REVIEW OF LITRATURE
DIABATIC RETINOPATHY

Diabetes mellitus is a disorder of carbohydrate metabolism characterized primarily by hyperglycaemia and glycosuria with secondary anomalies of the metabolism of proteins and fats.

Diabetes was known to mankind since antiquity. Thus a disorder with 'Honeyed urine' appears in ancient Sanskrit literature, while Egyptian papyri of ebers dating from 1550 B.C. contain dietary remedies for those passing abundant urine. The first clear account of diabetes is given in writings of Aretaeus of Cappadocia (170 A.D.) who described this mysterious affection as 'melting down of flesh and limbs into urine, life is short, disgusting and painful, thirst unquenchable and death inevitable' (Bloom & Ireland 1980).

Lot of work has been done on this disease and only in 1921 Frederick grant banting and Charles Herbert best in Toronto, discovered and isolated
insulin, the active principle of β cells of islets, of Langerhans and it's relationship to Pancreas was established (Duke Elder 1967). Many studies have subsequently thrown more and more light on this disease and it's complications but exact etiology is still not fully clear.

Diabetic retinopathy, an unfortunate complication of Diabetes mellitus, is becoming more and more common affecting all age groups, predictable but not preventable, relatively untreatable, chronic and progressive in it's course and leading to blindness in quite a large number of cases, has been known since last century (Duke Elder 1967). Von Jaeger (1956) was first to describe fundus changes in retina in sufferers of diabetes mellitus and Hira Chberg (1990) observed many changes and described them in detail in his paper.

The microangiopathic lesions in diabetes in the form of alterations in the walls of capillaries accompanied by deposits of hyaline from which the typical appearance of microaneurysms, haemorrhages and exudates results, were described by Ballantyn and Lovenstein (1943).
PREVALENCE

According to Duke Elder (1967) the reported incidence of diabetic retinopathy in diabetic patients depends upon the age of onset of diabetes, the length of it's duration, the control of glycosuria and above all the diligence of observer in searching for early lesions. Before the introduction of insulin, many diabetics died before the occurrence of retinopathy. But now the survival rate in diabetics is steadily growing, hence the frequency of diabetic retinopathy is increasing and statistics gathered before insulin are irrelevant from a comparative point of view.

RELATION BETWEEN INCIDENCE OF RETINOPATHY, AGE OF ONSET AND DURATION OF DIABETES

Burditt et al. (1968) have described the prevalence of retinopathy (Percentage) as under:

<table>
<thead>
<tr>
<th>Age at diagnosis(yrs.)</th>
<th>0-9 yrs.</th>
<th>10-14 yrs.</th>
<th>15 yrs. and above</th>
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<tr>
<td>Less than 30</td>
<td>10</td>
<td>45</td>
<td>73</td>
</tr>
<tr>
<td>30-99</td>
<td>30</td>
<td>53</td>
<td>68</td>
</tr>
<tr>
<td>60 and above</td>
<td>37</td>
<td>44</td>
<td>79</td>
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They observed that:

i) **Age at diagnosis and duration of diabetes** are the main determinants of the frequency of retinopathy. Progression is commoner and regression less common with advancing age at diagnosis except that the development of neovascularisation, glial proliferation or vitreous haemorrhage is rare in patients over 60 at the time of diagnosis.

ii) A lower glycosuria percentage (2% or less) is associated with lower frequency of retinopathy in patients under sixty at diagnosis but not with any difference in the behaviour of established retinopathy.

iii) Reduction in the subsequent chance of development of retinopathy is especially associated with a lower glycosuria percentage in the first five years after diagnosis of diabetes.

This was further supported by Kahn and Brodley (1973) the relationship of diabetic retinopathy to duration of diabetes was observed by Delandeva et al. (1981) who studied 797 cases of diabetes amongst whom 105 patients (25.3%) had
diabetic retinopathy. In their study prevalence of retinopathy rose from 6% in diabetics lasting less than one year to 75% in diabetics followed up for more than 15 years. The average interval between diagnosis of diabetes and occurrence of diabetic retinopathy was 7.3 years.

In a survey by Klein R. (1987) (The epidemiology of diabetic retinopathy, findings from wisconsin epidemiologic study) more than 10,000 patients were surveyed and it was found that despite medical management, nearly all younger onset diabetics who take insulin have diabetic retinopathy after 25 years of disease.

Those develop diabetics later do not have as high a incidence. Children have comparatively low risk but after puberty risk increases with duration of diabetes.

Those having increased blood glucose level along with a higher blood pressure showed increased presence and severity of diabetic retinopathy.

According to Rodansky et al. (1982) poor glycemic control is the most important factor
in causing diabetic retinopathy. They further clarified that diabetologists have abandoned the familiar text book classification of diabetes into Juvenile onset and maturity onset. The currently used classification in U.K.; which had been adopted by American diabetes association and W.H.O. (National diabetes data group11979), divides the majority of diabetics into two groups.

