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THE SKIN RESPONSE OF FISH  
TO ULTRAVIOLET RADIATION.  
A HISTOLOGICAL STUDY

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for the degree of Doctor of Philosophy

by

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To Joan, who never fails to keep me on course  
through life's squalls.

## PREFACE

Since its inception, scientific research into the impact of solar ultraviolet (UV) radiation has been confined largely to studies related to the harmful effects of the sun's rays upon the skin of higher animals, principally that of man. In recent years such investigations have received a new impetus with the realisation that man's technical capacity has developed to the point where his ability to modify substantially the stratosphere can no longer remain in doubt. Anthropogenic disturbances of the stratospheric ozone layer, a delicate gaseous interface between stratosphere and troposphere which shields life on earth from the more extreme effects of solar radiation, could result in an increase of UV reaching the earth with profound effects upon its inhabitants.

The role of UV radiation in aquatic ecosystems both at current and at potentially enhanced levels has excited the attention of biologists and physicists over the past decade. This increased level of endeavour has done much to increase our understanding of such matters. It was the paucity of literature pertaining to UV induced dermatopathies in fish when compared to that published on higher animals which prompted this investigation/

investigation and which focuses attention upon an environmental factor which, hitherto, has largely been ignored in relation to fish cultivation.

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## ABSTRACT

A histological investigation of the effects of ultraviolet (UV) radiation upon the skin of four species of teleost fish was conducted in an attempt to define the sequential pathogenesis of the UV induced lesion. The species used in the experiments, namely plaice *Pleuronectes platessa* L., turbot *Scophthalmus maximus* (L.), rainbow trout, *Salmo gairdneri* Richardson and Atlantic salmon, *Salmo salar* L. represent species reared commercially and thus likely, in the artificial environment of a fish farm, to receive levels of solar radiation in excess of those encountered in nature.

It was found that plaice were the most susceptible species to UV radiation whilst turbot were marginally less so. Rainbow trout and Atlantic salmon showed similar levels of susceptibility when compared with each other but, in addition, had significantly higher tolerance thresholds than either plaice or turbot.

Whilst the use of artificial radiation sources allows for more accurate control over irradiance than would natural sunlight, the need to recognise the limitations of such sources has been emphasised. In addition the importance of incorporating filters to remove the shorter wavelengths which would not be encountered at the earth's surface has been demonstrated. The photoreactive capability/

capability of fish skin to repair cell damage initiated by UV radiation has been shown by the use of variable light input in the visible spectrum.

The use of scanning electron microscopy to examine the surface topography of the radiation lesion confirmed the observations made by light microscopy and demonstrated the vulnerability of the damaged skin surface to the invasion of opportunistic bacteria.

The importance of recognising sunlight as an environmental factor in the initiation of dorsal skin damage is no longer in doubt; experimental evidence and clinical observations on high altitude fish farms confirm this. Consideration is also given to the implications of prolonged exposure to sunlight upon fish following transfer and handling. The possible role of sunlight in the aetiology of certain bacterial skin diseases in farmed and wild fish and in dorsal skin neoplasms in wild fish is discussed.

SECTION I

INTRODUCTION AND REVIEW

OF THE LITERATURE

## HISTORICAL OVERVIEW

Although a relatively young science, photobiology, the study of the effect of non-ionising radiation on living systems, had its beginnings at least two thousand years ago when the practice of exposing one's skin to sunlight for therapeutic gain was commonplace. Over the ensuing centuries the beneficial effects of such exposure gained credence as a wide diversity of ailments were claimed to respond effectively to sunlight. Many of the therapeutic applications are still today the subject of considerable controversy. Some of the beneficial results have a sound physical basis. Some would appear to be psychological.

In 1803 Ritter discovered the ultraviolet region of the solar spectrum by demonstrating that 'a chemical action was induced by some form of energy within the dark portion beyond the violet'. Many years were to pass before the importance of this region of the spectrum was to be recognised. Through the work of Cauvin (1815), Home (1820), Bostock (1825) and Davy (1839) some understanding of the response of skin to sunlight was gained. It was not, however, until 1893 when Niels Finsen, now recognised as the father of modern phototherapy, published his first report, *On the influence of light on the skin*, that the true significance of/

of the UV region of the solar spectrum was publicly recognised as being responsible for sunburn and not the radiant heat (infrared) which, until that time had been commonly accepted as the causative agent. In addition to being a research scientist Finsen was also a clinician and thus able to apply his findings to medicine. Using ultraviolet phototherapy his research into the treatment of *lupus vulgaris*, or tuberculosis of the skin, a condition at that time prevalent throughout much of Northern Europe, produced dramatic results and for his pioneering work in this field he was awarded the Nobel Prize for Medicine in 1903. Some recognition for his success must be apportioned to Downes & Blunt (1877) who demonstrated that bacteria were killed by exposure to sunlight. Although unable to determine which part of the spectrum proved most potent, their findings nevertheless laid the foundation for Finsen's work.

