David McLeod

- Training in Ophthalmology in Edinburgh and at Moorfields Eye Hospital in London.
- Appointed Consultant Surgeon at Moorfields in 1977 where he helped to develop the Ultrasound, Vitreoretinal Services and clinical and laboratory research.
- Chair in Ophthalmology at Manchester University.
- Honorary Consultant to the Royal Eye Hospital, First Clinical Director (1991-4).
- Area of Research: Diabetic Retinopathy.
- First generic Masters Degree Course in the ophthalmic sciences worldwide.
- Vice President of the Royal College of Ophthalmologists from 1997 till his resignation in 2001.
MREH 200

“The enigma of retinal capillary closure”

David McLeod
Looking back ... 

... historical context

... different perspective
RCC predicted by Isaac Michaelson (Glasgow) 1948

• mechanism of retinal neovascularisation in the mature eye extrapolated from vascularisation of the developing retina

• quote: “Just as the metabolic needs of embryonic retinal tissue demand closer proximity of capillary vessels, so does the disturbed metabolism of certain retinal diseases call for the accession of vessels to insufficiently or non-perfused situations, intraretinal, preretinal or vitreous”.

• biochemical mediator or “vessel growth promoting factor” (VGPF) = VEGF
RCC discovered by Norman Ashton 1950/53

- the first description of retinal capillary closure was in a paper in BJO 1953 entitled: “Arteriolar involvement in diabetic retinopathy”.
- India ink injected by microcannula into the CRA + CRV in excised post-mortem eyes of known diabetic patients
- findings suggestive of occlusion at pre-capillary level (not venous occlusion) consistent with current leucocyte adhesion theory.
- well-cited paper but not the first documentation of diabetic RCC
RCC discovered by Norman Ashton 1950/53

- RCC first illustrated in BJO 1950 – in Ashton’s methodology paper updating Michaelson’s technique for India ink injection of the retinal circulation
- injected retina was isolated and placed inside a glass hemi-sphere
- absence of ink from much of the capillary bed was attributed to incomplete filling (i.e. artefact), and not to diabetic pathology. Why
- Ashton was looking for retinal microaneurysms after their recent re-discovery by Ballantyne in Glasgow (working with Michaelson)
RCC discovered by Norman Ashton 1950/53

- description of RCC in "Arteriolar involvement in diabetic retinopathy"
  - arteriolar obliteration "leads to the disappearance of entire capillary bed"
  - "Arteriolar involvement ... would appear to be almost entirely responsible for the complete obliteration of the capillary blood supply to the retina."
  - "arteriolar changes merely represent a late stage in the development of the retinal disease"

- relationship of RCC to neovascularisation was apparently overlooked because many of the eyes with RCC had no neovascularisation! ? Why
RCC discovered by Norman Ashton 1950/53

• puzzle = why was relation of RCC to NV not initially realised?
  – ? many eyes of elderly diabetic patients have PVD, so no disc or preretinal neovascularisation develops (integrin-dependent “coupling” of NV to ECM)
  – ? vitreous new vessels were physically “delaminated” from retinal surface during specimen preparation (“frozen” vitreous removed, as in Ashton’s kitten ROP model)
  – ? counterintuitive, since the non-perfused tissue was assumed to be anoxic/necrotic, not “hypoxic”; no histopathology reported in area of RCC
Well-known relationship of RCC to ocular neovascularisation aided by introduction of FFA

- **Nature of relationship**
  - hypoxia via VEGF & HIF-1
  - proportionate/quantitative/cumulative
  - proximate/local & remote NV
  - directional/chemotactic response
Well-known relationship of RCC to ocular neovascularisation - aetiology

- ROP/OIR, PDR, RVO, (RAO), ocular ischaemic syndrome, HbSC
- similar FFA appearance whatever the aetiology
Well-known relationship of RCC to ocular neovascularisation – sites of NV

- NVE, NVD, (AHFVP)
- rubeosis iridis is common final path, but differences in time course
Well-known relationship of RCC to ocular neovascularisation – mechanisms of closure

- **ROP/OIR** - vaso-obliteration of immature vasculature from oxygen “flooding” into inner retina from choroid as pO2↑; then vasoproliferation as pO2↓
- **PDR** – leucocyte adhesion & arteriolar obstruction
- **RVO** – conversion from “non-ischaemic” to “ischaemic” ? via intracapillary stagnation thrombosis or ? (myogenic) arterial constriction
- **RAO** – upstream occlusion with no recanalisation
- whatever the mechanism, same “hypoxia-related” behaviour of NV eg proportionality, chemotaxis etc
How is “hypoxic” tissue generated?