**Type I** Insulin dependent diabetes mellitus (IDDM).

**Type II** Noninsulin dependent diabetes mellitus (NIDDM).

Abundant literature shows that these two polar forms of diabetes have separate etio-pathogenesis.

Bedansky et al. (1982) further clarified that proliferative retinopathy was associated with type I diabetes and diabetic neuropathy with type II diabetes. Reason for this segregation of retinal lesions with type of diabetic syndrome is unknown. It is possible that excursions in blood glucose level are wider and more erratic in type I than type II diabetics and this together with other more severe metabolic deviations may influence the retinal pathology. However older age of patients with type II diabetes
may it self be important; as macular disease in general is more common in older age group. This study also showed that the proliferative and macular lesions may each occur in both forms, duration of diabetes on presentation with retinopathy was longer in type I.

**RELATIONSHIP OF RETINOPATHY TO SEVERITY AND CONTROL OF DIABETES**

There is no constant relationship between severity of diabetes and occurrence of degree of retinopathy (Duke Elder 1967). The retinal lesions are observed in mild as well as severe cases. Prolonged glycosuria and poor control of diabetes may be more important factors, rather diet rich in fat and carbohydrates. But Carpenter and Taylor (1963) showed that along with duration and age at onset of diabetes, diet and number of injection of insulin per day were statistically significant.

West et al. (1988) showed that most important risk factor was duration of diabetes but retinopathy was related to plasma level of glucose also. Poor metabolic control considerably increases frequency of retinopathy. Valone et al. (1976) found thirteen
cases with poor diabetic control, of fourteen cases of proliferative diabetic retinopathy studied by them. In a study by Kingslay et al. (1983), six out of nine cases of adolescent diabetic retinopathy had a long standing history of poor diabetic control.

CLASSIFICATION OF DIABETIC RETINOPATHY

Hirschberg (1990) recognised three clinical types of diabetic retinopathy

1) Inflammatory
2) Haemorrhagic
3) Pigmentary

Ballantyn and Michaelsen (1946) divided it into five stages.

1) Microlesions
2) Macrolesions
3) Vascular changes
4) Destructive changes
5) Mixed form

Scott (1951) divided diabetic retinopathy into four stages:

Stage 1  a) Capillary microaneurysms  b) Changes in veins.

Stage 2  a) Punctate haemorrhages with or without exudates, b) Large round haemorrhages with confluent exudates.
b) Haemorrhages into the vitreous.

Stage 4 Retinitis proliferans, detachment of retina
and gross degenerative changes.

Alports and Slosses (1957) classified diabetic
retinopathy into six stages with perivascular, vascular
exudative changes.

1. Segmental dilatation of veins.
2. Microaneurysms of posterior pole with
   punctate exudates.
3. Small microthrombotic haemorrhages with
   large exudates.
4. Segmental dilatation of larger venous
   trunks with plaques of large exudates.
5. Retinitis proliferans, vascular proliferans
   with large spreading exudates.
6. Retinal detachment, glaucoma and degeneration
   of retina.

Lee and his colleagues (1966) recognised four types.

1) Angiopathy
2) Presence of exudates
3) Proliferative retinopathy
4) Vitreous haemorrhage
But the most followed system of classification has been that adopted by Duke Elder (1967), who divided diabetic retinopathy into three stages.

1) Pre retinopathic stage
2) Simple diabetic retinopathy (S.D.R.)
3) Proliferative diabetic retinopathy (P.D.R.)

**Pre Retinopathic Stage**

There is a uniform distension or turgescence of the larger veins and their main branches without any other visible evidence of diabetic retinopathy and with normal retinal function except that in E.R.G. and E.O.G. there is decreased electrical activity. The condition is also called as 'fundus diabaticus'.

A less frequent early phenomenon is distension of small venules draining, the paramacular region (Alaerts and Slesse 1957).

**Simple Diabetic Retinopathy**

In this the following finding may occur ophthalmoscopically in retina singly or in groups of 2 or more or all of them.
1) Capillary microaneurysms
2) Retinal haemorrhages
3) Retinal exudates
4) Late changes in the retinal veins
5) Hypertensive and arteriosclerotic lesions.

PROLIFERATIVE DIABETIC RETINOPATHY

In this there is new vascular formation
(Neovascularization) with attendant connective tissue
development from the retinal vessels.

This has been further divided into 3 stages.

Stage I  Stage of naked vessels.

Stage II Condensation of connective tissue around the
new vessels.

Stage III Stage of cicatrization, there is a gradual
regression in size and number of new vessels
and connective tissue surrounding them increases
in density and contracts into sheets or bands.
The traction bands thus formed may cause
traction detachment of retina.

Role of fluorescein angiography in study of
diabetic retinopathy has been highlighted by many
workers and proved it superior to colour fundus
photographs and biomicroscopy(Brennick et al., 1977,
Scott et al. 1967) (The diabetic control and complication trial research group 1987) Frank et al. (1980) noted that fluorescein angiograms positive or negative gave more information about the diabetic changes than the colour photographs.