During this period, when ultraviolet phototherapy was being publicly acclaimed as being of considerable benefit, disturbing reports began to appear in the literature. Unna (1894), a German dermatologist, noted a high incidence of skin tumours in the skin of sailors, whilst Dubreuilh (1896; 1907) showed that skin tumours on the faces of Bordeaux vineyard workers could be prevented by the shade of peasant headdress. Shield (1899)/

(1899) established a distinct correlation with such tumours and long term exposure to sunlight thus providing an insight into solar induced carcinogenesis.

Thus, by the end of the 19th Century, the basis of research on skin photobiology had been firmly established and certain factors recognised. Sunburn was elicited by that part of the UV spectrum which did not pass through window glass, with the infrared region playing little part in the reaction. Skin pigmentation protected against severe sunburn. Some exposure to sunlight was essential to the health of the skin and the body as a whole and UV radiation therapy could be used to treat certain skin and systemic disorders.

During the early part of this century the therapeutic role that sunlight played in the prevention of rickets, a vitamin D deficiency syndrome, was established (see review by De Luca, 1971) and the effectiveness, in relative terms, of different wavelengths of the UV spectrum (*viz.* the erythema action spectrum) was ascertained (Hausser & Vahle, 1922). This led the way to a plethora of studies on the photobiological effects of sunlight on human skin and in successive decades research by such workers as Coblentz & Stair (1934); Bachem (1955) and Everett *et al.* (1965) on the action spectrum, Hamperl *et al.* (1939); Rabbiosi (1962) and Daniels/

Daniels et al. (1961) on histological and histochemical responses and Jausion & Pages (1933); Epstein et al (1964) and Magnus et al. (1961) on clinical photopathology, brought into focus the wide variety of noxious effects that can be induced by UV radiation.

Although the vast bulk of the earlier literature relates to the effects of UV radiation upon human skin, the constraints applied to conducting long term experiments, particularly in relation to UV induced carcinogenesis were soon apparent. Accordingly attention was focused upon animals such as rats and guinea pigs (Logan & Wilhelm, 1963) and mice (Johnston, 1965).

Despite reports in the literature as early as 1820 suggesting a correlation between dorsal skin damage in fish and ultraviolet light, it was not until the 1960s when the pioneering work of Jerlov & Steeman Nielsen on UV penetration in oceanic waters (*vide infra*) was published that the potential hazard afforded by solar UV within the aquatic ecosystem was recognised.

## 2. ULTRAVIOLET RADIATION AND FISH

In a communication to the Royal Society in 1820, based primarily upon his observations of the protection afforded by the pigmentation of negroid skin from the sun's/

sun's rays, Sir Everard Home appears to provide the first authoritative account of sunburn in fish, the matter having been brought to his attention by no less an authority than the President of the Society. Thus, from such an auspicious source the first recorded case of sunlight affecting fish skin was documented (Home 1820).

No less fascinating, although somewhat more eccentric, were the observations of Page (1885) that sunlight enhanced the hatching rate of shad eggs, his experiments, in the main, being initiated within the confines of Central Station, New York. From there, he despatched batches of eggs arranged in such a manner that the youngest would receive full sunlight during transportation whilst the older eggs were kept in subdued light. By doing so he found the subsequent hatching rates for both groups to be equal.

In 1930 Crowell & McCay reported deaths in brook trout *Salvelinus fontinalis* (Mitchill) following exposure to ultraviolet light. They postulated that UV phototherapy might prove useful in the control of certain fish diseases and that irradiation of this type might afford a unique opportunity to eradicate potential pathogens simultaneously from both fish and water. The purpose of their study was to determine whether normal healthy fish could withstand levels of radiation that might otherwise be lethal to/

to bacteria or parasites either within the water or upon the fish. Their results showed that trout could be killed by one long period of irradiation or by a series of short periods at daily intervals. However, no quantitative data was presented on dose intensity or spectral distribution of the light source used. It seems most probable that the resultant mortalities may have been induced by levels of UV in excess of that encountered in nature in association with the probability of some extraneous UV-C emission from the lamp source used. Nevertheless the results obtained showed that trout were capable of withstanding a certain amount of radiation without injury although no reliable comparison could be made between the sensitivity of fish and other vertebrates.

It was not until 1950 when Bell & Hoar undertook a detailed quantitative study on the effects of UV radiation upon the eggs and alevins of the sockeye salmon *Onchorhynchus nerka* (Walbaum) that the injurious potential of UV radiation in aquatic ecosystems was fully realised. Their radiation source consisted of a UV lamp emitting in the UV-B spectrum (280-310 nm), control groups being irradiated through heavy plate glass, thus eliminating UV-B. They used as their standard unit of measurement the Finsen, a unit of erythemal flux intensity equal to one erythemal unit, viz. 10 microwatts per homogeneous radiation/

radiation of  $2967 \overset{0}{\text{A}}$  or 296.7 nm and subjected eggs and alevins to doses ranging from 1-35.5 Finsens. Even at the lower dose rates of 2-3 Finsens mortalities of up to 50% of the eggs occurred within ten days whereas deaths within the control groupings were relatively few. Mortality curves plotted from the results showed a rapidly increasing percentage of deaths within the eggs when the dose exceeded three Finsens with the steepness of the curves clearly dependent upon the intensity of the irradiation. Alevins irradiated at dose rates ranging from 0.5-8 Finsens showed a similar trend. In each group mortalities were directly related to dose intensity. At lower doses (0.5 and 1 Finsen) irradiated alevins appeared less active than the controls and by 15 days post irradiation mortalities were approximately 75% at 0.5 Finsen and 98% at 1 Finsen, at increasing dose rates of 2, 4 and 8 Finsens 100% mortalities were apparent within 7, 3 and 2 days respectively.

