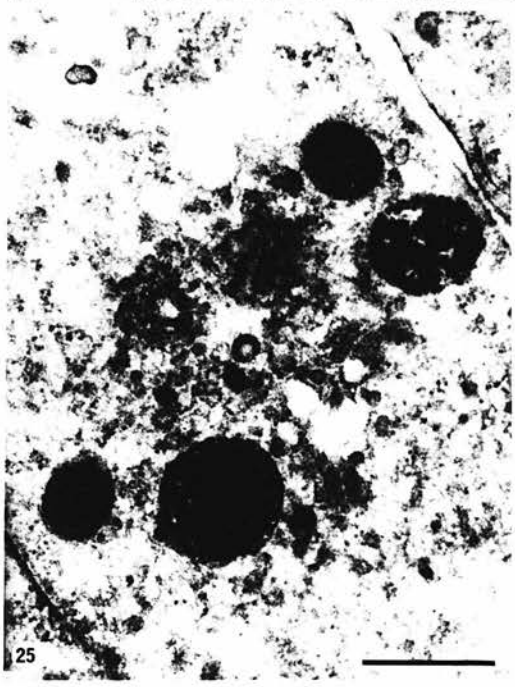
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Figs 26-28 Melanogenisis in Postmigratory Crest Cultures

Fig 26

Eumelanogenisis in amelanocyte within the explant. Most melanosomes are densely melanised after 4 days of culture. The less melanised organelles have a distinct filament array. (scale bar = 0.5μm).

Fig 27

Intermediate type pigmentation is present in cells migrating from the explants after 4 days of culture. Elongate densely melanised organelles co-exist with less dense granular type structures. (scale bar = 1μm).

Fig 28

A mixture of granular melanosomes and early melanosomal stages are present in a cell which differentiated at the periphery of the outgrowth. Even after 18 days the organelles are less dense than those found within the explant. (scale bar = 2μm).

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.).

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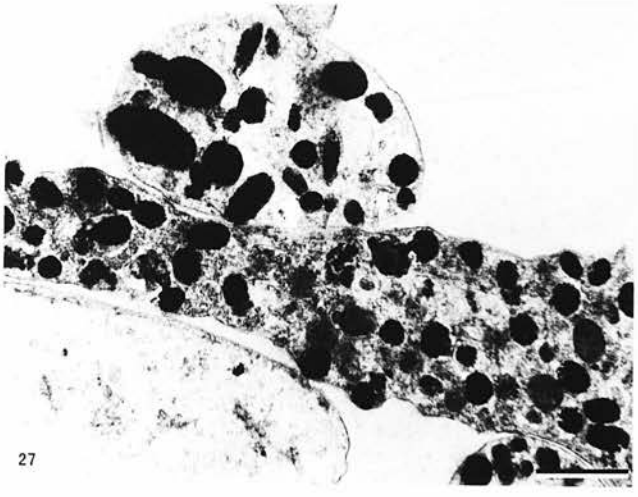

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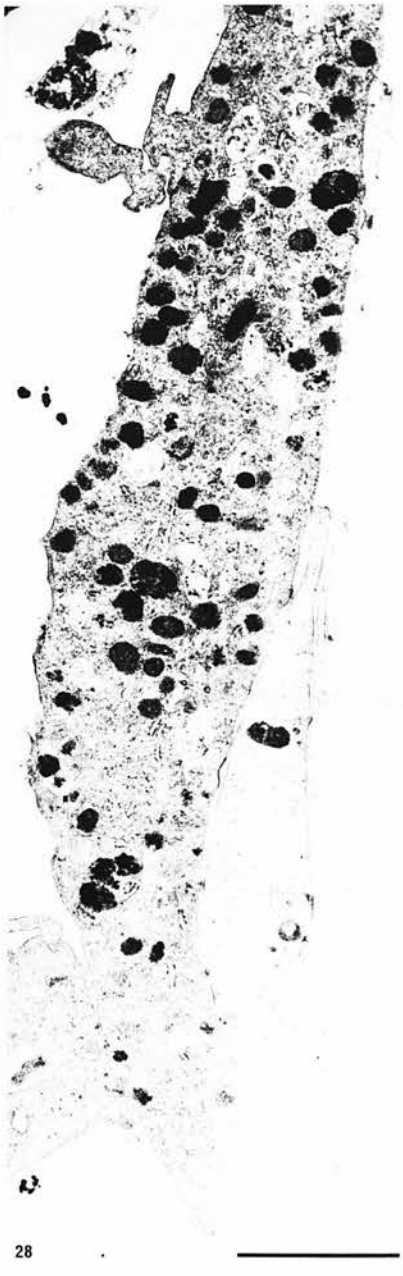