Beaumont and Hollows (1972) showed that fluorescein angiography make it possible to study anatomy and function of capillaries in living diabetics. It may show that a young diabetic with little retinopathy upon ophthalmoscopy may have extensive capillary disease which can be easily diagnosed by fluorescein angiography.

Morton and Gutman (1965) studied diabetic retinopathy by fluorescein angiography and made useful observations.

They observed small areas of fluorescence not on F.A. despite normal retinal examination quite frequently in diabetic patients. They concluded that this was due to drusen lying deep to retina as areas of increased transmission of choroidal fluorescence caused by changes in the over lying pigment epithelium. Drusen fluoresced with early arterial phase and there
was no extravasation of dye.

MICROANEURYSMS

Microaneurysms were initially noted by Mackenzie and Nettleship (1877) and attention was first drawn by Ballantyn and Lowenstein (1943) towards them as a significant sign of diabetic retinopathy. At first they are few in number and obscure without any other sign of retinopathy. Eventually their number increases but they tend to fade and disappear.

Microaneurysms vary considerably in size and may be as large as 75–100 microns (Ashton 1949). They have to be 20–30 microns to be visible ophthalmoscopically, but with fluorescein angiography a tenfold increase can be seen in their number (Hedge and Delliery 1964).

Bresnick et al. (1977) showed that retinal capillary microaneurysms was the earliest change on F.A. and presented a variety of appearances. Most of the microaneurysms fluoresced, as distinct round, regular spots in early phases of F.A. Many of the older hyalinised microaneurysms, fluoresced as
irregular fluorescent spots, with indistinct margins. While small microaneurysms fluoresced as tiny spots with either distinct or indistinct margins. A number of microaneurysms appeared as dark silhouettes, some of which had fluorescent capillaries or halos. The microaneurysms with this appearance were probably packed with stagnant erythrocytes. The fluorescent capillaries or halo may have been caused by, flow or diffusion of, fluorescein into layer of plasma over the surface of stagnant mass of erythrocytes or may have been due to leakage of fluorescein into wall of aneurysms. The dark appearance may also be explained by haemorrhage into the wall of microaneurysms. Some of microaneurysms fill during the early venous phase and advancing bolus of dye can be seen to pass along the afferent vessel to fill the microaneurysms and continue along the afferent vessels. The intermittent filling of microaneurysms has been noted based on the observation that they disappear and reappear in serial angiograms. This intermittent filling had been attributed to reversible plugging of the neck of microaneurysms with erythrocyte aggregates.
CAPILLARY OBSTRUCTION

Capillary obstruction is noted as one of early changes of diabetic retinopathy and can be appreciated on fluorescein angiography when some of the capillaries fail to fluoresce. Earliest capillary obstructions are in patchy fashion (Levene Horton and Corn 1966). Generally obstructed capillaries are located next to dilated, patent capillaries which can be seen on F.A. The dilated capillaries have abnormal permeability and leak fluorescein and have been referred by Cogan and Kuwabara (1968), as shunt vessels Algvere and Gjottabere (1974) showed that F.A. was very useful in finding these shunt vessels.

When perifoveal capillary arcade is affected it leads to enlargement in perifoveal, capillary free zone which was observed by Bresnicht et al. (1975). They had cases in which it was 4-5 times enlarged. They also observed that capillary obstruction leads to retinal ischemia which was accompanied by pallor of the disc and wide spread capillary and arterioles non perfusion areas are seen on fluorescein angiography.
Larger areas of capillary obstruction occur because of occlusion of, precapillary arterioles and it is seen as nonperfusion of precapillary arterioles and as multiple fluorescein stumps along larger arteriole on fluorescein angiography (Bresnick 1980).

**LARGER ARTERIOLE OBSTRUCTION**

It occurs as a late finding in diabetic retinopathy. Ashton (1953) observed them as sclerotic thread like arterioles. Bresnick et al. (1975) observed that some patients had severe form of arteriolar occlusion and large areas of nonperfusion were seen on F.A. They carry a poor prognosis.

**RETINAL HAEOMORRHAGES**

Retinal haemorrhages in diabetic retinopathy are typically round and deep, may occur singly or in clusters. Typical forms are dark red, 'dot and blot' and lighter 'sponge mark' haemorrhages. Superficial haemorrhages are relatively uncommon. Individual haemorrhages, fade after 6-8 weeks (Duke Elder 1967).

On F.A. they fail to stain but retinal vessels around them frequently leak resulting in late staining of the surrounding retina.
Roy and Macculloch (1982) noted small haemorrhages in periphery of retina next to ora serrata in cases of insulin dependent diabetes mellitus of long duration with minimal signs in posterior pole, which was confirmed by fluorescein angiography.