Behavioural observations during all irradiations showed that swimming activity was impaired and that alevins actively crowded into the corners of the holding chambers. During long exposures some fish became visibly weakened and sank to the bottom occasionally making sporadic bursts of activity to the surface only to fall back and remain quiescent. These less active fish/

fish were the first to die. In addition to swimming impairment other abnormalities were noted. These included restriction of lower jaw movement in respiration, distension of the vitelline veins, accumulation of serous fluid below the lateral line near the attachment of the yolk sac and an apparent irregularity in heart beat. A significant feature in terms of skin colouration was the delay of guanine formation of around seven days in those fish that survived the lower (0.5-1 Finsen) irradiations. Histologically skin damage was specific to those areas of skin directly exposed to UV. Flank and ventral skin remained undamaged.

Prior to Bell & Hoar's study the literature contains only brief reports on the susceptibility of fish skin to UV radiation. Dunbar (1959) stated that within three days of exposure to bright sunlight rainbow trout fingerlings transferred from a hatchery to outdoor ponds developed necrotic areas behind the head and around the base of the dorsal fin. He conducted further studies using separate artificial light sources emitting in the UV-B and UV-C bandwidths. By varying both distance from light source to fish and exposure times, lesions morphologically identical to those observed under natural sunlight conditions were induced. The germicidal (UV-C) wavelengths were reported to have a more noxious effect.

Subsequent/

Subsequent to Dunbar's report, De Long, *et al.* (1958) described similar findings related to sunlight damage among chinook salmon *Oncorhynchus tshawytscha* (Walbaum) and attempted to relate the condition to a nutritional deficiency. They noted from the work of Smith & Ruffin (1957) that pellagra, a chronic skin disorder in man, resulted from a diet deficient in niacin, and was greatly exacerbated by sunlight. De Long *et al* proposed therefore that a similar response might occur in fish. In order to test their hypothesis they fed a niacin deficient diet under maximum natural sunlight conditions over a 30-day period. The population was then split into two groups, one remaining on the niacin deficient diet the other on a complete diet. After 14 days their results showed an increase in mortalities in the niacin deficient group thus implying that enhanced dietary levels of niacin would afford some protection against sunburn. This theory was soon to be disproved, however, by Allison (1960). Citing the work of Phillips and Brockway (1947) which stated that trout having received their daily requirement of niacin (3.0-4.1 mg/kg of fish) excreted the remainder in their urine, he conducted a series of experiments using a niacin fortified diet of up to eight times the daily requirement. His results proved conclusively that enhanced niacin levels in the diet afforded no added protection to fish against sunlight./

sunlight. It is of some significance that his study was initiated by the annual appearance of skin lesions upon the heads of fingerling lake trout *Salvelinus namaycush* (Walbaum) a condition which he termed as 'baldspot'. Seasonality was an important feature, the lesions appearing from early June until August. By late September the condition had resolved.

Although evidence of the lethal and detrimental effects of UV irradiation upon fish at all stages of development, under the constrained conditions of aquacultural facilities in freshwater, was by now firmly established, little attention had been focused upon the role of solar UV in relation to natural fish populations, whether marine or freshwater. Marinaro & Bernard (1966) conducted a series of laboratory experiments on pelagic eggs of marine fish and established that sensitivity to UV was not limited to freshwater species. A similar observation was made by Pommeranz (1974) on the vulnerability of plaice eggs to solar UV.

The publication by Hunter et al. (1978) on the effect of UV-B irradiation on eggs and larvae of the northern anchovy *Engraulis mordax* Girard and the Pacific mackerel *Scomber japonicus* Houttvyn during the embryonic stage, and by Hunter et al. (1981) on the effects of solar and artificial UV-B radiation on larval northern anchovy represented a considerable advance in our knowledge of the/  
the/

the potential hazard that UV radiation might afford in nature.

When exposed to UV-B at irradiation levels based upon predicted UV-B increases resulting from anthropogenic diminution of the ozone layer, anchovy were found to be more sensitive to radiation damage than mackerel (Hunter et al., 1978). Their experiments related only to the first four days of larval life and showed also that a 50% mortality could be anticipated to occur at or near the ocean surface over that period assuming a diminution of between 25% and 50% of the ozone layer. Radiation damage included lesions in the retina and brain, marked retardation of growth and development and abnormal dispersion of melanosomes. The fate of surviving larvae was not studied.

