THE USES AND LIMITATIONS OF INTRAOCULAR BIOPSY

WALLACE S. FOULDS
Glasgow

SUMMARY

Based upon the author's considerable experience of trans-scleral resection of malignant melanoma of the choroid, a technique has been developed for the biopsy of tissues of the posterior segment of the eye. Its use in the management of atypical malignancy posing diagnostic difficulty and in the investigation of selected case of acute retinal necrosis, uveitis and retinal pigment epitheliopathy is described. In 34 trans-scleral biopsies of choroid, RPE and in some cases, retina, an adverse result occurred in only one case, this it was thought being due to not including pars plana vitrectomy as part of the biopsy technique. Pars plana vitrectomy is now regarded as an integral part of this form of biopsy.

Although intraocular surgery is an everyday event for the ophthalmic surgeon, techniques for the biopsy of intraocular tissues are not well developed and diagnostic biopsy of intraocular lesions is not commonly performed. This is in contrast to the external eye or adnexa where tissue is readily available and diagnostic biopsy presents few difficulties.

Our knowledge of the pathology of intraocular lesions is usually gained from 'end-stage' disease in enucleated eyes when either the basic pathology is advanced or complications have supervened, necessitating enucleation of the eye. Much of the pathology in enucleated specimens is secondary and not directly representative of the primary abnormality and there is no doubt that, were it possible to have ready access to intraocular tissues early in the pathological process, our understanding of many obscure entities would be enhanced and our ability to treat such conditions greatly improved.

Attempts to obtain information of diagnostic value by sampling intraocular tissues have included aqueous and vitreous tap, fine needle biopsy, and transvitreal or trans-scleral formal tissue biopsy.

AQUEOUS TAP

Aqueous tap has most commonly been used in the investigation of intraocular infection or of anterior uveitis.[1,2] Occasionally cellular infiltration in the anterior chamber, while mimicking inflammation, may actually represent neoplastic infiltration. Thus retinoblastoma may present as an acute anterior uveitis (Fig. 1). Although retinobastoma presenting in this way can be diagnosed by recovery of neoplastic cells from the aqueous or the vitreous,[3,4] there is a possible risk of extraocular dissemination of tumour if the wall of the eye is breached[5,6] and for this reason many would hold that any form of intraocular biopsy is contra-indicated in cases of suspected retinoblastoma.[6,7]

BIOPSY OF IRIS OR CILIARY BODY

Formal tissue biopsy of lesions in the iris or ciliary body may be readily performed and in many instances, as the lesion is small, the biopsy takes the form of an excision biopsy in which the whole lesion is removed for pathological examination. In the majority of such cases the lesion is a neoplasm affecting the iris or anterior ciliary body. For such tumours the surgical approach has much in common with trabeculectomy, a half-thickness sclerocorneal flap being raised and the deep corneo-scleral lamella being removed together with the affected iris or ciliary body tissue with a surround of healthy iris or ciliary body. In the case of iris tumours it is commonly recommended that these should be kept under observation and surgery only resorted to if significant documented growth is seen. This, however, may be dangerous advice. Iris tumours may be very slow growing (Fig. 2) and as a result loss to follow up is a very real risk and the author has personally seen three cases of iris tumour lost to follow up who eventually required enucleation, in one case of an only eye when what had been an easily treated iris tumour at the outset had converted to an inoperable widespread malignancy (Fig. 3). In the case of iris tumours, in the majority it is not too difficult to make a clinical diagnosis of malignancy with a high degree of accuracy and the author has never regretted carrying out an excision biopsy of an iris lesion for so far in some 30 cases none has proved to be other than a malignant melanoma.

BIOPSY IN THE POSTERIOR SEGMENT OF THE EYE

For lesions in the posterior segment the technical prob-

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Correspondence to: Professor Wallace S. Foulds, CBE, Ross Hall AMI Hospital, 221 Crookston Road, Glasgow G52 3NQ.

lems of biopsy are much greater. A variety of approaches has been used, diagnostic vitrectomy for cases in which the pathology has been largely in the vitreous, fine needle biopsy by either the transvitreal or trans-scleral route, and formal tissue biopsy usually via the trans-scleral approach but occasionally by transvitreal surgery.

DIAGNOSTIC VITRECTOMY

Just as retinoblastoma may present as a uveitis so also may malignant disease in the eye present as a vitritis. This is particularly true of the large celled malignant lymphoma, so-called reticulum cell sarcoma, and in such cases the diagnosis may be made by vitreous aspiration or by formal diagnostic vitrectomy.[4,8–10] In cases of vitritis of possible neoplastic, infective or non-infective inflammatory aetiology, diagnostic vitrectomy is a useful and relatively safe way of making a definitive diagnosis thus allowing appropriate therapy to be instituted.[4,11,12] Vitreous (and aqueous) aspirates are often almost cell free and diagnostic vitrectomy results in a better harvest of cells[12] although these may have to be concentrated for study by cyto-centrifugation or by filtration through a micropore filter. In the author's experience vitreous biopsy has been useful in the identification of malignant lymphoma (reticulum cell sarcoma) (Fig. 4), secondary melanoma from skin (Fig. 5) and in the identification of pathogenic organisms particularly Candida (Fig. 6).

FINE NEEDLE BIOPSY

Fine needle biopsy of suspected malignant lesions in the posterior segment of the eye can provide a sufficient number of cells to allow a cytological diagnosis[13–16] but gives no information about the vascularity or tissue architecture of the tumour. Additionally, fine needle biopsy requires access to a cytology laboratory skilled in the necessary cytological techniques. In spite of these drawbacks fine needle biopsy has proved very useful in the diagnosis of malignant melanoma of the choroid, particularly where treatment with radiation is contemplated and confirmation of the nature of the lesion sought prior to treatment.[17]

Although there is a theoretical risk of tumour dissemination along the needle track, no instance of this adverse outcome has been documented where a 25 gauge needle (or finer) has been used for the biopsy[17] nor has vitreous haemorrhage or retinal detachment been a problem.[16,17] In spite of this some workers advise caution in the use of fine needle biopsy recommending that the technique should be used 'only in special circumstances'.[18]

FORMAL TISSUE BIOPSY OF RETINA AND CHOROID

The author's interest in the biopsy of lesions of the retina, RPE or choroid stems from an interest in the management of malignant melanoma of the choroid and, particularly, from experience extending over 20 years of treating such cases by local surgical resection of the tumour.[19–23]

This type of surgery in which complete removal of the tumour from the eye is aimed at can be considered a form of excision biopsy but it is not difficult to modify the technique in appropriate cases so that only a portion of a lesion is removed rather than the whole.

EXCISION BIOPSY

Technique

The technique for the local resection of choroidal melanomas has been previously described.[24] In summary, haemostasis is achieved by profound hypotensive anaesthesia and the tumour is approached by way of a partial thickness lamellar scleral dissection, the tumour eventually being removed with the deep scleral lamella and a surround of healthy choroid, leaving the adjacent retina intact.

In recent years the technique has been modified by the inclusion of a pars plana vitrectomy at the start of the operation and the use of a temporary fluid/silicone oil exchange in cases where a breach in the retina has occurred as a result, for example, of tumour adhesion to retina or where a very large resection of ciliary body has resulted in a significant anterior disinsertion of the retina. Vitrectomy allows the volume of the globe to be reduced during surgery by the withdrawal of fluid by way of a three-way tap in the infusion line or restoration of the volume by a suitable disposition of the infusing reservoir. By making use of volume reduction during surgery, access to posteriorly placed tumours is greatly aided and even tumours abutting the optic disc can be successfully resected (Fig. 7). Additionally, any tendency of the retina to prolapse into the operation site during scleral closure can easily be controlled by volume reduction.

RESULTS

In Glasgow* we now have experience of local choroidal resection in more than 300 cases and our experience confirms that removal of even large amounts of choroid is compatible with retention of good vision, provided the submacular choroid is spared and that such complications as vitreous haemorrhage or retinal detachment do not supervene. Overall 60% of eyes undergoing choroidectomy for malignant melanoma retain useful vision and 25% good vision. Even when vision is poor because of macular involvement or gross pre-operative retinal detachment, a cosmetically satisfactory eye is retained in 80% of cases.

Although it is not strictly relevant to the topic of intraocular biopsy, it is worth noting that a comparison of mortality after local resection with that after enucleation in two matched series has shown no significant difference in survival rates in the two groups when risk factors have been equalised by the use of the Cox's proportional hazard model[25] (Fig. 8).

It is of relevance to a consideration of biopsy that local spread to the orbit has not been a major problem in our large series of cases of malignant melanoma treated by local surgical resection.

*Professor W. S. Foulds, Dr B. E. Damato.

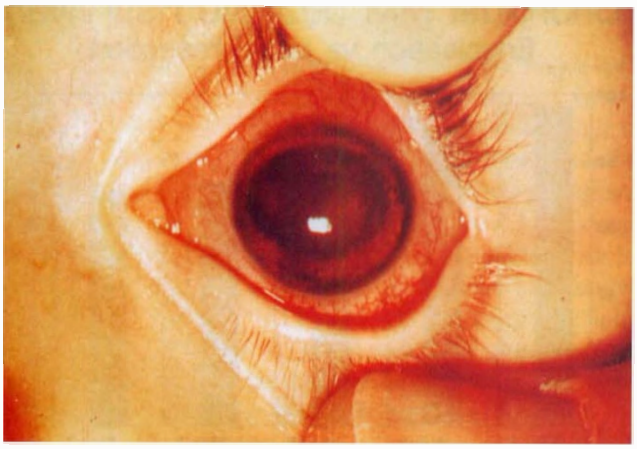

Fig. 1. *Advanced retinoblastoma presenting as acute anterior 'uveitis'.*

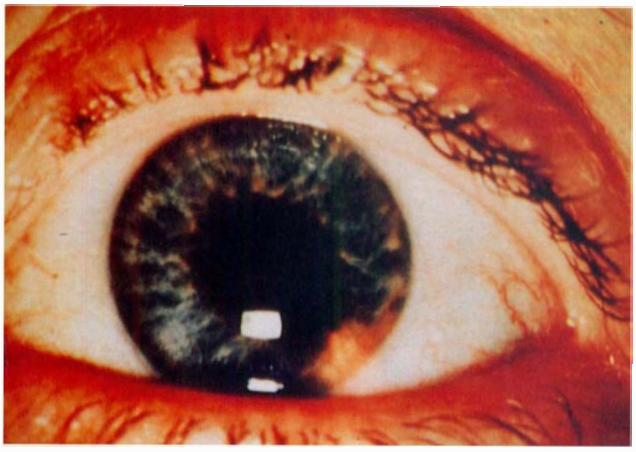

Fig. 2a.