28

Figs 29-36 Electron Probe Microanalysis of Melanosomes

The 'spectra' shown opposite record the frequency (vertical axis) versus energy (horizontal axis) of X-ray emission from the samples. Frequency is depicted on an arbitrary scale (with variable expansion) in order to allow comparison of peak-to-background ration in each sample. The spectrum consists of a broad background of 'Bremsstahlung' or 'continuum' radiation, and peaks of discrete elemental spectra. The intensity of the 'continuum' is related to the mass of the volume anlysed (Moreton, 1981). Peak-to-background ratio was used as a simple measure of elemental concentration which is presented numerically in table 5/1.

Figs 29-30

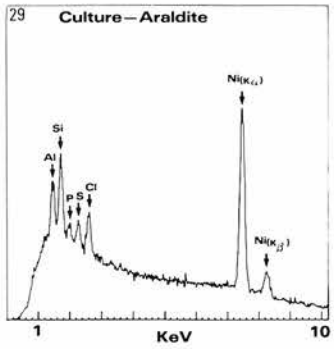
Show the background spectra of the araldite, cytoplasm and premelanosome.

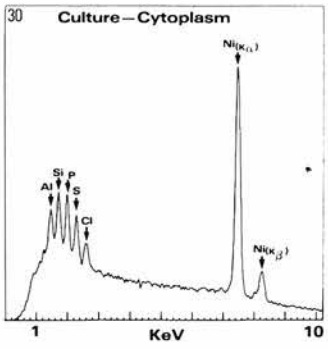
Figs 32-34

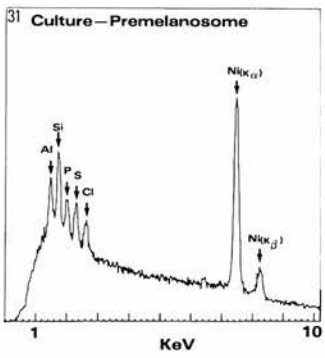
Show the spectra of 12 day retinal and neural crest melanosomes in vivo.

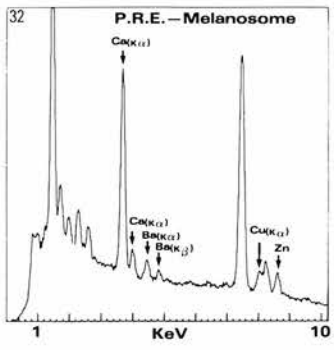
Figs 35-36

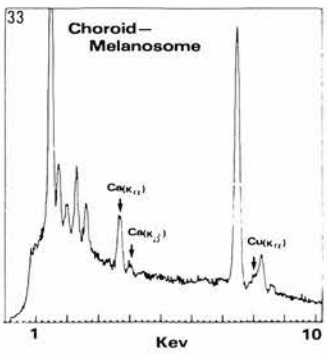
Show the spectra of melanosome from premigratory neural crest cells after 20 days in culture.