In a further series of experiments (Hunter et al., 1981) batches of anchovy larvae were exposed to various doses of both natural (solar) and artificial UV-B energy over a 12-day period. Both groups suffered significant mortalities and indicated that biologically adverse conditions existed near the sea surface. All surviving larvae were smaller. Using an action spectrum modified from Green & Miller (1975) 50% of the larvae were shown to survive a cumulative dose of  $605 \text{ J.m}^{-2}$  (bio eff) or  $50 \text{ J.m}^{-2} \text{ day}^{-1}$  (bio eff). This latter figure represented the mean daily dose of UV radiation measured at La Jolla, California/

California (33°N 118°W). It is significant to note, however, that the daily UV fluence ranged from 25 J.m<sup>-2</sup> (bio eff) in December to 215 J.m<sup>-2</sup> (bio eff) in July. Dose reciprocity did not hold. Larvae exposed for four days to a cumulative dose of 642 J.m<sup>-2</sup> (bio eff), a figure close to the LD50 dose for 12 days showed a 26.7% survival by 12 days. Thus, only half the expected number of larvae survived when an LD50 dose for 12 days was given at a high dose rate for four days. Dose rate, as well as cumulative dose, thus proved to be important variables.

Despite the large amount of literature by now accruing on the implications of solar UV in environmental carcinogenesis with resultant neoplastic tumours in humans (e.g. Epstein et al., 1969) there appear to be no reports in the literature of UV induced tumours in fishes. Hart & Setlow (1975) however described for the first time the potential afforded by fish in the study of carcinogenesis. Using the hypothesis that a specific test for the biological role of UV induced pyrimidine dimers in DNA is photoreactivation (PR) they irradiated cell suspensions of tissues (liver, heart and thyroid) of the Amazon molly *Poecilia formosa* (Girard) with 254 nm (germicidal) UV for short periods then injected the irradiated suspensions into isogenic recipients. An incident fluence of 20 J/m<sup>-2</sup> on the suspension resulted in 10% of the recipient fish developing large granulomas whilst/

whilst 100% developed thyroid carcinomas. If the irradiated cell suspension was illuminated with PR (visible) light *before* injection, the yields of both types of lesions were reduced ~10 fold. If, however, PR light was given before the UV exposure and not subsequent to it, there was no reduction in the numbers of growths. Their experiments proved conclusively that the development of pyrimidine dimers within nuclear DNA could lead to neoplastic transformation.

One of the many theories of ageing is the so-called "Orgel hypothesis" (Orgel, 1963) which proposes that senescence is directly related to the accumulation of damage in cellular DNA as a result of various environmental insults. Regan *et al* (1982) investigated the photoreactive capabilities of UV induced DNA damage in two closely related species, the tautog *Tautoga onitis* (L.) and cunner *Tautoglabrus adspersus* (Walbaum) both marine fishes of the family Labridae, but with markedly different longevities. The tautog has an average life span of around 34 years attaining sexual maturity at 3-4 years of age. The cunner lives to about 5-6 years of age and is sexually mature at about one year. Their results revealed marked differences in the rate of monomerization of UV induced pyrimidine dimers from their DNA between the two species. In the tautog half the dimers were removed within a period of 10 min of exposure/

exposure to PR light whereas a 50 min exposure was required to photoreactivate dimers to this same level in the cunner. These findings therefore suggest that long lived animals may be far more capable of repairing UV damage to their DNA than short lived ones. It is within this latter category that many species of mariculture potential lie.

### 3. *THE EFFECTS OF ULTRAVIOLET RADIATION ON THE CELL*

#### 3.1 Principles of radiation damage

When cells are exposed to sunlight, they absorb some ultraviolet radiation, primarily within the UV-B bandwidth. The cell's nucleic acids and proteins take up most of the radiation, which, in turn, often produces photochemical changes. After exposure, the altered molecules may affect one or more of the cell's functions. Biological damage can range from slight effects to death of the cell. For example, radiation might impair the function of a molecule e.g. an enzyme, but cause no discernible effect on the cell's activities because other undamaged molecules take over. Alternatively, radiation might damage a few or many critical molecules and temporarily delay the cell's progression through/

through the mitotic cycle (cell division delay) until the damaged molecules can be repaired or replaced. Radiation damage may induce a permanent mutation in the genetic material (DNA) of the cell which may result in neoplasia. Usually mutations are detrimental to the cell in that they affect the control of protein synthesis. Under some circumstances, such a loss in capacity to perform a vital function, leads ultimately to death of the cell. Radiation can so damage the cell that it becomes unable to undergo successful cell division yet can still function adequately for several days; the cell is thus metabolically active but reproductively 'dead'. High doses of UV-radiation can cause severe damage to cells, and thus kill them outright (interphase death) with, for example, such damage leading to rupture of the cell's outer membrane.