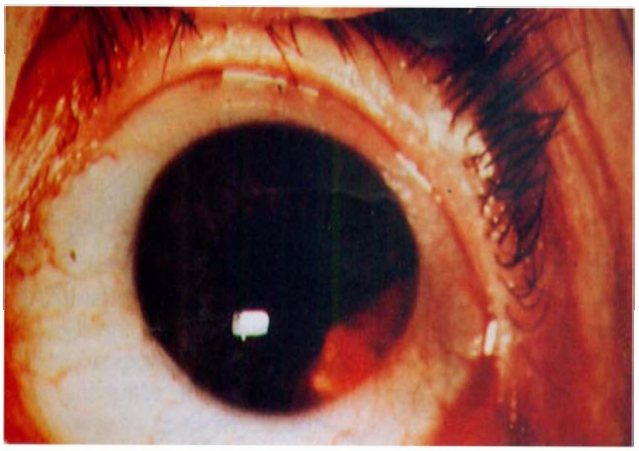

Fig. 2b.

Fig. 2. *Illustrating the slow rate of growth of some iris melanomas.*
(a) Appearance of iris melanoma at presentation
(b) Same tumour eight years later
The tumour was successfully excised.

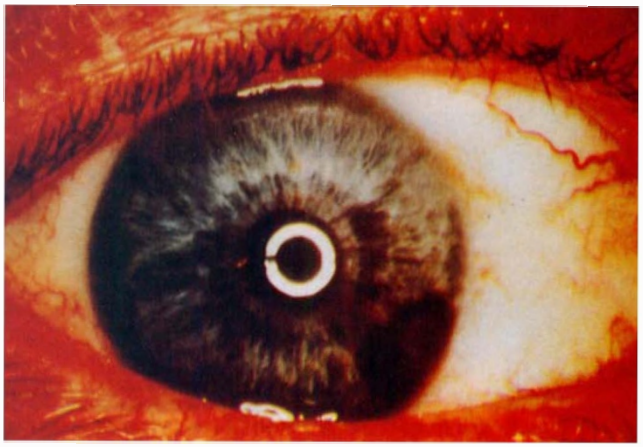

Fig. 3a.

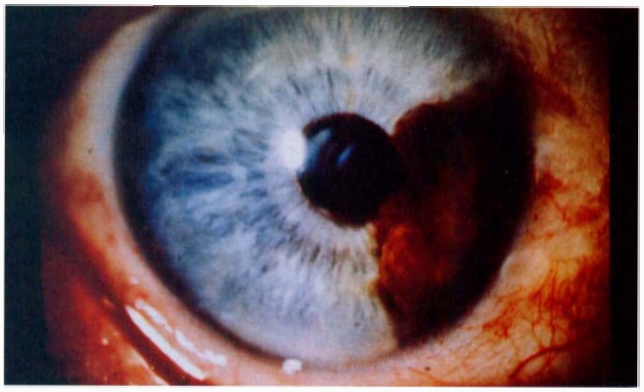

Fig. 3b.

Fig. 3. *(a) Easily resectable iris tumour at presentation in an only eye.*
(b) Same eye five years later. The tumour has invaded the canal of Schlemm leading to inoperable secondary glaucoma.

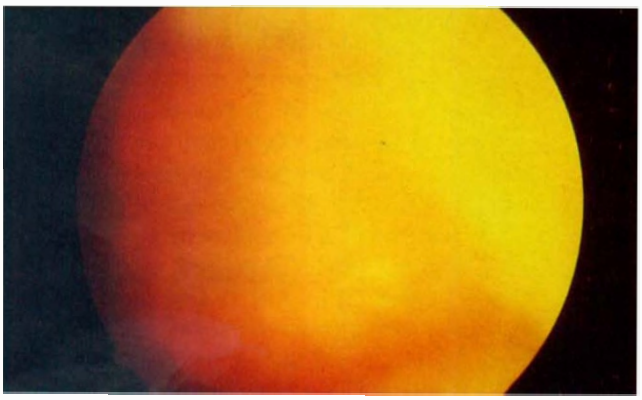

Fig. 4. *Malignant lymphoma (reticulum cell sarcoma) presenting as vitritis.*

Fig. 5. *Vitreous pigmentation due to malignant melanoma metastatic from skin.*

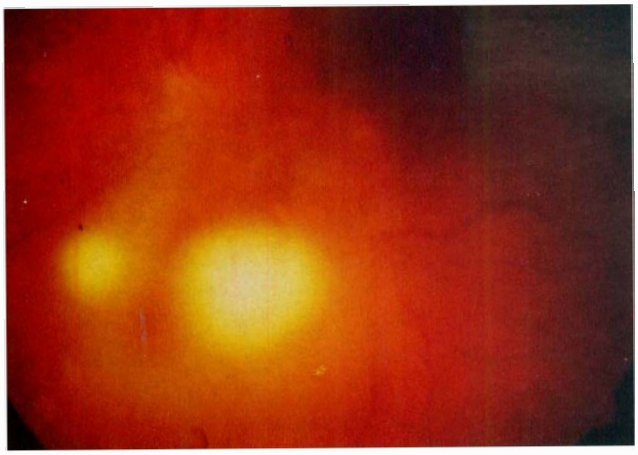

Fig. 6. *Retinitis and vitritis in a patient with metastatic Candida infection.*

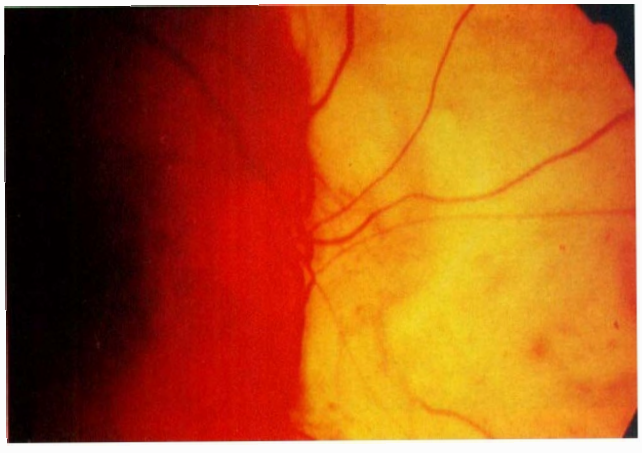

Fig. 7. *Appearance of a posteriorly placed melanoma treated by surgical resection.*

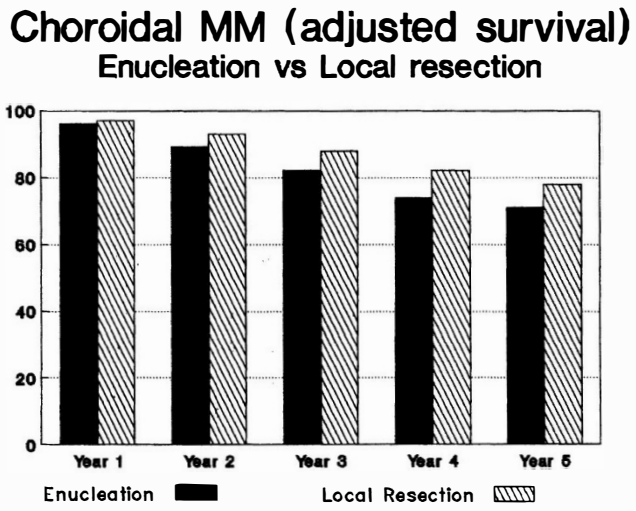

Fig. 8. *Histogram comparing survival rate in patients treated for melanoma of the choroid by either enucleation or local resection. Risk factors in the two series have been equalised using the Cox's proportional hazard model. There is no significant difference in survival between the two series.*

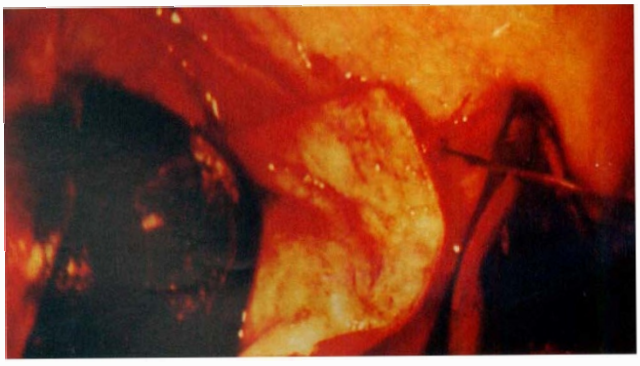

Fig. 9. *Appearance of cyanoacrylate glue when it has polymerised in contact with moist exposed choroid during choroidal biopsy.*

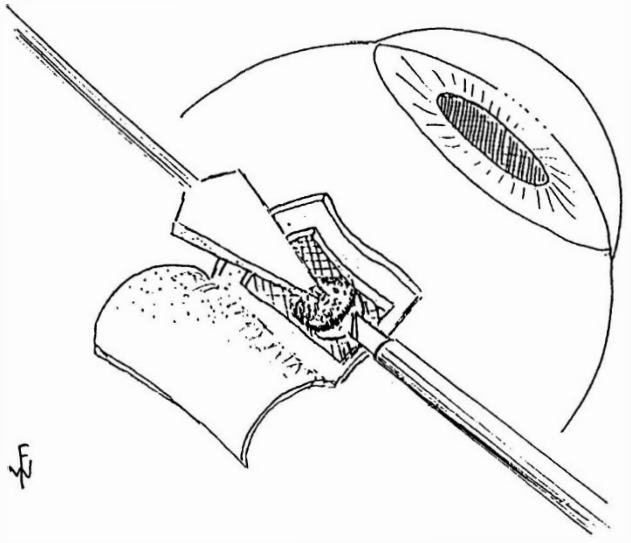

Fig. 10. *Diagram to illustrate the application of cyanoacrylate glue to the choroid by means of an arrowhead sponge. The glue adheres to the choroid and the handle of the sponge provides a useful tool for holding the biopsy specimen of choroid and RPE.*

DIAGNOSTIC BIOPSY OF RETINA, RPE AND CHOROID

Local resection of a choroidal melanoma is a major surgical intervention and not without risk to the eye. In the case of a malignant tumour where the survival of the eye is already compromised the additional risk to the eye can be accepted but, where disgnostic biopsy of a non-malignant lesion in the posterior segment of the eyes is contemplated, the biopsy technique must carry a very low risk to the eye and, additionally, not worsen the pathology under investigation. The obvious drawbacks to biopsy of lesions in the posterior segment are the vascularity of the choroid and the risks of vitreous haemorrhage or retinal detachment if a full thickness retinal biopsy is performed.