- the territory of an artery can be represented as a “cone” with apical penetration by the artery which then ramifies as the cone expands
- in thin organs like retina, a tissue section or “wedge” is the equivalent model of an arterial territory supplied with nutrients and oxygen
- complete arterial occlusion results in necrosis involving its entire territory of supply ( = “physiological end-artery”)
- **progressive arterial occlusion** has a **graduated effect** on tissue perfusion and metabolism → ordered normoxic, *hypoxic* and anoxic compartments
How is “hypoxic” tissue generated?

- complete arterial obstruction with proximal arterio-arterial collaterals of varying capacity will have the same graduated effect on tissue perfusion within cone – same order of compartments as partial arterial occlusion
  – eg optic nerve pial plexus after orbital CRAO

- complete arterial occlusion with distal collateral supply of blood circulation to, or oxygenation of, the non-perfused tissue
  – reverse order of compartments generated
  – eg leptomeningeal anastomoses in cerebral cortex after cerebral arterial occlusion/stroke
  – eg after CRAO and in areas of retinal capillary closure, the choroid is able to supply oxygen and nutrients to the inner retina under certain conditions and act as a distal collateral oxygen resource
How is “hypoxic” tissue generated?

• in most retinal neovascular disorders, it isn’t the graduated reduction of inner retinal perfusion that determines the generation of “hypoxic” tissue
• rather, the tissue receives no blood circulation from the intended source – the retinal capillaries are completely closed whether permanently or reversibly
• the degree of retinal hypo-oxygenation depends on the capacity of the distal collateral supply from the choroid
• that is to say, the “essential driver” of vitreous and iris neovascularisation is the choroid
What are the properties of hypoxic neural tissue?

- hypo-oxygenated cells (tissue pO₂ ≈5mmHg)
- hypometabolic cells, but in a state of energy homeostasis (ie ATP stores conserved)
- “electrically silent” neurons, but in a state of ionic homeostasis (ie cells remain polarised)
- rapid electrical/functional recovery on being reperfused eg by arterial recanalisation
- secretes VEGF etc if ischaemia persists (pO₂ sensing by HIF-1) ie angiogenic propensity
- in cerebral stroke, the hypoxic compartment (ischaemic penumbra) self-destructs in a matter of a few hours (by an apoptotic cell-death pathway) and no longer stimulates angiogenesis
Molecular signature of hypoxia on histology

- *in situ* hybridisation of tissue sections using VEGF riboprobes
- Overexpression of VEGF mRNA gives white signal in dark field
- Middle-retinal hypoxic tissue localised by gene expression in OIR, RVO etc
What does “hypoxic” retina look like?

- after nearly half a century of looking at the retina (including during vitrectomy procedures), I freely admit that I’m not at all sure
- “normal” appearance? swollen? semi-translucent? OCT correlate
- this question has never been answered (and is hardly ever asked)
- an ongoing enigma since 1948!
We all know what **anoxic** retina looks like!

- normoxic and anoxic retina eg in cilioretinal infarction and CRAO
What can central retinal artery occlusion tell us about retinal hypoxia?

• ischaemic anoxia of inner retina
  = “cloudy swelling”/oncosis
  = irreversible “membrane failure”/“anoxic depolarisation” once survival time (2 hr) expires
• opacification extends beyond macula as translucent retina to include a circular area >30 degrees in radius from fovea
• post-equatorial and peripheral retina remain transparent
What can central retinal artery occlusion tell us about retinal hypoxia?