### 3.2 The photon/target molecule reaction

If we take as an example a photobiological event in which a single cell is exposed to a beam of solar UV-B radiation, then it becomes clear just how complex a system of events, highly dependent upon each other, might occur before cell death takes place.

From/

From a beam of solar UV-B radiation an exceedingly large number of photons are emitted. However, only that portion of the beam striking the cell can induce a response. The photons of the effective portion of the beam can be reflected from the surface of the cell, scattered out of the beam by cellular structures and by macromolecules in suspension in the cell, or may be absorbed by nontarget molecules. Those photons that reach the vicinity of the target molecule do not necessarily strike it unless they have a precise energy output to match some permissible energy state in the target molecule. Alternatively they may be absorbed and the target molecule excited. Even so, the excitation energy may be degraded to harmless heat rather than produce a chemical change. Even if such an absorption should produce a chemical change in a target molecule this may not manifest itself as permanent biological damage the molecule may continue to function, and if a target molecule is permanently damaged it may not necessarily be detrimental to the cell in that it may be necessary to inactivate more than one target to yield a measurable effect. Furthermore, should even a sufficient number of target molecules be damaged the cell might not manifest an effect, because/

because the cell may be able to repair or replace the damaged targets. It follows, therefore, that of the exceedingly large number of potentially damaging photons in a beam of UV radiation, only a very few are actually responsible for a biological effect having overcome a quite remarkable sequence of events in order to induce cell damage.

### 3.3 Molecular changes in cell damage

It is generally accepted that ultraviolet radiation damages cells by interfering with macromolecular (DNA, RNA and protein) syntheses. DNA appears to be the primary target with these cells in the S phase of the mitotic cycle being most at risk (Giese 1976). Cellular DNA may be altered in any one of a number of ways. These are

- 1) by the addition of water to thymine or cytosine bases (hydration).
- 2) by linking thymine to thymine (T-T), cytosine to cytosine (C-C), or thymine to cytosine (T-C) on the same strand of DNA, (dimer formation).
- 3) by linking these bases across complementary DNA strands.
- 4) by breaking phosphate bonds in the backbone of a DNA molecule.
- 5)/

- 5) by linking some protein to the DNA.
- 6) by denaturation, the breaking of hydrogen bonds. Of these, the formation of dimers between thymine bases in DNA is usually the most frequent.

In RNA, changes similar to those that occur in DNA probably exist also, but because there are many more molecules of each kind of RNA than DNA in the cell, many more molecules must first be altered before the effect of irradiation interferes with cell function. Similarly, proteins may also be denatured during irradiation, but because large numbers of each kind of protein molecule are present in the cell, these effects do not interfere with function until a large number of molecules have been denatured. Furthermore, as long as DNA remains intact, more RNA and protein can be synthesised, replacing damaged RNA or protein.

### 3 4 Cellular repair of radiation damage

The sensitivity of a cell to radiation is determined largely by its ability to repair radiation damage to its DNA. Furthermore, certain chemical types of damage to DNA can be repaired by several separate repair systems, each of which has optimum/

optimum conditions for its action. Currently three systems are known for the repair of base damage

- 1) photoreactivation (the *in situ* enzymatic cleavage of cyclobutane-type pyrimidine dimers mediated by visible light).
- 2) excision (dark) repair (in the absence of light, the damaged bases are cut out of the DNA and are replaced with undamaged material).
- 3) postreplication (dark) repair (in the absence of light, the damaged section of DNA is not directly repaired but rather is bypassed during replication the missing section of the newly synthesised DNA is replaced subsequently by enzymatic processes).

Photoreactivation - To a considerable extent the damage inflicted by exposure to UV-B and UV-C rays can be reversed by simultaneous or subsequent cellular exposure to short wavelength visible light (Cook, 1970). This process occurs in all cells with photoenzymes. The enzyme responsible for this process appears to act only on cyclobutane-type pyrimidine dimers. In studies with extracted DNA it has been shown the enzyme attaches to the base dimers/

dimers (mainly thymine) of the UV radiation treated DNA. Neither cultured DNA nor photoenzyme alone absorbs radiation in the photoreactivating part of the spectrum, but the complex of the two apparently can, resulting in the splitting (monomerization) of the dimer, thus returning DNA to its original condition. Because the photoenzyme attaches only to dimers in DNA, it fails to reverse UV induced damage of other types to DNA or to RNA or to proteins. Photoreactivation is never complete in that some damage to DNA never undergoes repair, its backbone may become irreparably damaged, or linkage between DNA and protein may occur, or alternatively vital proteins may be harmed.

Excision repair - In this class of dark repair, often termed the 'cut and patch' mechanism (Regan et al , 1968) a piece of the DNA, consisting of several nucleotides including the dimer, is excised under the influence of an enzyme. The excised portion of the strand is then replicated under the guidance of DNA polymerase from information given by the complementary DNA strand. This new piece is then bound to the loose end of the uninjured portion of the DNA by the action of a binding enzyme. Ultimately, DNA is restored to its original form.