It has been shown in experimental animals[26–28] that a full thickness trans-scleral biopsy of choroid, RPE and retina is relatively easy to accomplish and that in the rabbit, dog or non-human primate, vitreous haemorrhage or retinal detachment is not a problem. Initially, published experience with trans-scleral choroido-retinal biopsy in humans was limited to experiments on eyes that were about to be enucleated.[29,30]

In the author's experience full thickness retinal biopsy in the human may be followed by vitreous haemorrhage except in cases such as acute retinal necrosis where the affected retina is avascular. Although pre-operative diathermy or laser photocoagulation can reduce the risk of haemorrhage, the use of diathermy or laser may complicate the histological picture and is probably best avoided.

Except in a few selected cases, full thickness retinal biopsy is probably too risky to be regarded as a routine diagnostic procedure and certainly risks to the eye have to be carefully weighed against possible benefits to the patient. In many cases, however, the pathology we are interested in is restricted to the choroid, the RPE or the outer retina and in these cases biopsy restricted to these tissues may be relatively safely accomplished and often provides information of diagnostic value.

TECHNIQUE

Trans-Scleral Approach

The technique used is similar to that for local resection of choroidal melanoma but obviously the scleral trapdoor is not so large. Having said this, there are advantages in making the trapdoor larger than might be thought necessary and currently a minimum opening in the sclera of 10×10mm is routinely used.

In the human the sclera is not very adherent to the choroid and any attempt to use the deep scleral lamella to manipulate the choroidal biopsy merely results in the mobilised piece of sclera separating from the choroid. If a biopsy of exposed choroid is attempted there are considerable technical difficulties because of the soft and friable nature of the tissues, while additionally any such biopsy is likely to be extremely distorted making histological interpretation difficult. Both problems can be overcome by the use of cyanoacrylate tissue glue applied to the exposed choroid.[31] The glue polymerises when in contact with moist tissues (Fig. 9) and adheres firmly to the choroid provided that this has not been dried prior to the application of the glue. The glue tends to spread on the choroidal surface and there is a risk of its adhering both to the choroid and to the edges of the scleral opening. This problem can be obviated by the use of a relatively large scleral opening and by applying the glue to the choroid using an arrowhead sponge (Fig. 10). The sponge adheres to the choroid and its handle makes an excellent tool for the manipulation of the biopsy specimen during dissection. With care it is easy to dissect free a specimen of choroid with attached RPE and outer retina. Using this technique there is strictly no necessity to approach the choroid via a lamellar dissection. The advantages of a lamellar approach rather than a full thickness scleral approach, however, are that the pathologist is provided with a specimen of sclera with no additional risk to the eye and, additionally, a lamellar dissection allows a stepped closure of the scleral opening which may help to prevent incarceration of uveal tissue, or even retina, in the scleral incision during closure.

As with choroidal resection for tumours, haemostasis is achieved by systemic hypotensive anaesthesia although in some cases adequate haemostasis can be obtained by the careful use of peroperative diathermy to the exposed choroid, or even argon green laser if this is available in theatre.

Initially believing that choroidal biopsy was a less extensive procedure than local choroidectomy for melanoma, pars plana vitrectomy was not included as part of the operation. Without vitrectomy there is a tendency for the retina to bulge into the scleral opening during dissection of the biopsy specimen or during closure of the scleral opening. Pars plana vitrectomy is now regarded as an integral part of the procedure which greatly increases its safety, allowing one to control easily any tendency of the retina or vitreous to prolapse into the surgical site.

TRANS-VITREAL APPROACH

Some surgeons have favoured a trans-vitreal approach to the biopsy of lesions in the retina or choroid[32–34] and this approach has occasionally been used by the author. An example is a patient from the Middle East with a melanoma close to the optic disc for which enucleation was advised but refused (Fig. 11). The lesion was treated with long-exposure low-energy laser using a technique previously described [35] (Fig. 12). Some months later the lesion became extremely necrotic and shed clumps of pigment into the vitreous (Fig. 13). It was not certain whether these clumps were melanin, melanomacrophages, or malignant cells. A vitrectomy and endo-removal of the necrotic tumour was carried out. The specimen revealed that in addition to melanin and melanomacrophages there were indeed active tumour cells present (Fig. 14) and the patient agreed to undergo enucleation.

INDICATIONS FOR BIOPSY OF RETINA/ CHOROID

Under what circumstances would one have recourse to

intraocular biopsy? There are a number of requirements. The patient must be suffering from a sight-threatening (or possibly life-threatening) disorder. There must be a reasonable expectation that biopsy will provide information which will be of direct value to that particular patient. Biopsy of intraocular tissues cannot be lightly undertaken and cannot be used as a research tool to further knowledge of disease processes except as part of a manoeuvre designed to improve the management of the patient being investigated.

The commonest reasons for considering biopsy of the posterior segment are:

(1) The differentiation of malignant from non-malignant lesions.
(2) The differentiation of primary from secondary malignancy.
(3) The investigation of a clinically diagnosed secondary in an attempt to help to locate the primary.
(4) The investigation of inflammatory or other diseases of the posterior segment of the eye to identify the pathological process and if possible the aetiological agent.

THE DIFFERENTIATION OF MALIGNANT FROM NON-MALIGNANT DISEASE

In most cases of intraocular malignancy in an adult the diagnosis of malignant melanoma is easily made on clinical grounds. In some cases however the patient is reluctant to consider appropriate treatment, particularly when this involves enucleation, without confirmation of the diagnosis. In other cases the tumour may be atypical and the diagnosis somewhat uncertain and an exact diagnosis can indicate the appropriate management strategy (Figs. 15, 16). Because our clinical diagnostic accuracy is now so high, supported as it is by angiography, echography and biomicroscopy, the need for diagnostic biopsy in suspected malignancy is rare and to date in the author's own experience has only been required in 20 cases, 18 of whom proved to have malignant and two non-malignant neoplasms including a benign haemangioma (Fig. 17) and a schwannoma.

THE DIFFERENTIATION OF SECONDARY MALIGNANCY FROM PRIMARY

Second malignancies are not uncommon in patients with neoplastic disease and in some eight per cent of patients presenting with an intraocular mass there has been an earlier history of primary malignancy elsewhere, raising the possibility that the intraocular lesion might be metastatic.

The management of primary or of secondary malignancy in the eye is so different that in cases of doubt confirmation of the diagnosis by biopsy may be useful. In most instances we are dealing with an amelanotic lesion in the fundus of the eye in a patient who has had earlier treatment of some other malignancy such as carcinoma of breast or of lung. In the majority, differentiation of primary from secondary malignancy is easy on clinical grounds allowing the institution of appropriate therapy. In a few cases, however, the differentiation is difficult. For smaller lesions which might be either primary or secondary, a beta-emitting ^{106}Ruthenium/^{106}Rhodium plaque is placed over the lesion immediately after the biopsy and the length of time that the plaque is left *in situ* is determined according to the histological diagnosis so that in the case of a secondary tumour a radiational dose of around 3,000cGy is delivered and, in the case of a melanoma, around 9,000cGy to the apex of the tumour (or more usually a scleral dose of 50,000cGy). In the case of larger lesions, if the diagnosis proves to be metastatic whole eye irradiation or chemotherapy is used while, occasionally, a 60 Cobalt plaque is used if the biopsy reveals a primary melanoma. In the latter case surgical local resection of the tumour may be appropriate. Illustrative examples are:

Case 1: A woman of 60 years of age who some five years earlier had undergone mastectomy for carcinoma of breast presented with a relatively flat amelanotic mass in the posterior pole of the left eye reducing vision to 6/60 (Fig. 18). Differentiation between primary or secondary malignancy was difficult on clinical grounds and a trans-scleral choroidal biopsy was carried out with the placement of a ^{106}Ruthenium/^{106}Rhodium plaque. Histology of the biopsy specimen revealed primary melanoma and the plaque was left *in situ* for sufficient time to give a tumouricidal dose of 9,000cGy at the apex of the tumour. The tumour responded well to therapy with satisfactory regression of the lesion (Fig. 19).

Case 2: A man of 55 years who had previously undergone pneumonectomy for carcinoma of bronchus presented with a large amelanotic mass in the temporal fundus of the left eye (Fig. 20). Clinically the diagnosis was thought to be a malignant melanoma although a metastatic lesion could not be ruled out. A biopsy was followed immediately by the placement of a 60 Cobalt plaque (as it was considered that local resection under hypotensive anaesthesia was contra-indicated because of poor lung function even if the biopsy result had shown primary melanoma). As expected the biopsy confirmed a diagnosis of primary malignant melanoma and five years later, although there is still a residual mass in the eye (Fig. 21), the patient is alive and well.

Biopsy has been used very infrequently to differentiate primary from secondary malignancy (four cases). In the two patients quoted above the diagnosis was primary melanoma while in a further two cases confirmation of secondary tumour was obtained.

In patients with a typical metastatic lesion in the eye and a known primary, biopsy of the ocular lesion is not called for. Some patients, however, present with a fundus lesion which is undoubtedly metastatic, but no recognisable primary has been located in spite of exhaustive investigations. In such cases the oncologist may seek help from the ophthalmologist, for useful diagnostic information may be obtained from a biopsy of the ocular lesion.

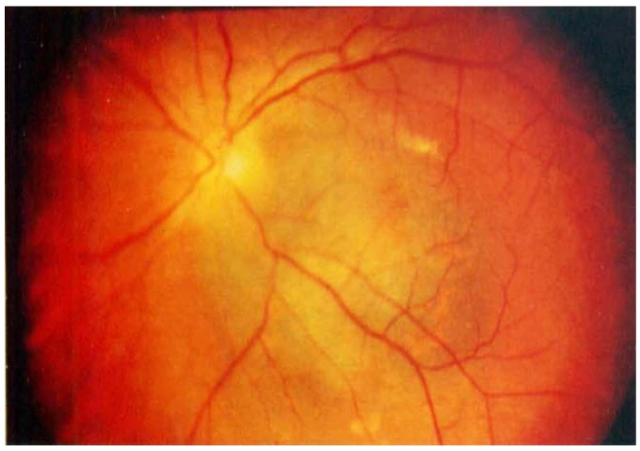

Fig. 11. *Appearance of a juxta papillary choroidal melanoma in a patient from the Middle East who refused enucleation.*

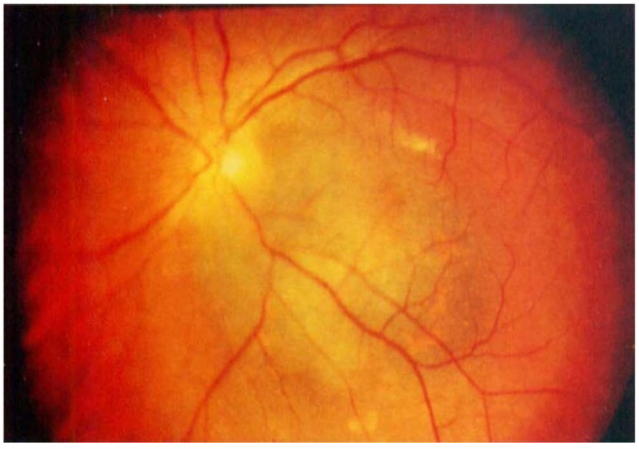

Fig. 12. *Same patient as Figure 11 after treatment of the lesion with low-energy long-exposure laser.*