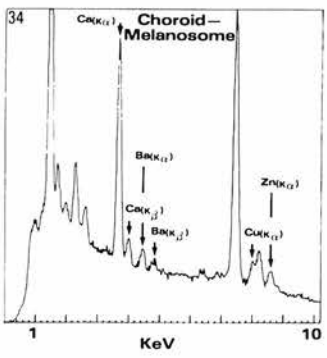

29
Culture—Araldite
Ni(Kα)
Si
Al
P
S
Cl
Ni(Kβ)
1
KeV
10

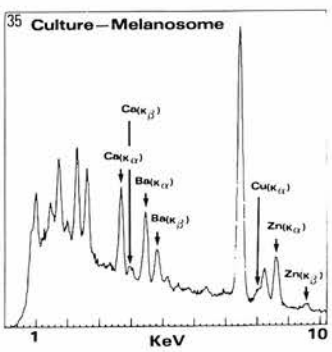

30
Culture—Cytoplasm
Ni(Kα)
Si
Al
P
S
Cl
Ni(Kβ)
1
KeV
10

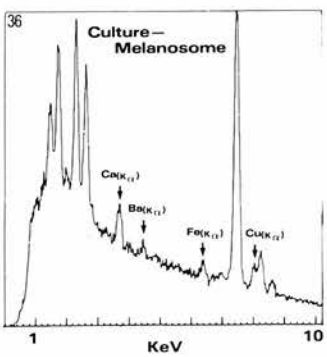

31
Culture—Premelanosome
Ni(Kα)
Si
Al
P
S
Cl
Ni(Kβ)
1
KeV
10


32
P.R.E.—Melanosome
Ca(Kα)
Ca(Kα)
Ba(Kα)
Ba(Kβ)
Cu(Kα)
Zn
1
KeV
10


33
Choroid—
Melanosome
Ca(Kα)
Ca(Kβ)
Cu(Kα)
1
Kev
10


34
Choroid—
Melanosome
Ca(Kα)
Ba(Kα)
Ca(Kβ)
Ba(Kβ)
Zn(Kα)
Cu(Kα)
1
KeV
10


35
Culture—Melanosome
Ca(Kβ)
Ca(Kα)
Ba(Kα)
Ba(Kβ)
Cu(Kα)
Zn(Kα)
Zn(Kβ)
1
KeV
10


36
Culture—
Melanosome
Ca(Kα)
Ba(Kα)
Fe(Kα)
Cu(Kα)
1
KeV
10

FIGURES ACCOMPANYING CHAPTER 5

Figs 1-11 Explantation of Corneas around the Time of Stromal Colonisation

Fig 1

An unpigmented stage 29 cornea after 24 days in culture, showing prolific fibroblast outgrowth with a necrotic phase dense explant (arrow) and large fibroblast aggregates. (ph, scale bar = 100um).

Fig 2

An exceptional stage 28 explant after 1 week in culture containing dense brown pigment (although no dendritic melanocytes are visible), and a pigment clump (arrow). (ph, scale bar = 65μm).

Fig 3

A stage 27 outgrowth, after 20 days in culture, containing many stellate melanocytes as well as more fibroblastic cells. (ph, scale bar = 40μm).

Fig 4

A stage 28 outgrowth after 20 days showing the largest group of melanocytes observed in such cultures. (si, scale bar = 65μm).

Fig 5

A stage 27 outgrowth after 20 days in culture, showing dendritic melanocytes overlapping each other in the process of aggregation. (si, scale bar = 40um).

Fig 6

The same field in phase illumination.

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.).