EXUDATES

Exudates are commonly of hard variety, white or yellow deposits. These occur in three forms which frequently co-exist.

i) A cluster of small deposits.
ii) A ring of creninate arrangement.
iii) A large waxy plaque of confluent deposits.

Cotton wool patches have also been seen, specially in initial stages of retinopathy but they are not typical of diabetes.

The cluster form gives a speckled appearance to a localised area in posterior pole, usually one half to one disc diameter in extent. These small white exudates often appear and disappear in a few months.
The ring type may occur in two forms. The more common is a small lesion from one half to one disc diameter across enclosing small areas of visible vascular disturbance such as microaneurysms or haemorrhages. The rings are often incomplete and are sometimes multiple when they may coalesce (Houston and Wise 1957). The larger type is a wreath-like structure some, two or three disc diameters across, usually single and enclosing the macula it is often incomplete. These ring forms, specially larger ones are not confined to diabetic retinæe and are frequently found in arteriosclerotic retinopathy. They are relatively stable and have a cycle of appearance and disappearance of 2-3 years or longer (Whittington 1957).

Large waxy plaques are found at the posterior pole and may be so extensive to cover it completely. But smaller plaques of half a disc diameter or so are frequently found at or close to macula. The plaques surrounded by the fluid have eroded margins, are very stable and may remain for years. But they tend to absorb slowly leaving deposits of shining cholesterol crystals which form within them. The larger
plaques cause serious visual disturbance producing scotomas (Larsen 1960) and even when they are absorbed the retina remains functionless over the site (King et al. 1963).

Cotton wool patches present the typical ophthalmoscopic picture of isolated white patches about a quarter of a disc diameter or less in size, with frayed edges merging into the surrounding retina. These are small infarcts around which the capillaries are often engorged and they tend to fade in 6-8 weeks leaving no trace.

Recently, it had been shown that cotton wool spots may be the earliest signs of diabetic retinopathy.

M.S. Roy and J.C. McCulloch (1986) had seen in five patients of insulin dependent diabetes mellitus, that they had only a few cotton wool spots either isolated or associated with fewer than ten microaneurysms. Significant biological abnormality in these were high levels of glycosylated hemoglobin and mild increase in thrombin generation indicating
slight activation of coagulation system.

Late changes in the retinal veins follow
the turgescence (as in prodromal stage) which in the
larger veins develop into an increased tortuosity
with variations in the calibre of the blood column.
The most typical change is a fusiform dilatation
involving the whole lumen and extending from half
to two disc diameters along the vein. Some times
several of these dilatations occur along a sector
of the vein giving a beaded or 'string of sausages'
appearance (Ballantyn 1939). Eventually these loops
may become engorged and twisted around its base and
strangulated and slough, in which event new vessels
may develop into the anoxic area(Ballantyn and Lowenstein
1943, Luntz and Nickley 1962).

PROLIFERATIVE DIABATIC RETINOPATHY (PDR)

Proliferative changes are generally superimposed on simple diabetic retinopathy. Lawrence
(1981) reported that PDR was a secondary phenomenon,
a response of damaged retina due to occlusion of
capillaries. Root et al. (1959) reported in a study
of 847 cases of diabetic retinopathy, that the incidence
of PDR was one in every five cases. In these cases in
which diabetes started before the age of 20 years
the average duration of diabetes before the
discovery of proliferative diabetic retinopathy
was 17.4 years and none developed this disease
in less than eight years.

P.D.R. is characterised by growth of
fibrous, glial and neovascular tissue presumably
in response to underlying retinal ischaemia. Associated
with these changes are alterations in the vitreous
which consists of vitreous contractions, detachment
of posterior hyloid surface and thickening of posterior
hyloid membrane (Bresnick 1980).

The visual symptoms of the proliferative
phase are the result of opacification of the
vitreous due to haemorrhage from new vessels and
dense fibroglial tissue proliferation.

Detachment of the retina is due to traction
from contraction of fibroglial tissue or contraction
of the vitreous or both.

Beetham (1963) observed that
1) About 50% cases with PDR had fairly good
vision.
ii) About 30% were legally blind, some becoming so with considerable rapidity.

iii) No more than 7% were totally blind.

iv) The prospectus were much worse visually speckining in maturity onset rather than juvenile onset diabetes mellitus.

v) 10% proliferative diabetic retinopathy.

Association of trauma with development of proliferative diabetic retinopathy in very short history of diabetes has been established by Alexander et al. (1979). They suggested that trauma caused changes in blood viscosity which led to precipitation of proliferative phase of retinal disease. Development of PDR has serious associated disorders. The average life span after onset of severe blindness due to PDR in a study by Joseph et al. (1963) was 5-8 years.

In a series of Oaklay et al. (1974) proliferative changes were first noted after a mean duration of diabetes of thirty two years in sixteen patients with diabetic retinopathy which is considerably longer than twenty years cited by Beetham (1963).
In a study by Oakley et al. (1974) on patients who survived more than 40 years of insulin dependent diabetes.