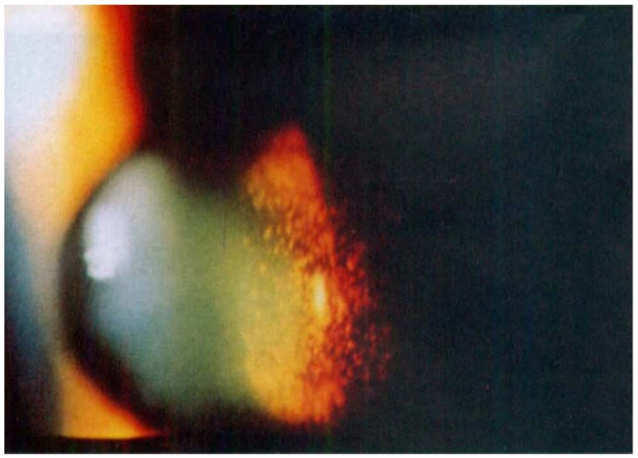

Fig. 13. *Same patient as Figure 11 showing clumps of pigment in the vitreous cavity after the treated tumour had become necrotic.*

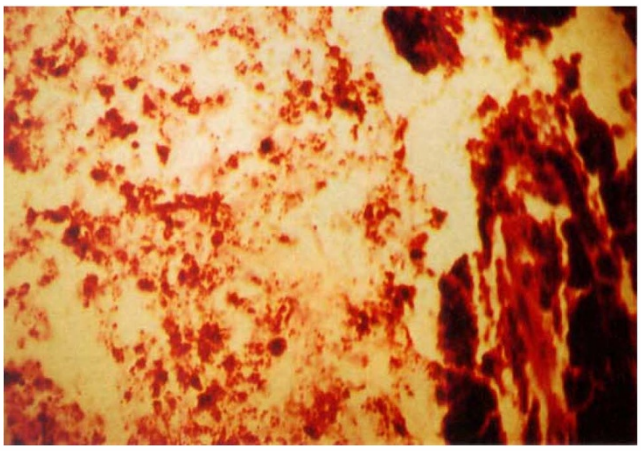

Fig. 14. *Specimen obtained from patient illustrated in Figure 11 after vitrectomy and endosurgical excision of necrotic tumour. Biopsy specimen shows free melamin pigment, melanomacrophages and active tumour cells.*

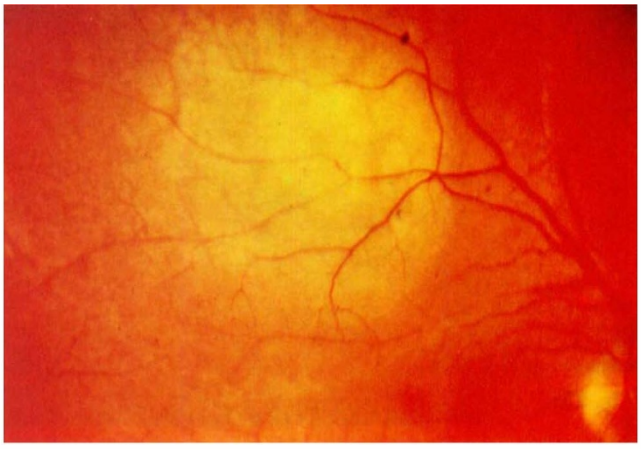

Fig. 15. *An amelanotic relatively flat choroidal tumour which proved on biopsy to be a malignant melanoma which was successfully treated with low-energy long-exposure laser therapy.*

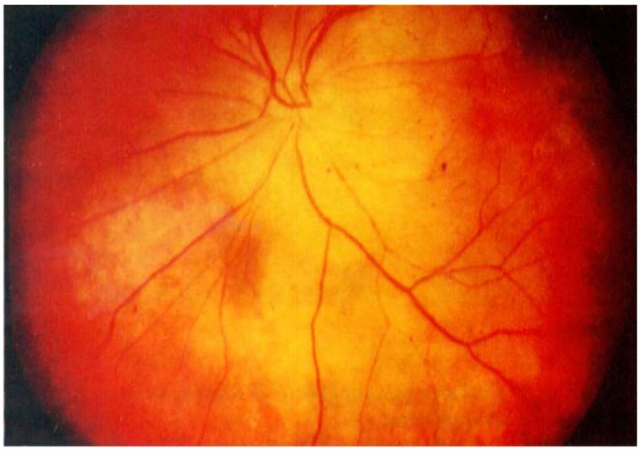

Fig. 16a.

Fig. 16. *(a) A flat infiltrative lesion of the choroid shown by biopsy to be a diffuse melanoma necessitating enucleation. (b) Appearance of the biopsy site shortly after surgery.*

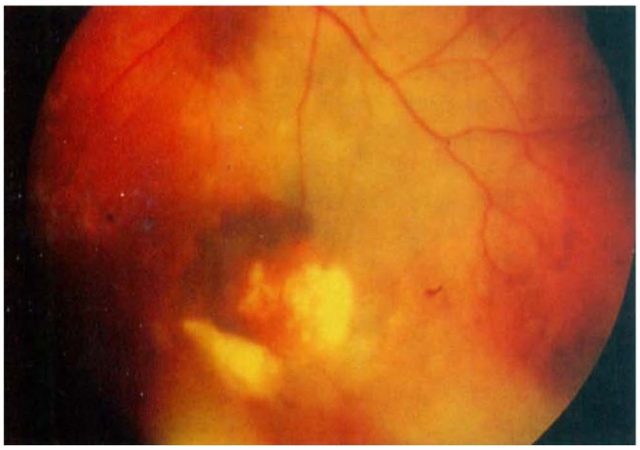

Fig. 16b.

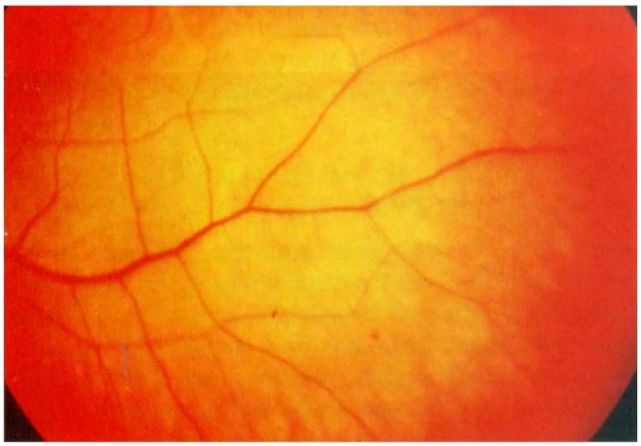

Fig. 17a.

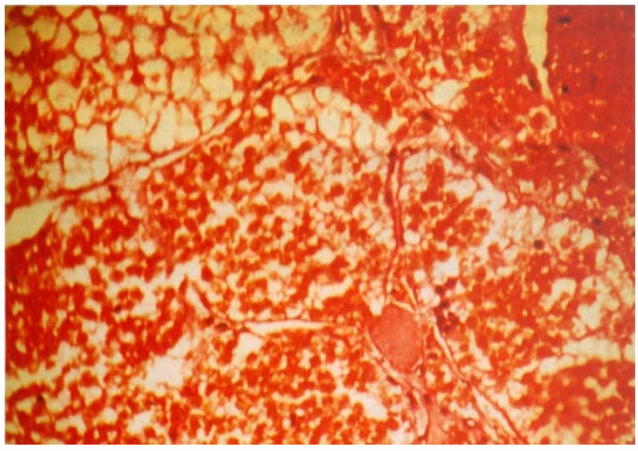

Fig. 17b.

Fig. 17. *A relatively flat amelanotic lesion (a) shown on biopsy (b) to be a haemangioma.*
The tumour responded well to ^{106}Ruthenium/^{106}Rhodium plaque therapy.

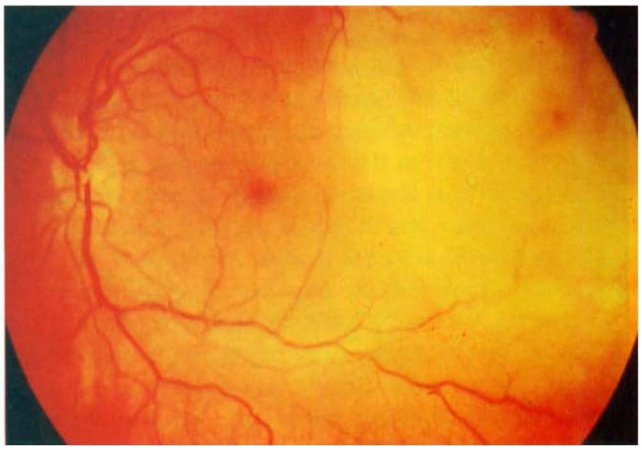

Fig. 18. *A flat amelanotic choroidal tumour in a patient who had previously undergone mastectomy. Biopsy showed primary malignant melanoma.*

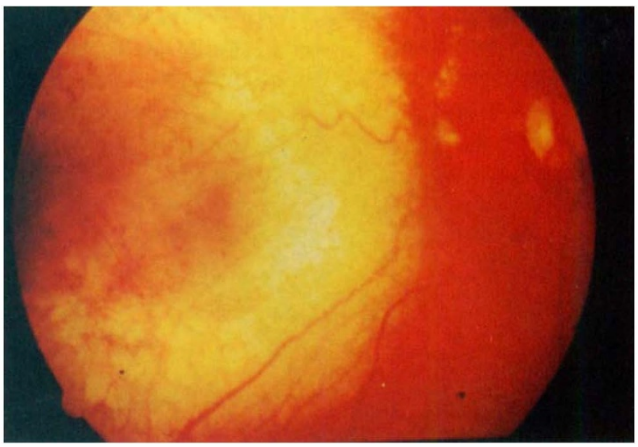

Fig. 19. *Same eye as Figure 18 after successful treatment with a ^{106}Ruthenium/^{106}Rhodium plaque placed at the time of biopsy.*