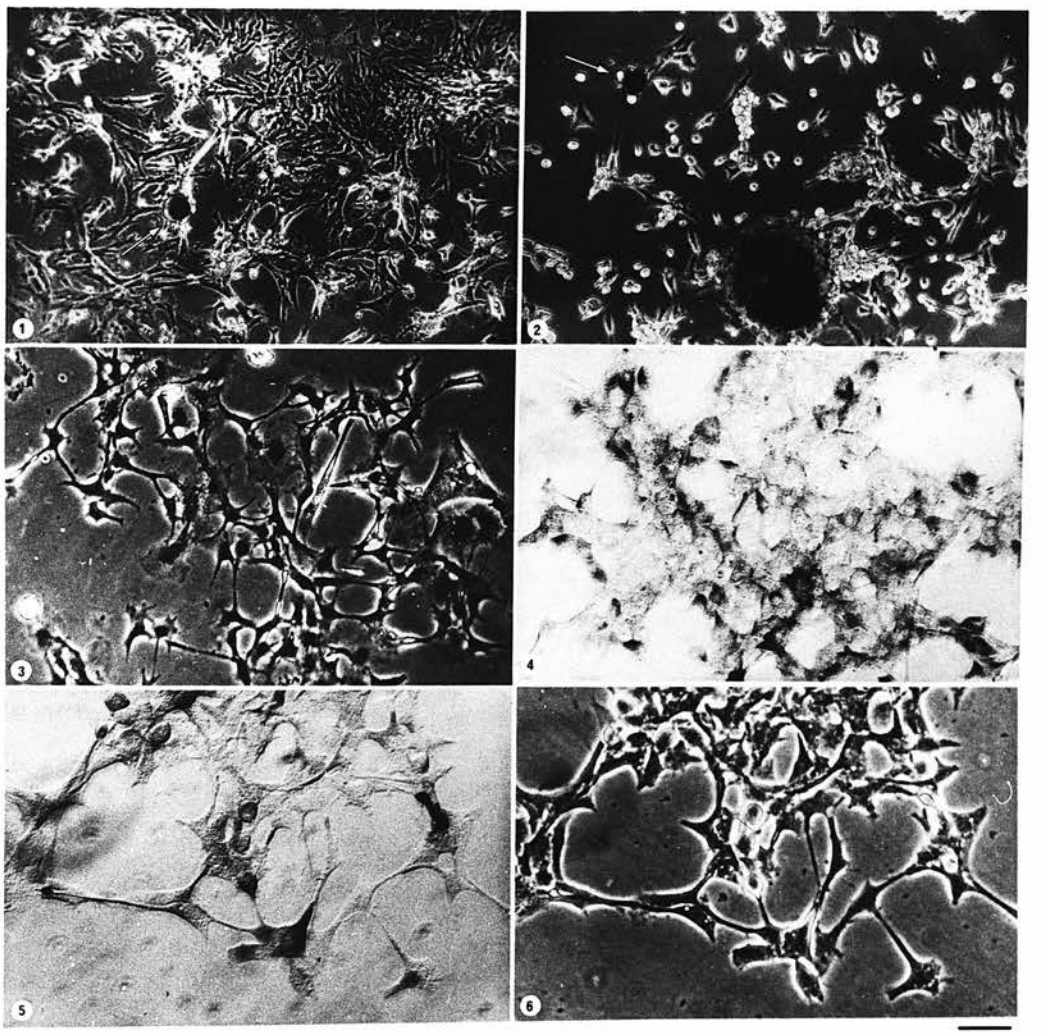

1
2
3
4
5
6

Fig 7

A stage 27 explant with an exceptional epithelial outgrowth after 3 days of culture. (ph, scale bar = 160μm).

Fig 8

The same culture 3 days later showing how the epithelial sheet became detached. (ph, scale bar = 160μm).

Fig 9

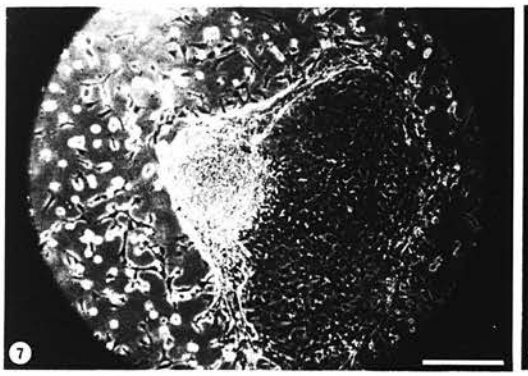
A histological section of a stage 28 cornea whose age was assessed using the same criteria as that in culture experiments. Not all of the corneal stroma is colonised at that stage. (scale bar, approx 100μm).

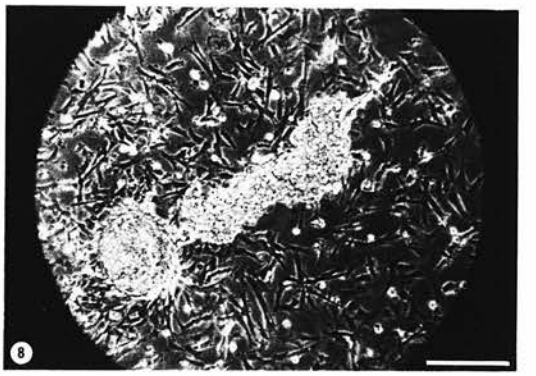
Fig 10

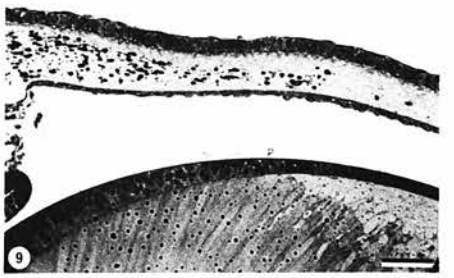
'Control' fibroblasts from a 6 day heart after 1 week in culture. (scale bar = 40μm).