1)  .25% had no signs of retinopathy.
2)  45% mild simple diabetic retinopathy.
3)  30% had proliferation disease, and only 7% developed legal blindness.

Fibrovascular proliferation tend to evolve in an orderly manner and has been divided by Dobree (1964) into three stages but separate lesions may be present at different stages in same eye or in two eyes.

1)  Stage of naked vessels.
2)  Stage of condensation of connective tissue, around the naked vessels.
3)  Stage of cicatrisation.

On F.A. all the new vessels fill during early venous phase and in the late phase they leak fluorescein which mask their fine cut lines. The new vessels break through internal limiting membrane and arborise at the inter surface between it and the posterior hyaloid membrane, and form dense adhesions with latter. But the new vessels which arise from
disc may penetrate into vitreous body.

Neovascularisation is almost always found posterior to the equator in diabetic retinopathy, common sites being at the disc and along the major vessels 1-3 disc diameters around the disc. New vessels patches occur preferentially near arteriovenous crossings with a predilection for the superotemporal quadrant (Taylor, 1970).

On fluorescein angiography the new vessels are revealed in detail. They have a haphazard arrangement and follow a tortuous course. When situated on the retina, they are associated with microaneurysms. New vessels show stasis of blood and transudation of the fluorescein (Scott et al, 1963).

Yuval Yassur et al. (1980) performed fluorescein angiography to demonstrate disc neovascular leakage, which occurred 1-2 minutes after injection and was evaluated into different stages. They showed that there is high incidence of vitreous haemorrhage, as well as fibrous tissue proliferation and traditional retinal detachment with disc neovascularisation.
Although foveal neovascularisation is very rare, Brian with Joondeph et al. (1987) had reported cases of PDR with foveal neovascularisation in their study.

John N. Williams and Robert Machemer (1988) had described ultrastructure of new vessels in PDR.

In their study they had included 23 vascularised preretinal membranes, removed during parsplana vitrectomy. They had categorised them as having either developing, mature or regressing characteristics. Developing in 10 of 23 (43%), mature in 19 of 23 (83%) and regressing in 15 of 23 (57%). Endothelial fenestrations and cell septations were rare. With three dimensional reconstruction it is found that new vessels often extended cytoplasmic processes into the extracellular matrix and that lumina were present even at the distal most tips of the vessels. Solid cords of endothelial cells were not seen. They concluded that in PDR new vessels develop by a process of focal extracellular matrix degradation, generalised and exuberant extracellular matrix production.
cytoplasmic microvillus extension into the extracellular matrix and active lumen formation.

ALTERATIONS IN VITREOUS BODY

Contraction of vitreous, detachment of posterior hyloid surface from the retina and thickening of posterior hyloid are, various changes in vitreous observed by Davis (1965).

HAEMORRHAGE INTRAVITREAL AND SUB HYLOID

Vitreous traction on the neovascular membranes, seems to be a substantial cause of vitreous haemorrhage during course of posterior hyloid detachment. With eye movements blood tends to mix with the fluid vitreous and obscure the view. If haemorrhage is in the subhyloid space it assumes a boat shaped appearance.

RETINAL DETACHMENT IN DIABATIC RETINOPATHY

This occurs in proliferative phase of diabatic retinopathy. This is non-rhegmatogenous tractional detachment initially but may become rhegmatogenous by formation of holes at a later stage in the disease. It occurs because of abnormal vitreo retinal adhesions and tissue shrinkage. It may progress slowly and in case it extends into macula it may cause serious loss
of vision. It may suddenly extend due to formation of holes and loss of vision may be rapid.

According to Tasman (1972), the duration before the retinal detachments occurs is quite long. In his series, this was eighteen years for maturity onset diabetics and twenty-four years for juvenile onset diabetics. Location of retinal breaks was close to optic nerve head and type of retinal breaks was oval or linear and these breaks seemed to be associated with vitreous traction especially in the area of proliferative membranes.

**FLUORESCEIN ANGIOGRAPHY**

Fluorescein angiography (F.A.) has attained a unique position in the diagnosis and understanding of numerous fundus lesions in a comparatively short time (about two decades). Melcan and Naunacee (1960) were the first to examine the fundus of patients with slit lamp and indirect ophthalmoscope after injection of 5% sodium fluorescein intravenously. Newotony and Alvis (1961) made the first successful photographs of the passage of dye through the retinal vessels with a modified fundus camera. Since then F.A. has developed into a very important diagnostic investigation
which has helped us to understand pathogenesis, pathology and course of number of lesions of ocular fundus.

**FLUORESCENCE**

The essence of fluorescein angiography is the visualisation of fluorescein in intravascular or extravascular spaces. Absorption of radiant energy is a prerequisite for the appearance of fluorescence and release of radiant energy in the form of visible light is the basis of fluorescence.