Postreplication/

Postreplication gap repair - This second class of dark repair occurs after replication has proceeded to either side of a damaged portion of a DNA strand leaving gaps (Smith, 1971). Repair then follows, after replication of the undamaged part, essentially by filling the gap(s) with the synthesis by DNA polymerase using the information on the complementary undamaged strand. This is followed by the insertion of the newly synthesised piece (or pieces) by a binding mechanism. Whilst the facts are clear, the way in which this type of dark repair works remains unresolved.

In both excision and postreplication gap repair the system is operational in visible light as well as in the dark, but in the light, photoreactivation probably occurs more rapidly, and thymidine dimers may be split and 'inactive' DNA reconstituted, even before the dark repair mechanisms involved have had much opportunity to operate. However, damages to DNA other than pyrimidine dimers such as simple strand breaks are also subject to dark repair, thus lending dark repair the advantage of generality over light repair.

### 3.5 The sunburn cell

Following a single exposure to short wavelength ultraviolet/

ultraviolet light numerous non-specific dermal and epidermal inflammatory changes are observed in human skin by light and electron microscopy (Montgomery, 1967; Nix, 1967).

Most notable amongst these cellular changes is the presence, often within six hours following irradiation, of a very distinctive cell type scattered randomly throughout the superficial epidermis and commonly referred to within the literature as the 'sunburn cell'. Numerous investigators have reported the presence of these cells, for example, Hamperl *et al.* (1939); Miescher (1957); Lorincz (1960); Daniels *et al.* (1961) and Olson *et al.* (1974). Based upon their morphological appearance under the light microscope they have been described as possessing a densely staining, glassy, homogeneous cytoplasm and pyknotic nuclei most often in association with a perinuclear or pericellular oedematous halo. A typical sunburn cell in irradiated human skin is shown in Section III, fig. 11a.

The piscine equivalent of the sunburn cell was first demonstrated histologically by Bell & Hoar (1950) in salmonid skin although no direct comparison was made by these workers to the cells prevalent in irradiated human skin. They describe briefly the nuclei of the superficial epidermal cells following irradiation/

irradiation as rounding up into one or two granules which stained variously with acid and basic dyes. A more recent study by Hunter et al. (1979) on the effects of UV-B on eggs and larvae of the northern anchovy and the Pacific mackerel during the embryonic stage has shown UV induced cell damage to occur in the brain and eye of both species. 'Lesions' in both organs were described as consisting of 'one or more spherical pyknotic nuclei surrounded by an extracellular vacuolated region frequently containing cytoplasmic debris'.

#### 4. SOLAR RADIATION

##### 4.1 Sunlight

The sun, the centre of our planetary system, is an incandescent mass 300,000 times greater than that of the earth, having a surface temperature of 6000° Kelvin, with a central temperature calculated to be around 20 million degrees. It is neither the largest nor the hottest star in our galaxy being placed by astronomers in the spectral class G<sub>0</sub> of yellow stars, the middle of the spectral range.

Within its core, thermonuclear reactions transmute about 564 million tons of hydrogen into 560 million tons of helium every second, with the resultant/