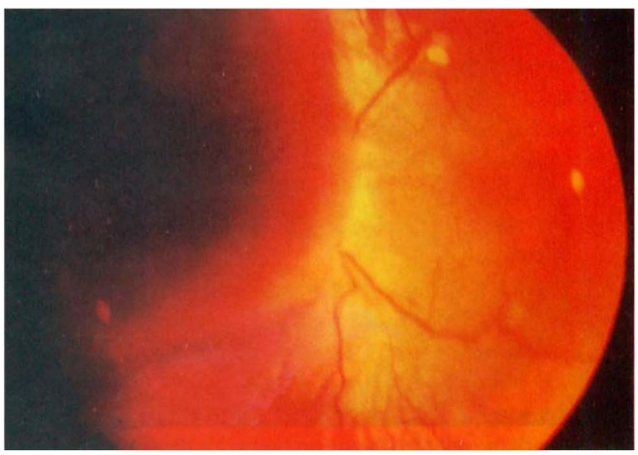

Fig. 20. *A large choroidal tumour in a patient who had previously undergone pneumonectomy for carcinoma of bronchus. Biopsy confirmed a diagnosis of malignant melanoma.*

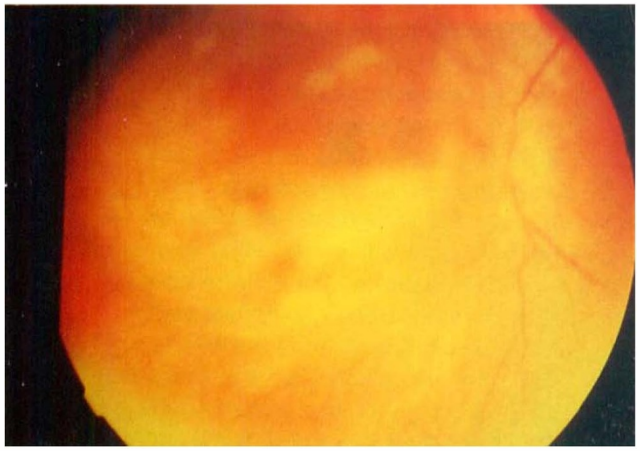

Fig. 21. *Same eye as Figure 20 three months after treatment with a 60 Cobalt plaque. The lesion has reduced somewhat in size. Five years later the patient is alive and well but there is still a mass present in the eye.*

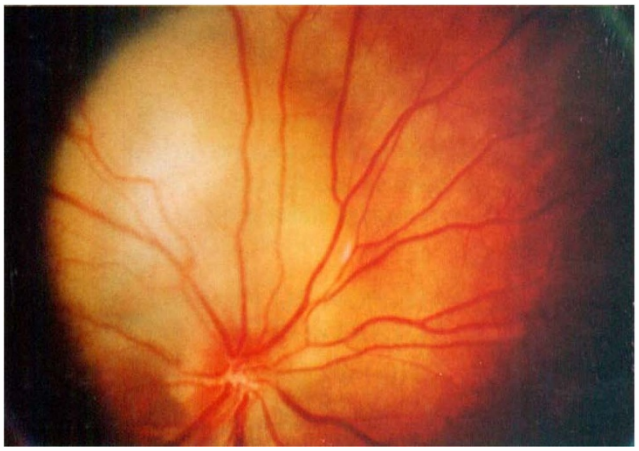

Fig. 22. *Juxta papillary mass in the right eye thought by the referring ophthalmologist to be an amelanotic melanoma on the basis of negative investigation for a primary systemic tumour.*

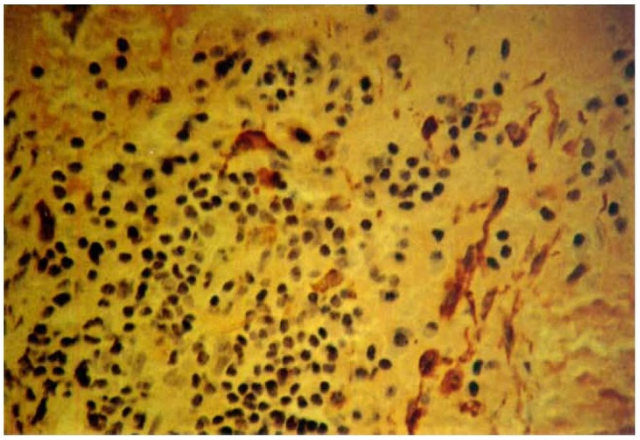

Fig. 23. *Same eye as Figure 22 immediately after choroidal biopsy which has resulted in localised choroidal haemorrhage. Histological diagnosis was metastatic mucopolysaccharide secreting adenocarcinoma.*

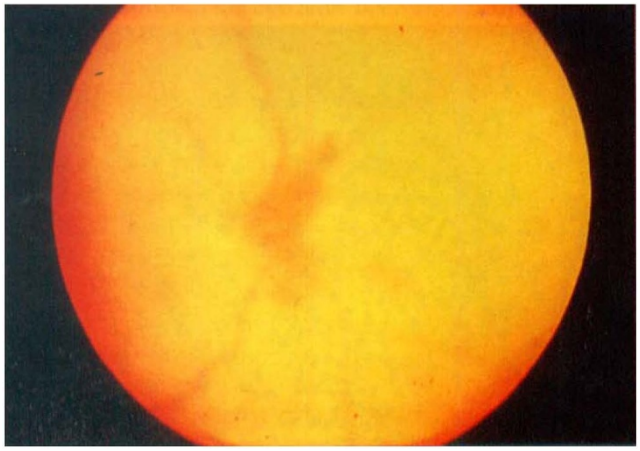

Fig. 24. *Fundus appearance in patient with the acute retinal necrosis syndrome. The necrotic haemorrhagic retina can be dimly seen through a marked 'vitritis'.*

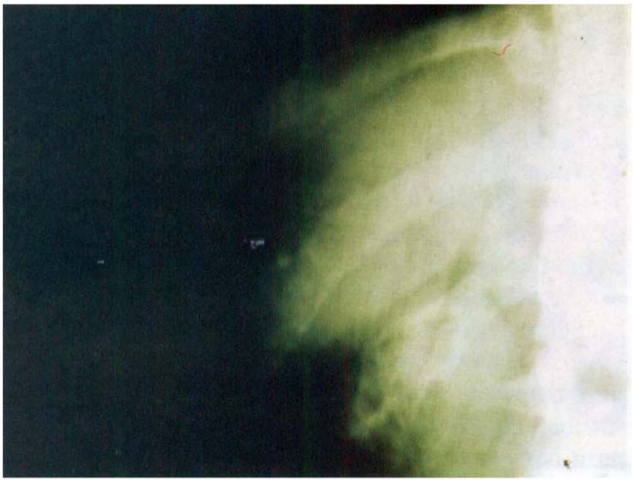

Fig. 25. *Osteoclastic rib lesions in patient illustrated in Figure 24 which raised the possibility of a lymphoproliferative disorder.*

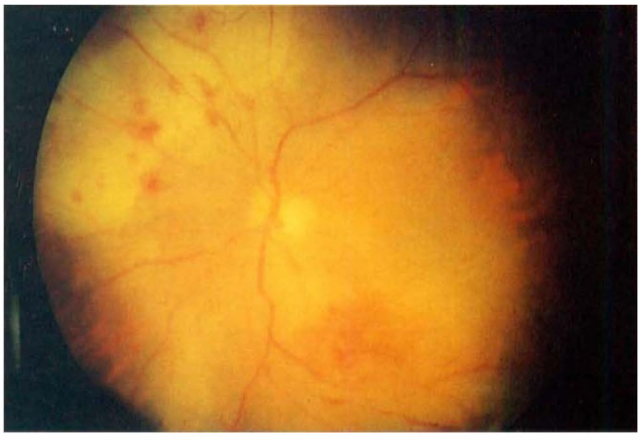

Fig. 26. *Choroidal/RPE biopsy from patient illustrated in Figure 24. The specimen is distorted as it was not stabilised on glue. There is a marked lymphocytic infiltration of the choroid.*

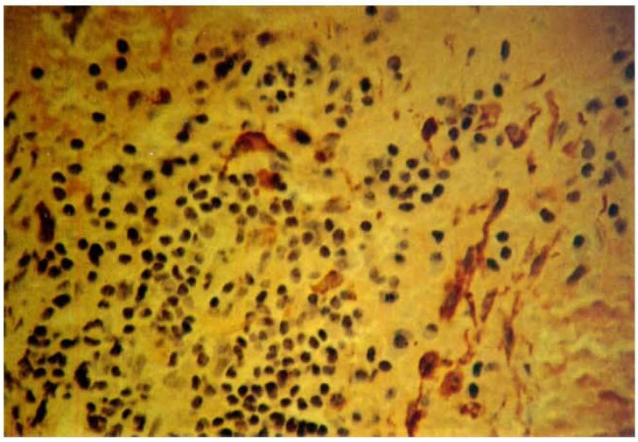

Fig. 27a.

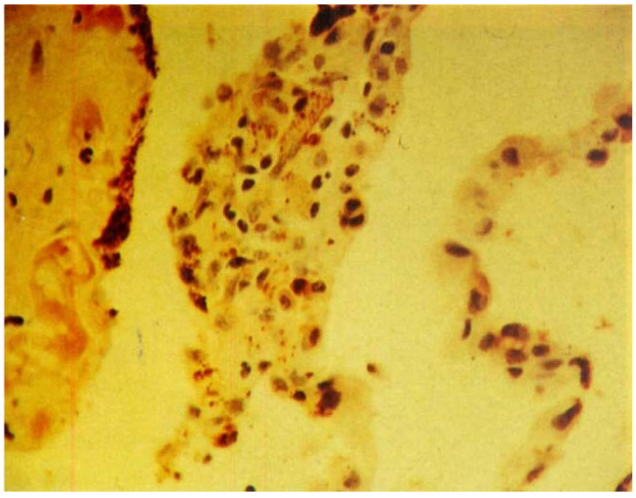

Fig. 27b.

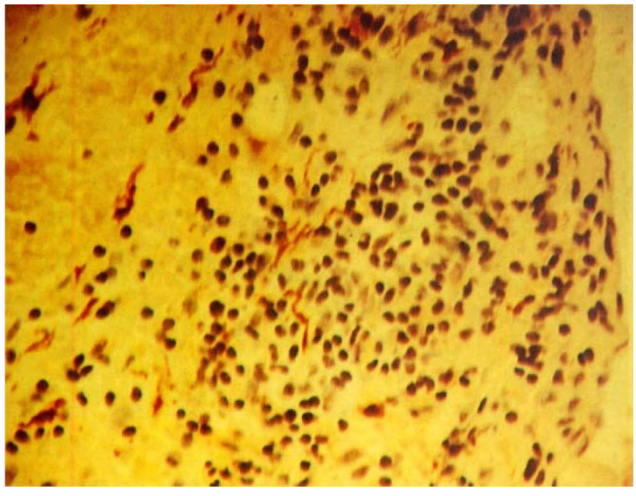

Fig. 27c.