**FLUORESCENIN**

Fluorescein is chemically related to phenolphthalein, results from interaction of phthalic acid anhydride and resorcinol which in an alkaline sodium salt solution forms sodium fluorescein ($C_{20}H_{14}O_5Na_2$). It has low molecular weight (367.27), which facilitates rapid diffusion through the body fluids. It is highly soluble in water. The yellow green fluorescence is visible in it's aqueous solution even in concentrations as low as 1:100,000.
The dye has remarkable fluorescent properties. The conversion of absorbed light to fluorescent light is almost complete. The maximum light absorption and excitation of fluorescein is found between 485 and 500 nm in aqueous solution and the peak emission lies between 525 and 530 nm, other weaker absorption and emission ranges have been demonstrated at wavelengths longer than those but they are clinically insignificant (Hedge and Clemett, 1966).

In blood, the fluorescein is bound to plasma albumins to a large extent and to globulins to a smaller extent. The quantitative estimation of protein bound fluorescein have ranged, from 40-85 percent (Lange and Boyd 1944). But this is readily reversible and all the dye can be dialysed off the proteins. The importance of protein binding is that only the free dye in plasma can diffuse across cell membranes. The fluorescein albumin conjugate demonstrates only about 30% of fluorescence of dye in aqueous solution because of reduced absorption of light at the peak excitation wave length of 490-495 nm. Thus the majority of fluorescent light comes probably from free fluorescein in the plasma, although more than half
is bound to proteins. The dye molecules also bind to blood cells, predominantly deposited on the surface of erythrocytes, accounting for 15–17% of the total fluorescein concentration. The intravascular fluorescein is mainly bound to the proteins thus reducing the fluorescence. The emitted light is also absorbed by haemoglobin. The extravascular fluids will have a higher proportion of free fluorescein dye not bound to proteins and which is not screened by haemoglobin, therefore the leaking points on the fundus from blood vessels may fluoresce more intensely than the blood vessels in vascular phase. Blood reduces the intensity of fluorescence. A concentration 20% greater than in saline being required to achieve a comparable fluorescent emission (Dellery et al., 1962).

Physiological changes can modify the duration and intensity of fluorescence in retinal vessels. The oxyhaemoglobin molecule has absorption peak at 445 nm and 577 nm and reduced haemoglobin at 555 nm, so that appreciable absorption takes place at the frequencies between 500–560 nm at which fluorescent light is emitted (Wessing 1969). If the patient is anaemic as in the case with many diabetics who have
renal disease and uremic bone marrow depression
the emitted fluorescence is greater and the necessary
film exposure less (Bresnick 1980).

After injection the dye rapidly diffuses
in all extracellular spaces except for potential
extracellular spaces of central nervous system and
retina. Dallery, Hodge and Bagal (1962) have found
that plasma concentration ten minutes after injection
was only one fourth of that of radiiodine labelled
albumen which had been mixed in the syringe. It is
excreted rapidly, mainly by the kidneys, where
clearance exceeds glomerular filtration rate and
a small amount is excreted by the liver in the bile.
The dye stains the skin and mucous membranes a yellow
green tint. Usually the skin stain fades with in
twenty four hours and urine ceases to be obviously
coloured after thirty six hours. Fluorescein is not
metabolised to any important extent and in consequence
the dye may persist for longer periods in plasma and
tissues of patients with severe renal failure.
PERMEABILITY OF OCCULAR TISSUES TO FLUORESCIN

CHOROID

Fluorescein does not penetrate, the major choroidal vessels to any marked extent. The chorial capillaries however contain multiple fenestrations and pores through which fluorescein passes into extracellular space (Cunna - Vaz, Shakib and Ashton 1966). As the dye accumulates into extravascular spaces of choroid it becomes, transiently bound to choroidal connective tissue and to inner scleral connective tissue which accounts for a portion of the background staining in the late phase of F.A. Bruch's membrane is also permeable to fluorescein dye through which it readily passes and a portion of it binds to inner and outer collagen layers of bruch's membrane.

RETINAL PIGMENT EPITHELIUM (RPE)

RPE is impermeable to the dye under normal conditions and forms a chorioretinal barrier (Shakib, Rutikowsky and Wiso, 1972). The dye stops at the level of semilae occludentes.
RETINAL VESSELS

Retinal vessels because of their inner lining of endothelial cells which are joined by specialised junctional complexes are impermeable to the dye (Cumah Vaz 1966) so, the endothelial cell complexes form blood retinal barrier to fluorescein dye. Some dye may adhere locally to endothelial cells particularly of veins which may give late angiographic appearance of vascular staining. Thus under normal circumstances there is no fluorescein within the retina during the early stages of angiography. During the late stages of dye transit some dye may conceivably diffuse into the retina from vitreous.

CILIARY BODY AND IRIS VASCULATURE

Ciliary vessels particularly capillaries are very permeable to fluorescein dye. Ciliary body stroma stains extensively and epithelium to a lesser extent by the dye (Grayson and Laties 1971). The dye reaches anterior chamber by means of ciliary vessels via epithelium of ciliary body. The iris vessels are likewise permeable to fluorescein.
VITREOUS BODY

Fluorescein remains in the vitreous gel for several days after angiography.