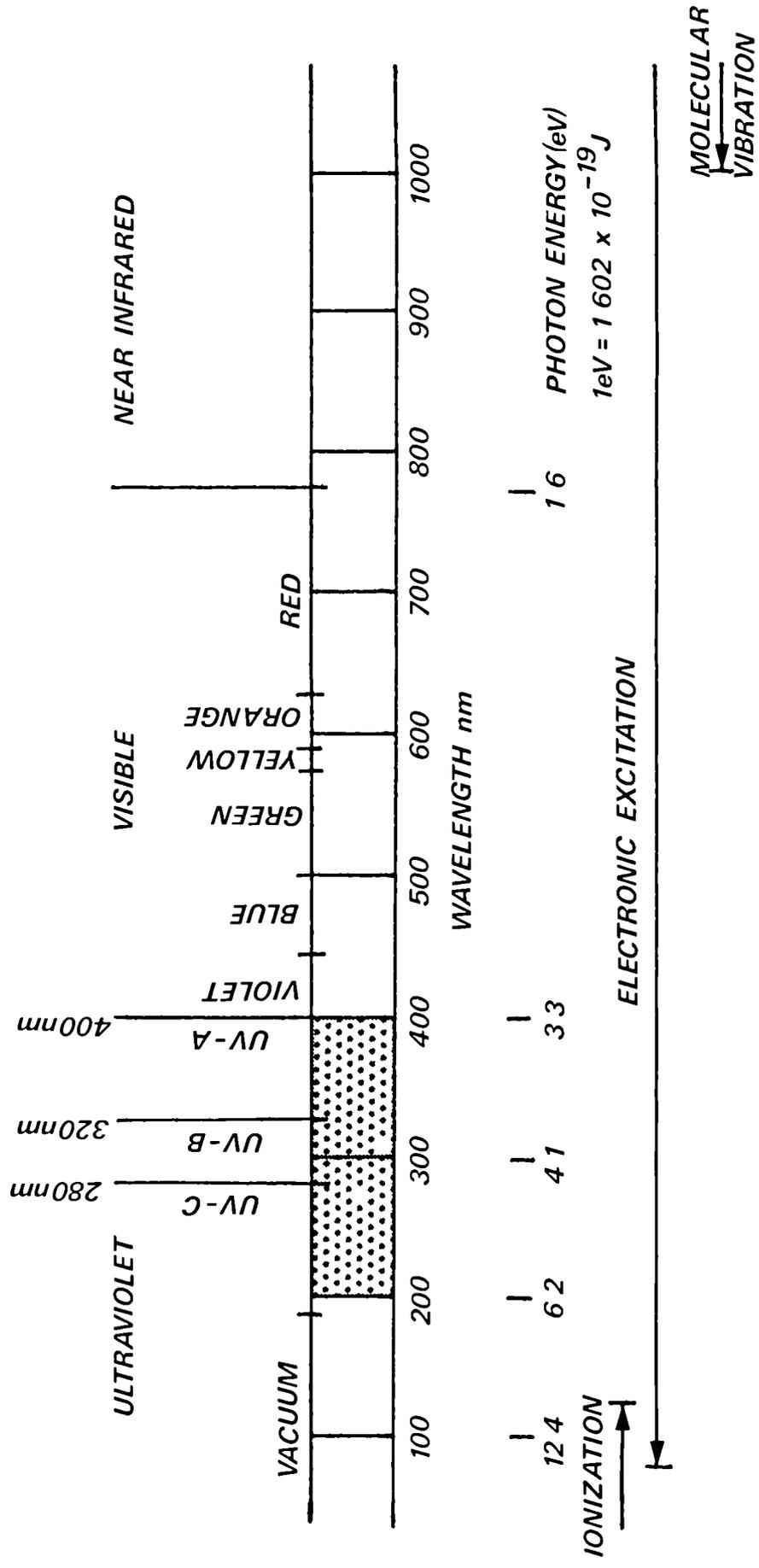
resultant conversion of four million tons of matter into radiant energy, approximately one-third being absorbed in the atmosphere. Without such energy the earth's surface would be cold, still and completely lifeless.

#### 4.2 The electromagnetic spectrum

Extraterrestrial sunlight is a relatively constant but wide spectrum of electromagnetic energy ranging from cosmic rays through to Hertzian waves. Such radiation exhibits both wavelike (oscillating field) and particle-like (discrete packet) properties. These discrete packets or quanta are termed photons. Photons follow "laws of motion" that can be described by wave equations. Thus we can speak of photons of different wavelengths. Figure 1 shows the relationship of the principal photons from the sun according to wavelength and energy. The number of oscillations per second (frequency) times the distance travelled through space per oscillation (wavelength) is the velocity of light. Because all photons travel through space at this velocity, wavelength and frequency are inversely proportional. Planck, in 1900, determined that the energy carried by a photon is directly proportional (Planck's constant) to its frequency. Therefore, shorter wavelength, higher frequency/

Figure 1

Fig 1 The electromagnetic spectrum



frequency electromagnetic radiations consist of photons of higher energy. It is characteristic of all wave motions that

$$v = \frac{c}{\lambda}$$

where  $v$  = frequency  
 $c$  = velocity of light  
 $\lambda$  = wavelength

Thus ultraviolet radiation, having shorter wavelength than visible light is more energetic than visible light, while infra red radiation, having longer wavelength than visible light, is less so. This indeed is suggested by the terminology used (ultra = beyond, transcending, infra = below).

The ultraviolet region of the spectrum is subdivided by photobiologists into three bands in order of decreasing wavelength, UV-A 400-320 nm, UV-B 320-280 nm and UV-C 280-200 nm and is based on a combination of the physical properties and biological effects of each of these wavelength regions (Parrish *et al* , 1978). Unlike the specific wavelength divisions applied to the electromagnetic spectrum, however, it is important to recognise that the biological effects of UVR do not terminate sharply at any given wavelength and therefore some overlapping of these bands is not uncommon throughout the literature with 315 nm often being selected as/

as the division between UV-B and UV-A. UV-C, although capable of inducing a sunburn response, does not reach the earth's surface because of absorption by ozone formed in the stratosphere.

#### 4.3 Air mass, absorption and scattering

As solar radiation passes through the earth's atmosphere it is modified by absorption. It is evident that the total absorption which occurs must be dependent upon the thickness of the layer of atmosphere through which the radiation passes. The relative amount of atmosphere traversed is termed the air mass and is taken as unity when the sun is in the zenith (Fig. 2). For any other position the air mass is greater than 1. In addition to the absorptive aspect of the air mass two other important factors must be taken into account, (a) attenuation in intensity due to true absorption in the atmosphere which results in a portion of the original radiation being changed into some other form of energy such as heat and (b) scattered radiation. Although lost to the original beam such radiation is not changed into some other form of energy but merely diverted from its original direction.