Fig. 27. *Immune peroxidase showed that the lymphocytic infiltration in the biopsy specimen of patient illustrated in Figure 24 was polyclonal (a) IgA; (b) IgG; (c) IgM.*

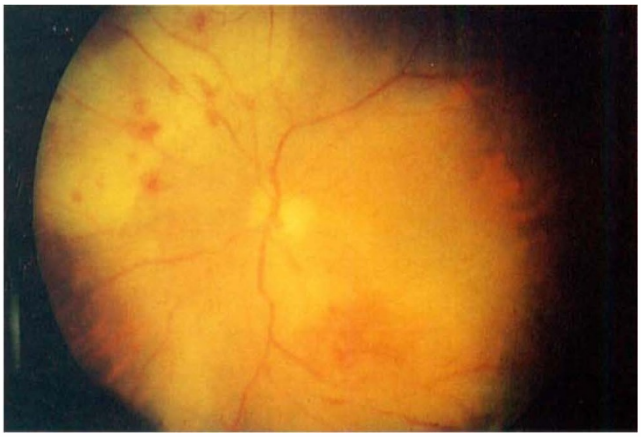

Fig. 28. *Fundus appearance in a patient with acute retinal necrosis due to herpes simplex Type 1. There are superficial haemorrhages and widespread opacification of the outer layers of retina and RPE.*

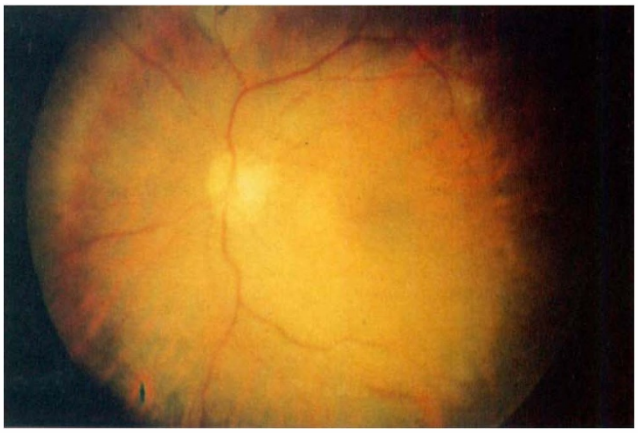

Fig. 29. *Same eye as Figure 28 two weeks after commencement of acyclovir therapy. There is a marked improvement in the appearance of the retina.*

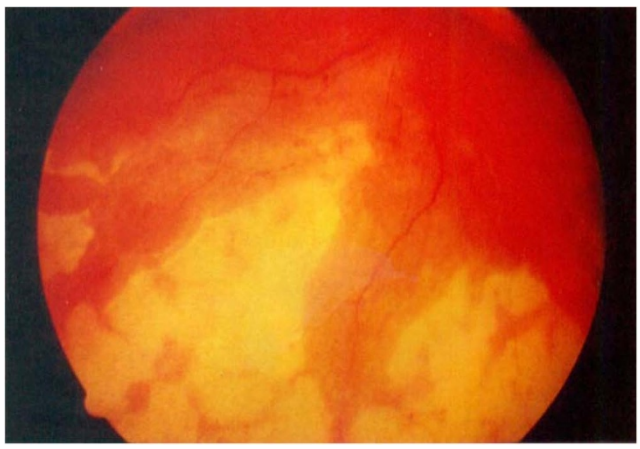

Fig. 30. *Fundus appearance in a patient with acute retinal necrosis due to herpes varicella zoster virus.*

Although such metastatic lesions are often de-differentiated, some clue to the likely site of the primary can be obtained from the architecture of the biopsy specimen be it, for example, adenomatous or squamous in appearance. An example is an Egyptian female patient of 40 years of age who presented to her ophthalmologist with a two weeks' history of reduced vision in the right eye (6/24 as compared with 6/6 in the unaffected left eye). An amelanotic mass was noted above the otpic disc (Fig. 22). Extensive systemic investigation failed to show any evidence of systemic malignancy and a tentative diagnosis of amelanotic melanoma was made and the patient referred for a second opinion. In addition to the main lesion two smaller amelanotic lesions were noted in the nasal periphery of the right fundus. Mammography and investigation of the lung fields had failed to show any sign of primary tumour in these sites and it was decided to carry out a biopsy on the nasal lesions. The biopsy confirmed that both lesions were metastatic adenocarconima in the chorroid (Fig. 23). The cells were noted as secreting acid mucopolysaccharide suggesting an origin in either lung or gastrointestinal tract. The ocular lesion responded well to whole eye irradiation but subsequently the patient developed pulmonary and bone metastases.

Again it must be stressed that as most primary or secondary malignancies in the eye can be diagnosed on clinical grounds, it is in only a small minority that a diagnostic biopsy is required.

INVESTIGATON OF NON-MALIGNANT DISEASE OF THE POSTERIOR SEGMENT

The third group of patients in whom choroidal/RPE biopsy has been found useful has been a small number of patients with inflammatory ocular disease or retinal pigment epitheliopathy.

ACUTE RETINAL NECROSIS

Biopsy has been used in the investigation of four cases of acute retinal necrosis. In this condition, because the retina is necrotic, any attempt at limiting the biopsy to the choroid and RPE fails and a full thickness biopsy always results unless the retina is already detached. Owing to the avascularity of the affected retina, however, intraocular haemorrhage is not a problem and additionally, since the aetiological agent is more readily identified in retina than in RPE, the availability of retinal tissue is of considerable advantage.

In one patient with acute retinal necrosis whose retina was detached prior to biopsy, the biopsy specimen showed a marked lymphocytic infiltration of the choroid while the RPE was also grossly abnormal (Figs. 24–26). Within the populations of lymphocyes some stained positively for IgA, some for IgG and some for IgM (Fig 27), indicating a polyclonal and thus, probably, an inflammatory rather than a neoplastic pattern (in this case there had earlier been a suspicion of a lympho-proliferative disorder on the basis of osteoclastic lesions in the ribs, suggestive of myeloma). The differentiation is not completely secure for lymphomatous tumours in the eye have been shown to be immunologically heterogeneous.[36]

In this patient no viral or other aetiological agent was identified although nowadays with the use of such techniques as the polymerase chain reaction, viral DNA might have been found for it is now well recognised that acute retinal necrosis is the result of infection of the retina with viruses of the herpes group.[37–40]

In three other cases of acute retinal necrosis, herpes simplex Type I virus was recovered by viral culture from the biopsy specimen in one case, herpes varicella zoster from a chorio-retinal biopsy in a second case, and cytomegalovirus in a third.

The patient with HSVI infection was a healthy Pakistani housewife of 62 years of age. Vision had been noted as poor in the left eye for four weeks (bare peception of light compared with 6/12 in the unaffected right eye). Fundus examination showed diffuse slightly swollen opacification of the outer retina and pigment epithelium with overlying blot retinal haemorrhages (Fig. 28). Extensive systemic investigations proved unhelpful.

A biopsy of the superonasal equatorial choroid, RPE and retina was carried out together with removal of a small portion of related vitreous. The tissue specimen was unstabilised and consequently distorted. The specimen was divided, one half for virological investigation and the other for histopathology. Histopathology reported only a scanty inflammatory cell infiltration of the choroid. No viral particles were identified in the retina. Virological studies were carried out separately on vitreous, sensory retina and choroid/RPE. No viruses were recovered from the vitreous or the sensory retina but HSV Type I, identified by restriction enzyme analysis, was recovered from the specimen of choroid/RPE. A course of systemic acyclovir[41] was followed by a prompt improvement in the fundal appearance (Fig. 29) although vision remained poor. Some weeks later marked vascular attenuation in the retina was noted together with marked optic atrophy. Three weeks after the initial presentation, vision in the right eye failed to counting fingers associated with mild uveitis and marked cystoid macular oedema. In the short term vision improved to 6/18 with a combination of acyclovir and systemic steroid therapy but subsequently vision in the right eye deteriorated to hand movements associated with retinal vascular attenuation and optic atrophy.

There are many interesting features about this case which demonstrated a common pattern of an initially good response to antiviral therapy with subsequent severe visual failure associated with retinal and optic nerve ischaemia probably secondary to obliterative vasculities.[41] The recovery of HSVI from the specimen of choroid and RPE and not from the sensory retina suggested that this may have been a primary HSV retinal pigment epitheliopathy rather than a retinitis.

The patient from whose biopsy herpes varicella zoster was recovered was more typical of the acute retinal necrosis syndrome, the left retina showing extensive areas of

necrosis and haemorrhage (Figs. 30, 31) The right eye was unaffected but vision in the left was reduced to less than 6/60. Vision in the left eye made a good initial response to intravenous acyclovir which was, however, complicated by temporary anuria. After two weeks of therapy vision in the affected eye was 6/9 but eventually vision failed again and the eye became blind and painful. The patient with CMV retinitis will be separately reported.

Now that we are aware that acute retinal necrosis is probably always a manifestation of infection of the retina with a virus of the herpes group, one might argue that antiviral therapy with acyclovir could be used without the need to demonstrate the infecting organism. Not all herpes viruses react similarly to acyclovir, however, and there is to the author's mind virtue in determining the exact nature of the causative organism in acute retinal necrosis. CMV retinitis is, of course, now being seen more commonly among patients infected with HIV and requires its own management strategy. Atypical forms of cytomegalovirus retinitis may occur.[42] Freeman *et al.*[34] recovered herpes group viruses by transvitreal biopsy of the retina in cases of acute retinal necrosis and strongly advocated biopsy proven diagnosis in this condition.

CHRONIC UVEITIS

Three patients with chronic sight-threatening uveitis of unknown aetiology were investigated by biopsy of the choroid/RPE. The first, a patient with bilateral intermediate uveitis was interesting from a negative point of view. The patient was a male of 47 years of age with a 20 year history of classical pars planitis with 'snowballs' in the peripheral vitreous, chronic cystoid macular oedema (Fig. 32) and anterior uveitis. Biopsy of the peripheral choroid and of the *pars plana ciliaris* (Fig. 33) showed no evidence of inflammatory cells suggesting that the condition might be a peripheral retinitis rather than a uveitis and certainly making the term pars planitis inappropriate.

The second patient was a man of 40 years of age with a chronic granulomatous mass in the upper temporal choroid, chronic recurrent anterior and posterior uveitis and secondary glaucoma. On biopsy the choroidal lesion showed only non-specific granulation tissue without evidence of a causative organism but the uveitis which previously had been persistent cleared up after the granulomatous mass was removed and the secondary glaucoma was controlled by a trabeculectomy operation carried out at the same operation as the choroidal biopsy.

The third case in this group was a male of 22 with a long history of bilateral chronic panuveitis of unknown aetiology. Vision in the right eye was reduced to hand movements and in the left to 6/36 from a combination of vitreous opacity and cystoid macular oedema. In the hope of obtaining information of diagnostic value a biopsy of choroid and RPE was carried out on the right eye. This was an early operation and a vitrectomy was not used as part of the procedure. Although a satisfactory specimen of choroid and RPE was obtained the retina was accidentally breached and the patient developed a significant post-operative vitreous haemorrhage which subsequently organised to a fully developed PVR. Pathology on the biopsy specimen revealed non-specific inflammatory cell infiltration of the choroid with no evidence of any aetiological agent.