OPTIC NERVE

The retinal capillaries of superficial, epipapillary layer and pre and post laminal capillaries of ciliary origin have shown to be impermeable to the dye (Anderson 1969, Grayson and Laties 1971).

Fluorescein leaks from peripapillary choriocapillaries staining connective tissue of optic disc and lamina cribrosa in centripetal fashion which accounts for the late fluorescence of optic disc.

SCLERA

The inner layers of sclera stains from choroidal dye and outer layers from dye extravasated through episcleral and orbital blood vessels.
MODE OF INJECTION OF FLUORESCEIN

To get best resolution of vessels in the fundus it is essential to cause a very small bolus consisting of as high a concentration as possible, to reach the dye giving a sharp and bright dye front, in the ocular vessels. This can be achieved by delivering a concentrated bolus of dye as fast as possible e.g. by intracarotid injection or by catheter intobig veins near thoracic inlet, (Dellery Hedge and Engel 1962) or a compromise by injecting through a large calibre needle (No. 18 or 19) in a big antecubital vein in shortest possible time (less than a second).

CONCENTRATION AND DOSAGE OF FLUORESCEIN

Following preparations have been used

1) 10 ml of 5% sodium fluorescein.
2) 5 ml of 10% sodium fluorescein.
3) 2.5-3 ml of 20% sodium fluorescein.

Concentration above 25% solution are not advisable as beyond this level fluorescein may crystallise out of solution. The last one is preferred because it gives a concentrated bolus
for a sharp contrast and better quality of angiograms
during initial transit and less complication of
nausea (Justice et al. 1977). The usual total dose
is 10 mg per kg body weight.

**TOXIC REACTIONS OF FLUORESCEIN**

Transient nausea and occasional vomiting
may occur in about 5-10% of cases but are of no
serious consequence. It may occur on the first
injection even with a dilute test dose on repeated
injections of fluorescein no nausea or vomiting
occurs (Hayreh 1968), so nausea or vomiting on
first injection is no contraindication for subsequent
injection.

Stein and Parke (1971) reviewed and summarised
the more serious side effects of intravenous
fluorescein reported in the literature and added nine
patients of their own making a total of fifty five
as follows:
### Side effects

<table>
<thead>
<tr>
<th>Side effects</th>
<th>No. of Pt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Urticaria and allergic skin reactions</td>
<td>36</td>
</tr>
<tr>
<td>2. Respiratory allergic reactions</td>
<td>05</td>
</tr>
<tr>
<td>e.g. wheezing, laryngeal oedema etc.</td>
<td></td>
</tr>
<tr>
<td>3. Hypotension/Shock</td>
<td>10</td>
</tr>
<tr>
<td>4. Cardiac</td>
<td></td>
</tr>
<tr>
<td>Fetal myocardial infarction</td>
<td>01</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>02</td>
</tr>
<tr>
<td>5. Basilar artery sclerosis</td>
<td>01</td>
</tr>
<tr>
<td>6. Pyrexia</td>
<td>06</td>
</tr>
</tbody>
</table>

Extensive extravasation of dye during injection may slough the overlying skin.

### EXCITOR AND BARRIER FILTERS

The quality of fluorescein photographs is greatly influenced by the choice of appropriate filters. Ideally filter pairs should produce high contrast between fluorescent and nonfluorescent structures and still preserve a maximum yield of fluorescence at the photographic plate. An excitor filter is incorporated before the flash source of fundus camera and a barrier filter in the camera back, just before the film plane. The excitor filter should have a maximum transmission
of between 465 and 500 nm where fluorescein has its maximum light absorption and like wise the barrier filter should have peak, close to where the fluorescein emission is at a maximum i.e. between 525 and 530 nm. A sharp cut off should exist between the range of transmission of exciter and barrier filters, so that exciting light reflected from the fundus through the barrier filter does not diminish the contrast on the photographic film. However wavelength transmissions overlap considerably in most filter pairs, so that the ultimate choice of filters is usually a compromise (Archer D.B. 1972). With most filter combinations and using light flash intensities, a very faint image of fundus may be discernible in the control photographs (Archer D.B. 1972). The most commonly used barrier filter combinations allow no reflected blue light to reach the camera, thus high flash intensities are needed (Haining and Lancaster 1969).

FILM

Weening (1969) has described in detail the proper film, correct developer, proper temperature, and time of developing to ensure best contrast. Black and white 125 ASA are generally recommended, but a
fast film like 400 ASA is definitely better for very rapid sequence photography.

The use of Polaroid films has been advocated by Allan et al. (1966) to obtain instant positive prints of angiograms which are a great advantage. However rapid sequence photography is not possible and quality of angiograms are not as good as can be obtained by conventional films.