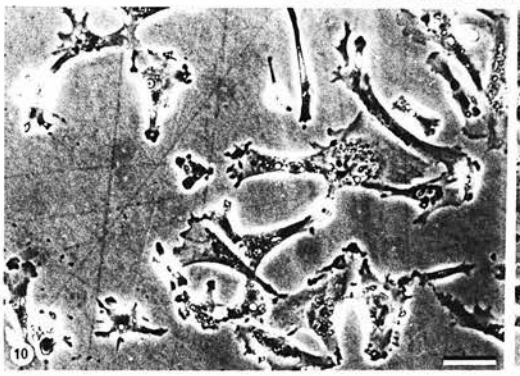
Fig 11

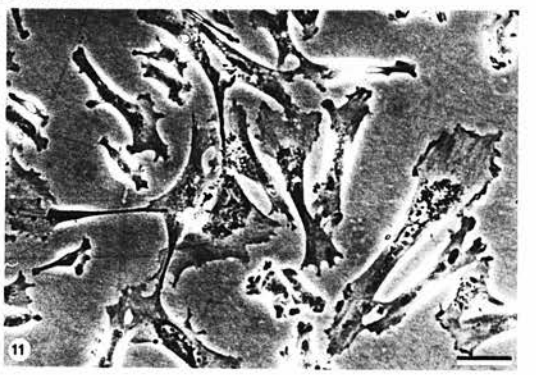
'Control' fibroblasts from 18 day cornea after 2 weeks in culture. (scale bar = 40μm).











Figs 12-13 The Ultrastructural Appearance of the "Corneal" Melanocytes

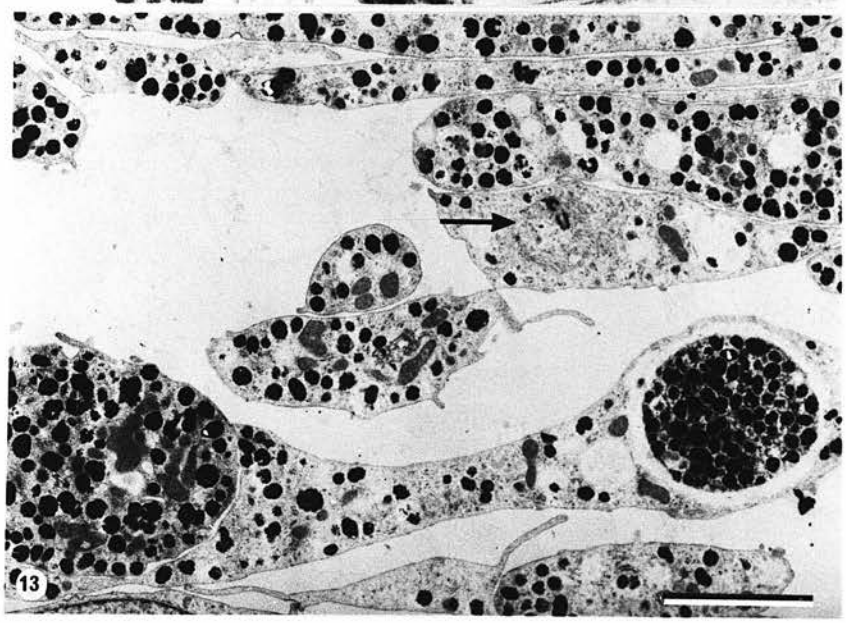
Fig 12

Granular, filamentous, and well-melanised organelles are present after 28 days in culture. (scale bar = 0.2μm).

Fig 13

Melanocytes have multilayered after 28 days, as shown in the light micrographs. Features typical of premigratory crest culture were observed, melanosome clumps (small arrow), and golgi apparatus plus centrioles. (scale bar = 3μm).

12



13

Figs 14-19 Cell "Coats"

Fig 14

Fibroblasts from the outgrowth of a stage 30 periorbital mesenchyme explant possess prominent halos after 9 days in culture. (si, scale bar = 50μm).

Fig 15

Phase illumination of the same field. (some RBCs have become suspended). (ph, scale bar = 50μm).