The patient's bilateral uveitis has been brought under control with systemic cyclosporin. It was experience with this patient that prompted the use of pars plana vitrectomy as part of the biopsy technique, for retinal damage in this case was the direct result of an unwanted bulging of the retina through the sclerotomy at the time of biopsy.

RETINAL PIGMENT EPITHELIOPATHY

Three patients with retinal pigment epitheliopathy have been biopsied and a possible aetiological agent identified in two.

The first case was typical of the presumed ocular histoplasmosis syndrome. She had already lost central vision in the left eye from a subfoveal disciform lesion and the right fovea was threatened by active extension of a parafoveal lesion (Fig. 34). Earlier success in identifying viral particles in biopsies of acute retinal necrosis suggested the possibility of identifying an aetiological agent in this case also but no viral particles or other aetiological agent were identified on electron microscopy. Viral and bacterial cultures were also negative. The biopsy specimen taken from what appeared to be an area of activity was suprisingly normal on light and electron microscopy although the specimen did contain giant drusen and Bruch's membrane was thickened and fragmented (Fig. 35) while the retinal pigment epithelium itself appeared 'active'.

The second patient with pigment epitheliopathy was a man of 42 with a rapidly evolving bilateral exudative retinal detachment and extensive focal areas of retinal pigment epithelial abnormality as seen on angiography (Fig. 36). Because vision failed to hand movements in each eye, a biopsy was performed on the poorer eye in an attempt to establish the nature of the disorder. Examination of the specimen showed no gross abnormality on light microscopy and cultures for bacteria or viruses were also negative. On electron microscopy, however, numerous inclusions were seen in the pigment epithelium (Fig. 37). These had many of the morphological characteristics of mollicutes[43,44] a class of organism to which the mycoplasmas belong. Treatment with Rifampicin was accompanied by a rapid resolution of the ocular lesions, a return to near normal vision in the less affected right eye, and recovery of visual field in the other which has exhibited subretinal macular scarrring prior to the inferotemporal biopsy.

A retrospective study of nine other biopsy specimens showed similar inclusions in one other specimen (Fig. 38) obtained from a young woman of 20 years of age with a pigment epitheliopathy with some of the features of the presumed ocular histoplasmosis syndrome with a central 'choroiditis' and two small foci of subretinal neovascular membrane (Fig. 39). By the time the inclusions were identified the ocular condition appeared to be quiescent and no treatment was offered.

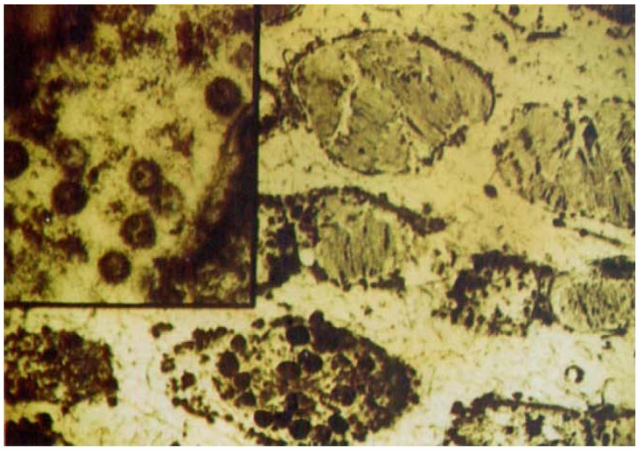

Fig. 31. *Electronmicroscopy of biopsy specimen from patient illustrated in Figure 30. Inclusions typical of herpes virus are present in large numbers and were subsequently shown to be herpes zoster.*

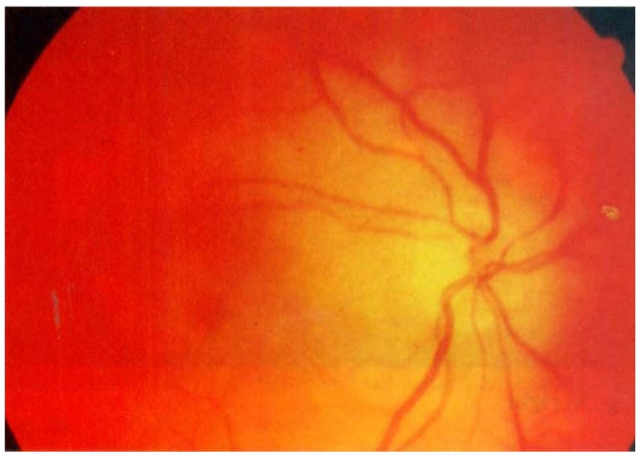

Fig. 32a.

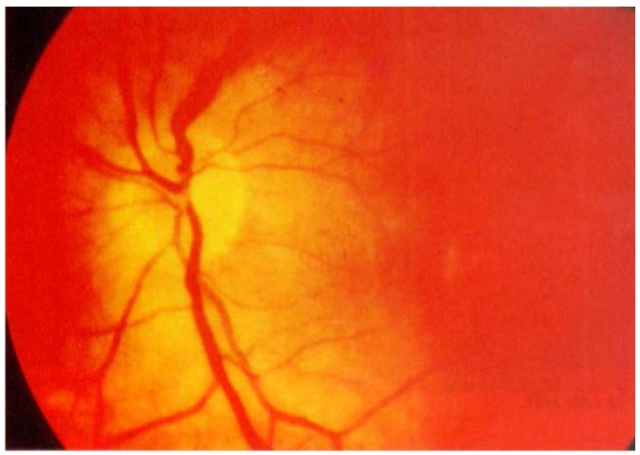

Fig. 32b.

Fig. 32. *Fundus appearance of patient with bilateral cystoid macular oedema (confirmed by angiography) associated with chronic 'pars planitis'.*

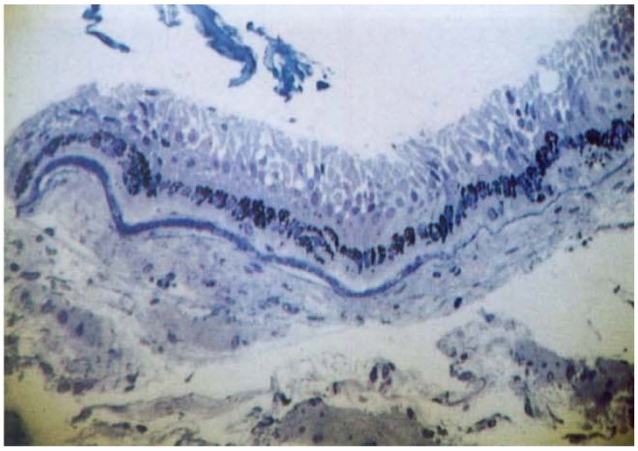

Fig. 33a.

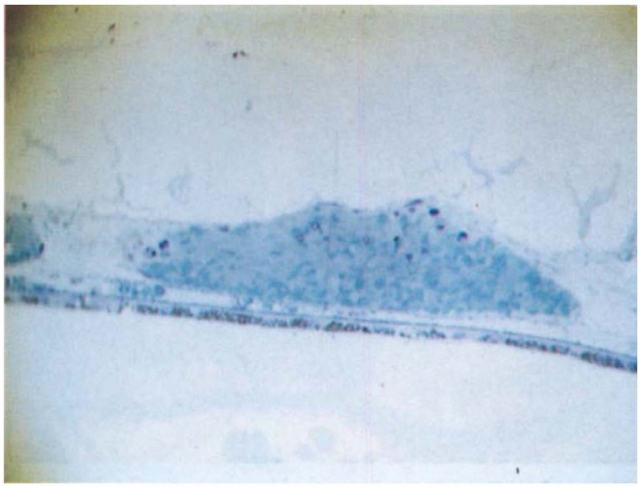

Fig. 33b.

Fig. 33. *Biopsy of (a) the pars plicata ciliaris and (b) the pars plana in the patient illustrated in Figure 32 revealed no inflammatory signs.*

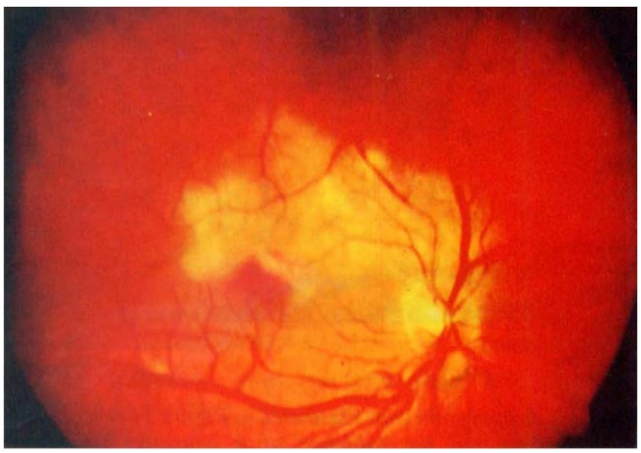

Fig. 34 right.

Fig. 34. *Fundal appearances in a patient with a pigment epitheliopathy resembling the presumed ocular histoplasmosis syndrome.*

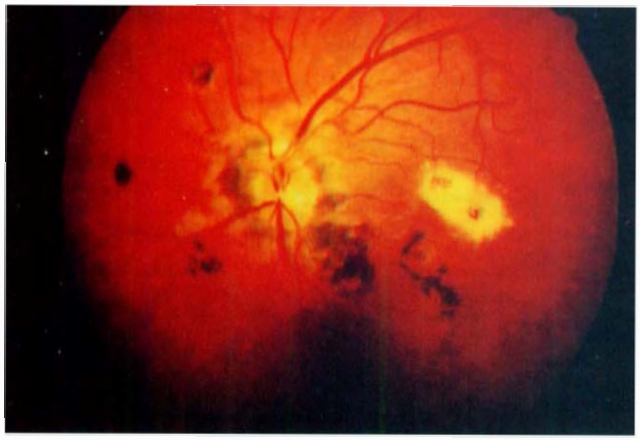

Fig. 34 left.

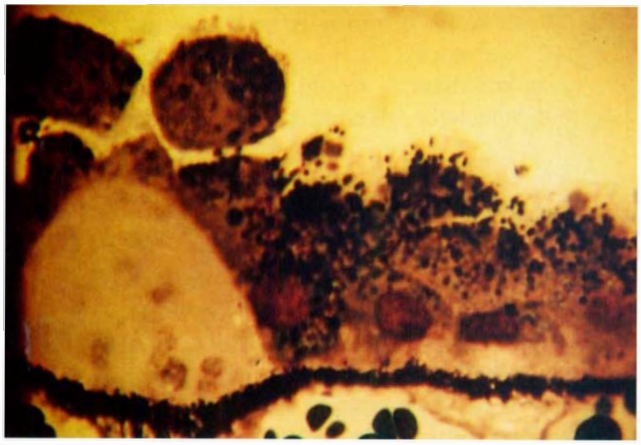

Fig. 35a.