NORMAL FLUORESCIN FUNDUS ANGIOPHAN ARM TO RETINA CIRCULATION TIME

Fluorescein appears in the chorio-capillaries within 6-40 seconds after injection into antecubital vein which is half to one second before the dye appears at optic disk. Along with chorio-capillaries, the cilioretinal artery (when present) also stands to fill. Arm to retina circulation time is the mean interval between injection and appearance of dye at optic disk which is 6.5 to 11 seconds. Blood volume, blood viscosity and caliber of carotid vessels may alter this value. The manner in which dye is injected matters a lot. Transit time is shorter when:
- Dye is injected rapidly.
- Large bore needle is used.
- Small bolus of highly concentrated dye is used.
A variation of up to 20% is within normal limits. Large differences may suggest carotid or ophthalmic artery disease (Auberg, 1980). Dollary and others (1962) and subsequently many other workers have used following terminology to denote the different phases in the transit of fluorescein in the fundus:

1. Choroidal flush.
2. Early arteriolar phase.
3. Late arteriolar phase.
5. Early venous phase.
6. Late venous phase.

As 3, 4 and 5 phases merge into each other and can not be differentiated, Hayreh (1974) has introduced following terminology.

A. Pre - retinal arterial phase (corresponding to 1).
B. Retinal arterial phase (Corresponding to 2).
C. Retinal arterio - venous phase (Corresponding to 3, 4 and 5).
D. Retinal venous phase.
E. Late venous phase about 10-15 minutes after the injection of dye or even longer.
Except in albinotics or very lightly pigmented fundi, the choroidal circulation is poorly visualized other than as diffuse fluorescent flush. Pigment epithelium acts as diffuse filter, reducing the intensity of fluorescence only when there is rarefaction in retinal pigment epithelium does underlying detail becomes well defined.

NORMAL CHOROIDAL VASCULAR PATTERN

Hayreh and Baines (1972) with F.A. have demonstrated segmental nature of arterial supply not only in main posterior ciliary arteries but all along their main subdivisions including chorio capillaries and no direct communication between adjacent sectors at any level.

Hayreh (1973) with F.A. has shown that border between neighbouring vessels from major posterior ciliary arteries down to terminal choroidal arterioles forms a water shed zone (Area of poor vascularity). The watershed zone between major posterior ciliary arteries usually passes through optic disc while water shed zone between individual short posterior ciliary arteries radiate out from optic disc or macula or both with in the zone of their parent posterior ciliary arteries
RETINAL ARTERIAL PHASE

Within a fraction of a second after choroidal phase, dye can be detected in retinal arteries. Initially only mid stream segment of arteries, fluoresce (Wessing 1969) and plasma adjacent to vessel wall stains later. Advancing dye is parabolic and receding is converse in shape. Once entire arteries or arterioles are filled it appears 10-25% wider (Allen 1969) than on colour photographs or ophthalmoscopy, veins appear 5-12% larger. According to Wessing (1969) this is due to the fact that plasma flowing adjacent to the vessel wall is not visible ophthalmoscopically although it carries fluorescein.

CAPILLARY PHASE

Capillary phase follows arterial phase. Peripapillary capillaries are radial and branch perpendicularly with few connecting loops. At macula there is avascular zone about one third to one half disc diameter. Usually round or slightly oval horizontally, surrounded by fine lazy network of capillaries. Background fluorescence as the dye diffuses from chorioscapillaries into extravascular space, increases as the retinal capillary net work fills.
RETINAL VENOUS PHASE

Onset of venous flow varies with the area of fundus. First peripapillary and macular veins fill followed by temporal veins. Flow in the veins is in the form of outer lamellar layer i.e. more in the periphery of veins.

Circulation time is the duration of time between the first detection of dye in arterial system until the detection of dye in the tributary venous system. For macular area it is 1.2 to 2.4 seconds, for temporal veins it is 2.6 to 3 seconds.

RECIRCULATION PHASE

It begins within first minute after injection observed as mild exacerbation of fluorescence in the arterial system when arteries and veins appear equal in degree of homogenous fluorescence. By 5-10 minutes dye is distributed equally in the entire blood but fluorescence becomes weaker. Late staining of vessels occurs as the dye adheres to endothelial cells and gives a weak fluorescence.

OPTIC DISC

Grayson and Laties (1971) observed that F.A. is invaluable in studies of blood supply of optic
disc. Its fluorescence can be divided into four stages.


2. Prelaminar capillary fluorescence: Occurs during choroidal and early arterial phase. Fluorescence is homogenous but individual capillaries can be made out.

3. Epipapillary capillary fluorescence: Peak fluorescence, of these occurs during the retinal venous phase. Epipapillary capillaries fill from central retinal artery and peripapillary capillaries from retinal arterioles. Former are prominent in papilloedema and later in occlusion of central retinal artery

4. Late fluorescence of disc is from:
   - Deep capillary plexus of disc.
   - Extravasation from choroid capillaries at disc margin.
   - Scleral fluorescence in lamina cribrosa.