Fig 16

Fibroblasts in a stage 27 outgrowth after 6 days in culture, showing retraction processes and coat material deposited on the substratum. (ph, scale bar = 100μm).

Fig 17

Fibroblastic cells from a 6 day dorsal root ganglian explant, after 28 days in culture. (ph, scale bar = 50μm).

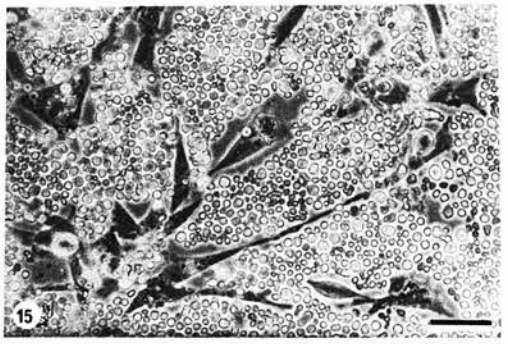
Fig 18

Neural crest melanocytes after 7 days in culture have no coats. (ph, scale bar = 50μm).

Fig 19

Unpigmented neural crest cells after 7 days in culture have no coats. (ph, scale bar = 50μm).


14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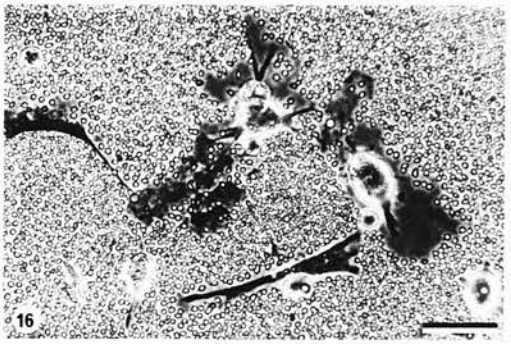
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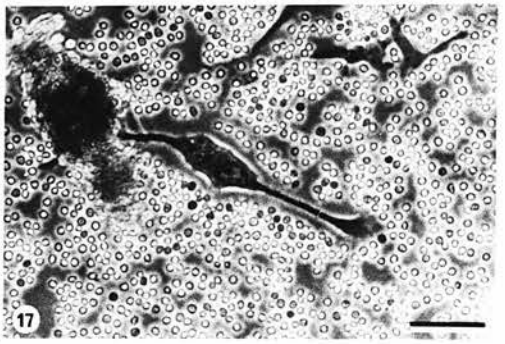
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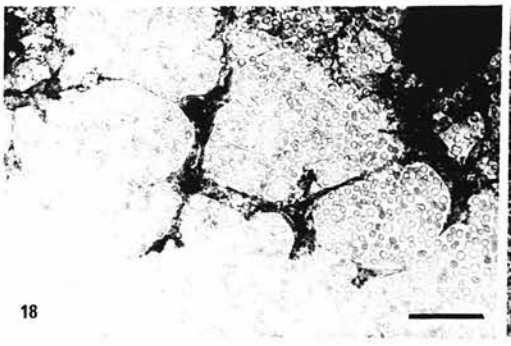
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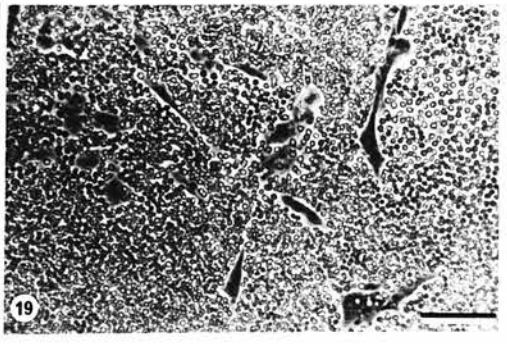

19

Fig 20

18 day Corneal Fibroblasts embedded within the dense collagenous stroma. Note prominent rough endoplasmic reticulum. (scale bar= 2μm).

Fig 21

Corneal Fibroblasts from a stage 28 embryo after 18 days of culture. Note the dense osmophilic lipofuscin-type vesicles, and the complex leading edge (or ruffling membrane) profile. (scale bar = 4μm).