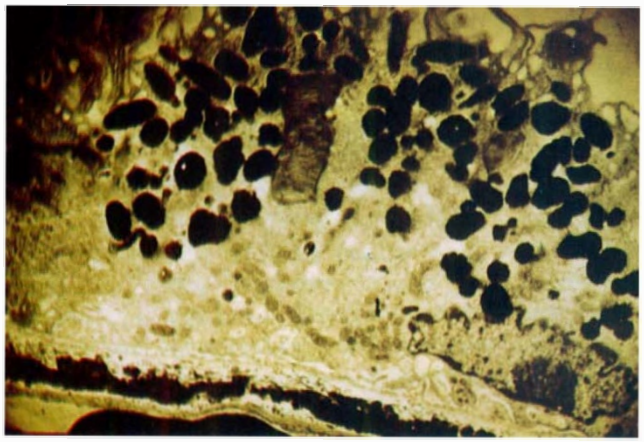

Fig. 35b.

Fig. 35. *Biopsy from case illustrated in Figure 34 revealed (a) giant drusen and the evidence of a causative organism; electronmicroscopy (b) showed a thickened and fragmented Bruch's membrane but a relatively healthy RPE.*

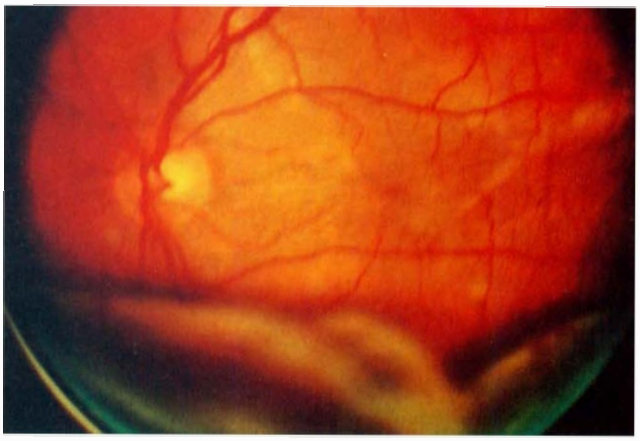

Fig. 36 right

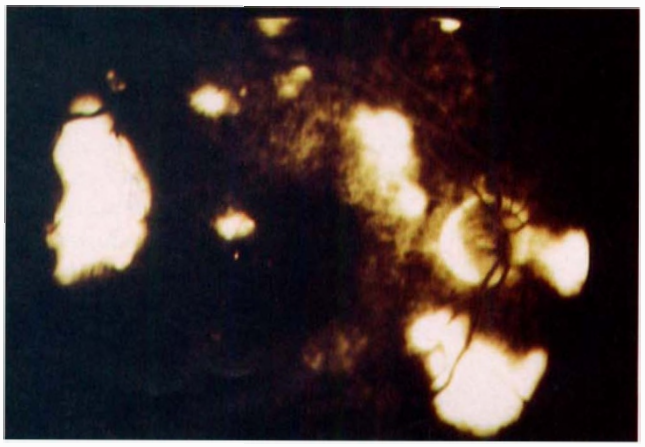

Fig. 36 right

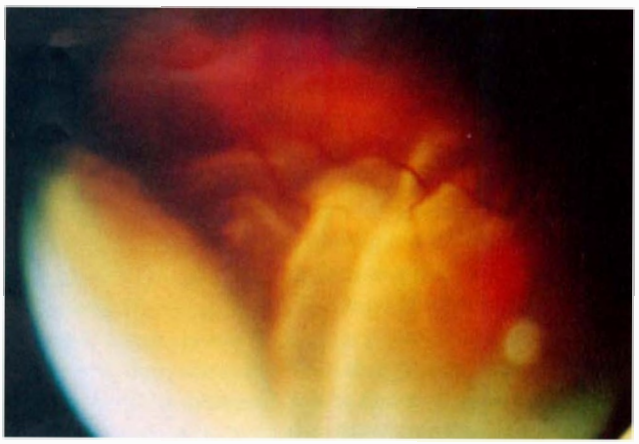

Fig. 36 left

Fig. 36. *Fundal appearances and fluorescein angiography of each eye in a patient presenting with a widespread retinal pigmen epitheliopathy complicated by bilateral inferior exudative retinal detachments.*

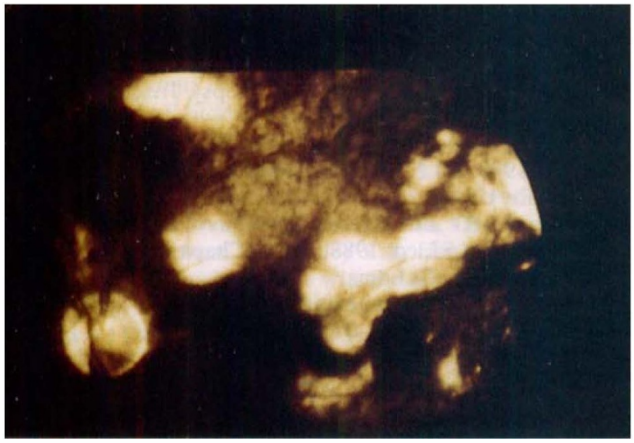

Fig. 36 Left

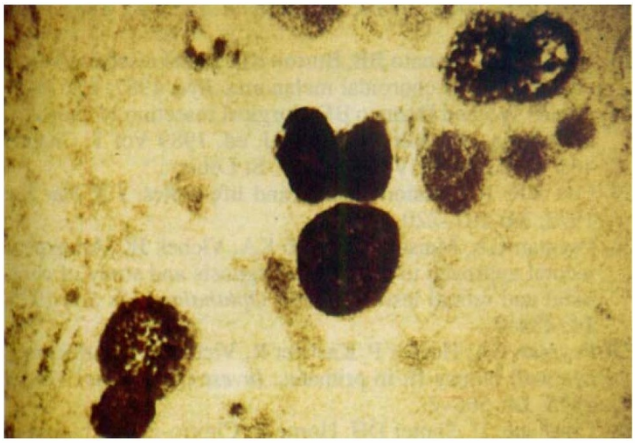

Fig. 37. *Electronmicroscopy of biopsy specimen from patient illustrated in Figure 36. There are numerous intracytoplasmic inclusions in the RPE (arrowed) with the characteristics of mollicutes.*

Although it has been claimed that mollicute-like organisms can cause ocular inflammatory disease[45,46] some doubt has been expressed as to whether mollicutes and can indeed cause human ocular disease.[47,48] Whether the inclusions found in the RPE in two of the three patients with retinal pigment epitheliopathy subjected to biopsy were of aetiological significance has not been established. The fact that no such inclusions were found in another eight biopsy specimens suggests that the inclusions were neither an artefact of the biopsy technique nor of the subsequent pathological processing. The first patient with inclusions in the RPE also had Crohn's disease and it is of interest that MLO parasitisation of vitreous leucocytes has been described in vitritis associated with Crohn's disease.[49] Additionally intracellular inclusions have been found in vitreous macrophages and in intestinal epithelial cells in Whipple's disease[50] a condition which can cause both inflammatory ocular disease and a gut disorder resembling Crohn's disease. Whipple's disease is responsive to treatment with antibiotics.

DISCUSSION

Although trans-scleral choroidal/RPE biopsy is relatively straightforward, the use of biopsy to investigate pathology in the posterior segment of the eye needs the ready availability of sophisticated laboratory back-up with immunohistochemistry, immuno-electron microscopy, cell culture, and advanced genetic and virological techniques. It is likely that such expertise will only be found in major academic centres and this together with the restricted indications for biopsy suggest that such investigations are best limited to major centres at present.

What complications have been encountered? These have been few. In one patient whose lesion proved to be metastatic, full thickness chorio-retinal biopsy without vitrectomy resulted in vitreous haemorrhage which required a subsequent vitrectomy and release of vitreous traction to avoid the complication of retinal detachment. In another case with chronic bilateral uveitis with severely compromised vision, full thickness chorio-retinal biopsy as already indicated was complicated by the development of proliferative vitreo-retinopathy. This was the only case with any long-term adverse effect from the biopsy and represented a complication rate of three per cent (one case out of 34 biopsies). With a careful choice of patient and a careful technique, together with suitable laboratory

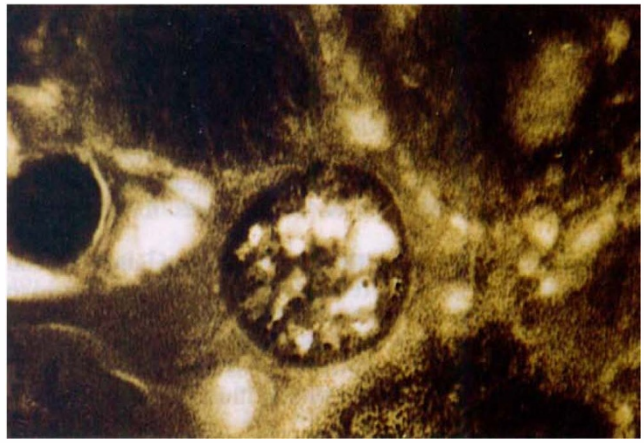

Fig. 38. *Intracytoplasmic inclusions in the RPE of a biopsy specimen from a patient with a retinal pigment epitheliopathy.*

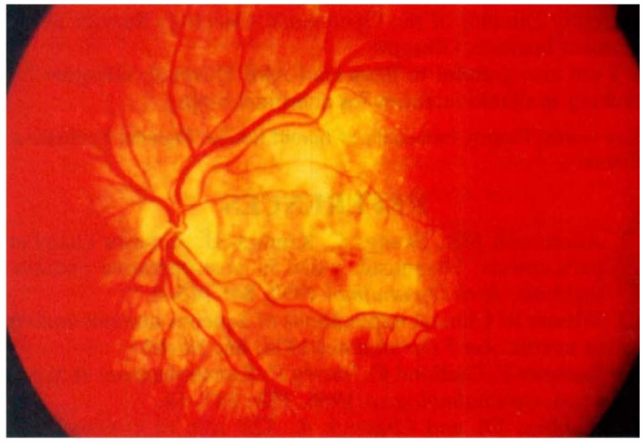

Fig. 39. *Fundal appearance of the eye from which the biopsy specimen illustrated in Figure 38 was obtained.*

back-up, in selected cases one may obtain information by biopsy of the tissues of the posterior segment of the eye which would otherwise be unobtainable and in some instances this information can directly influence the choice of management to the great benefit of the patient.

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