- **ERG in experimental CRAO** – 3 classic studies revisited:
  - Hamasaki & Kroll 1968: temporary CRAO in 9 squirrel monkey eyes
  - Hayreh & Weingeist 1980: “” in 63 young rhesus monkeys
  - Hayreh et al 2004: “” in 38 elderly arteriopathic monkeys
What can central retinal artery occlusion tell us about retinal hypoxia?

- **ERG in experimental CRAO**
- **scotopic conditions**
- **classic “negative” ERG**
  - major reduction in b-wave
  - a-wave is preserved (so rods must be functioning normally)
- **“electrical silence”** affects 75% of the middle-retinal tissue volume
- 25% of b-wave is preserved (so 25% of middle-retinal tissue volume must be functioning normally = “normoxic” tissue compartment
What can central retinal artery occlusion tell us about retinal hypoxia?

- **ERG in experimental CRAO**
- **recovery of b-wave after CRA reopens**
  - if CRA reopens after up to 1-1.5 hr of CRAO, the b-wave recovers to 100% of its pre-CRAO amplitude (= “TIA”)
  - if CRA reopens after > 2 hr of CRAO, the b-wave recovers from residual 25% to 50% of pre-CRAO amplitude in presence of permanent inner retinal infarction = anoxia “survival time” has been exceeded but only 50% of the middle-retinal tissue volume is dead
- **the anoxic tissue compartment in the posterior pole comprises 50% of the middle-retinal tissue volume**
What can central retinal artery occlusion tell us about retinal hypoxia?

• scotopic ERG in experimental CRAO
• 50% of b-wave represents middle-retinal tissue volume that is not functioning at all after 2 hr of CRAO ie “anoxic” tissue compartment in the posterior pole
• 25% of b-wave represents middle-retinal tissue volume that is functioning normally ie “normoxic” tissue compartment in the retinal periphery
• the remaining 25% of middle-retinal tissue volume recovers quickly on CRA reopening whatever the duration of CRAO = “hypoxic” tissue compartment in the retinal mid-periphery
• marginally oxygenated ischaemic penumbra
• NB penumbra provides angiogenic stimulus for rubeosis iridis within 1-3 months in 15-20% of eyes with CRAO if the CRA doesn’t recanalise and no collaterals develop
What is the mechanism of “marginal oxygenation" of mid-peripheral inner retina?

- Oxygenation of non-perfused inner retina by choroid is subject to a “metabolic oxygen barrier” imposed by several energy-expensive retinal layers.
- These include the photoreceptor inner segments and plexiform/synaptic layers – OPL & IPL (each layer staining heavily for cytochrome oxidase).
- The height of the barrier provided by rods and cones varies with eccentricity from the fovea, rod density reducing with increasing distance beyond the “rod ring.”
What is the mechanism of “marginal oxygenation" of mid-peripheral inner retina?

- choroidal oxygenation of the retina depends on the high pO$_2$ within the choroidal vessels (including arteries, choriocapillaris and veins)
- high volume flow in choroid and low oxygen extraction fraction (OEF) ensure that a high pO$_2$ is maintained throughout the extent of the choroid
- this contrasts with the reduction in the height of the metabolic barrier with increasing eccentricity from the fovea
What is the mechanism of “marginal oxygenation" of mid-peripheral inner retina?

- during CRAO or in areas of permanent capillary closure, a graduated flow of oxygen from the choroid penetrates the metabolic oxygen barrier
- in the **posterior pole**, no oxygen is available to supply the inner retina as it is entirely consumed within the photoreceptor inner segments and synaptic terminals in OPL
- in **mid-peripheral retina**, inner retinal neurons survive albeit they are hypo-oxygenated
- in the **retinal periphery**, oxygen penetrates the barrier with sufficient residual tension (pO2) to allow normal cell metabolism and function to continue
Implications for “smart” laser PRP of RCC

- therapeutic effect achieved through destruction of photoreceptor inner segments (i.e., focal reduction in the height of metabolic oxygen barrier)
- try to minimise local tissue damage and the inflammatory response arising
- only treat areas of capillary closure
- **only treat hypoxic/penumbral retina** within the areas of capillary closure
- ? how to identify hypoxic inner retina, especially given that the hypoxic zone can be predicted to migrate peripherally in case of choroidopathy e.g., in PDR
- an ongoing enigma
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