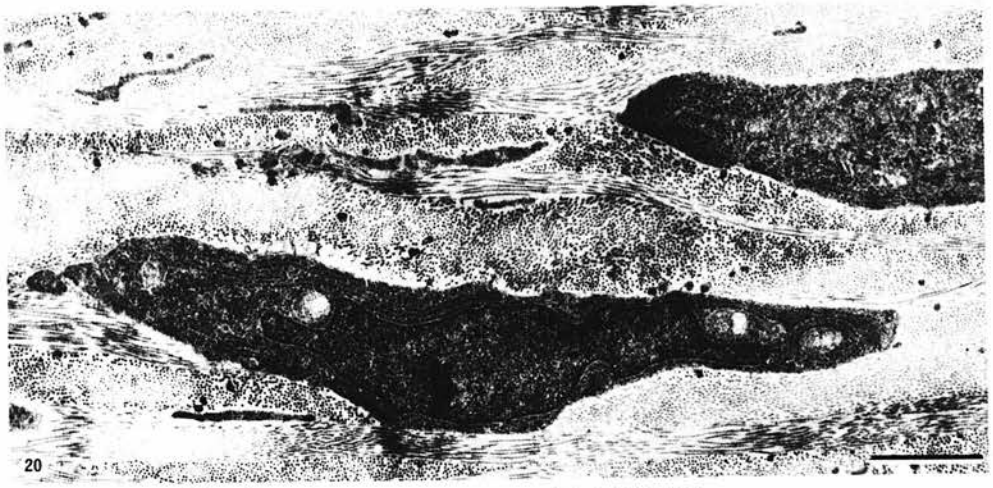
Fig 23

Ruffling membrane profile of the fibroblast (same source as above) with complex membrane folding and pronounced microfilament bundles. (scale bar = 4μm).

Fig 24

An apically running microfilament bundle traverses the cytoplasm and makes contact with basal membrane at a point of peripheral substratum attachment. (scale bar = 4μm).

(Cells were grown in medium supplemented with 10% F.C.S. + 5% C.E.E.



20

21

22

23

Figs 1-3 Figures Accompanying Chapter 6

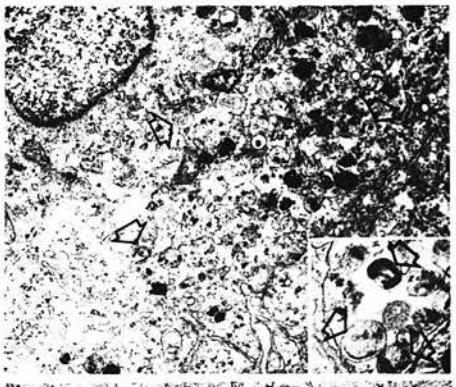
The Ultrastructure of Human Melanoma taken from Hunter et al. (1978).

Fig.1

Type I cell with numerous relatively normal ellipsoidal melanosomes. Occasional abnormal melanosomes (open arrows) are seen. Arrow points to autophagic vacuole (× 16,000).

Inset: Ellipsoidal melanosomes in Type I cell. Filaments show cross striation with periodicity of 80A (× 57,000)

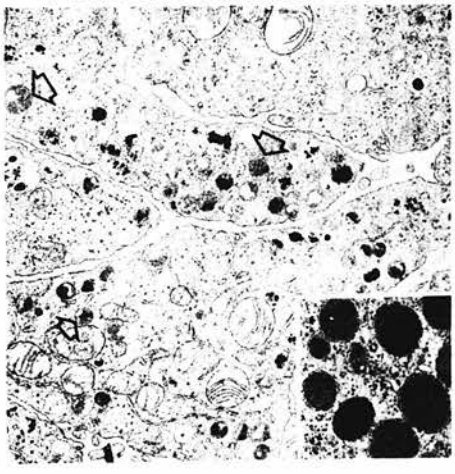
Fig.2



Type II cell containing numerous abortive melanosomes (open arrows). Filaments in these are orientated in a haphazard manner and there is no cross linking. Irregular pigment deposition is seen in many melanosomes (× 21,000).

Inset: Abortive melanosomes are arrowed (× 32,000)

F'g.3



Type III cell containing numerous granular melanosomes (arrows) as well as occasional abortive and lamellar organelles. Random pigment deposition is seen in many melanosomes (× 16,000).

Inset: granular melanosomes. There is pigment deposition in some (× 37,000).

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