Scattering is produced by small particles, such as dust and water droplets in the air, as well as/

as gas molecules of the atmosphere. Each of these particles acts as a centre from which radiation is scattered in all directions. It has long been known that the amount of energy scattered by particles much smaller than a wavelength in diameter is universally proportional to the fourth power of the wavelength. Accordingly, scattering is most pronounced for the short wavelength in the blue and ultraviolet. If light only reached the earth in the straight-line path from the sun, the sky would appear black since no light would come from it. Scattering, however, diverts some of the radiation from the straight-line path. This repeatedly scattered radiation thus appears to come from all parts of the sky and since the effect is most pronounced for the short wavelengths, the sky appears predominantly blue.

Ultraviolet wavelengths are scattered even more strongly than visible light. On a clear day, at some hours, the amount of ultraviolet falling on a horizontal surface from the sky may be greater than the amount directly from the sun. As the elevation of the sun becomes greater, both the sky and the direct components increase, reaching a maximum at noon and falling off again in the afternoon as the elevation of the sun becomes less and the air mass increases. The direct component changes/

Figure 2

Fig. 2 Atmospheric path of directly transmitted solar radiation as a function of solar zenith angle.

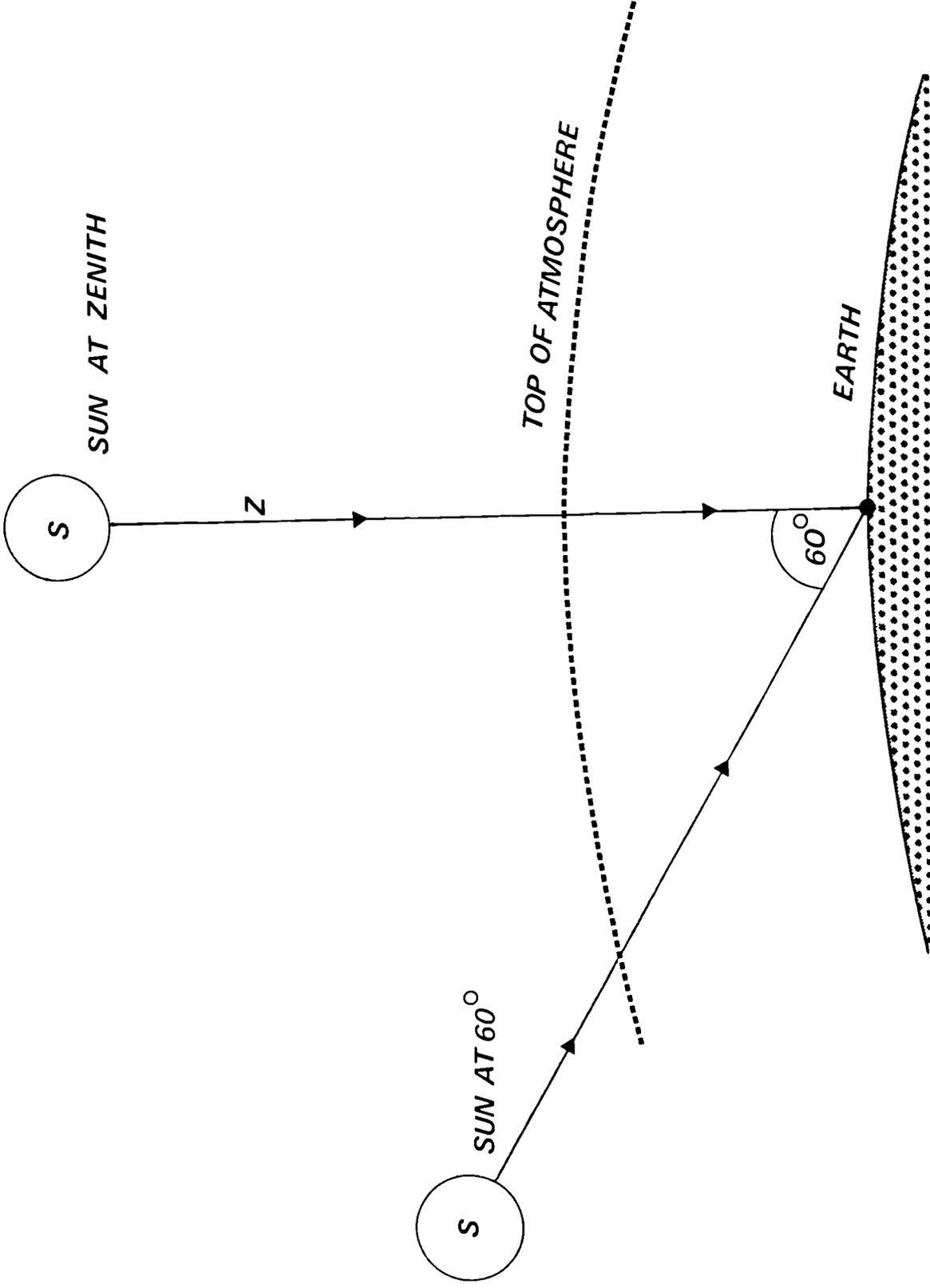
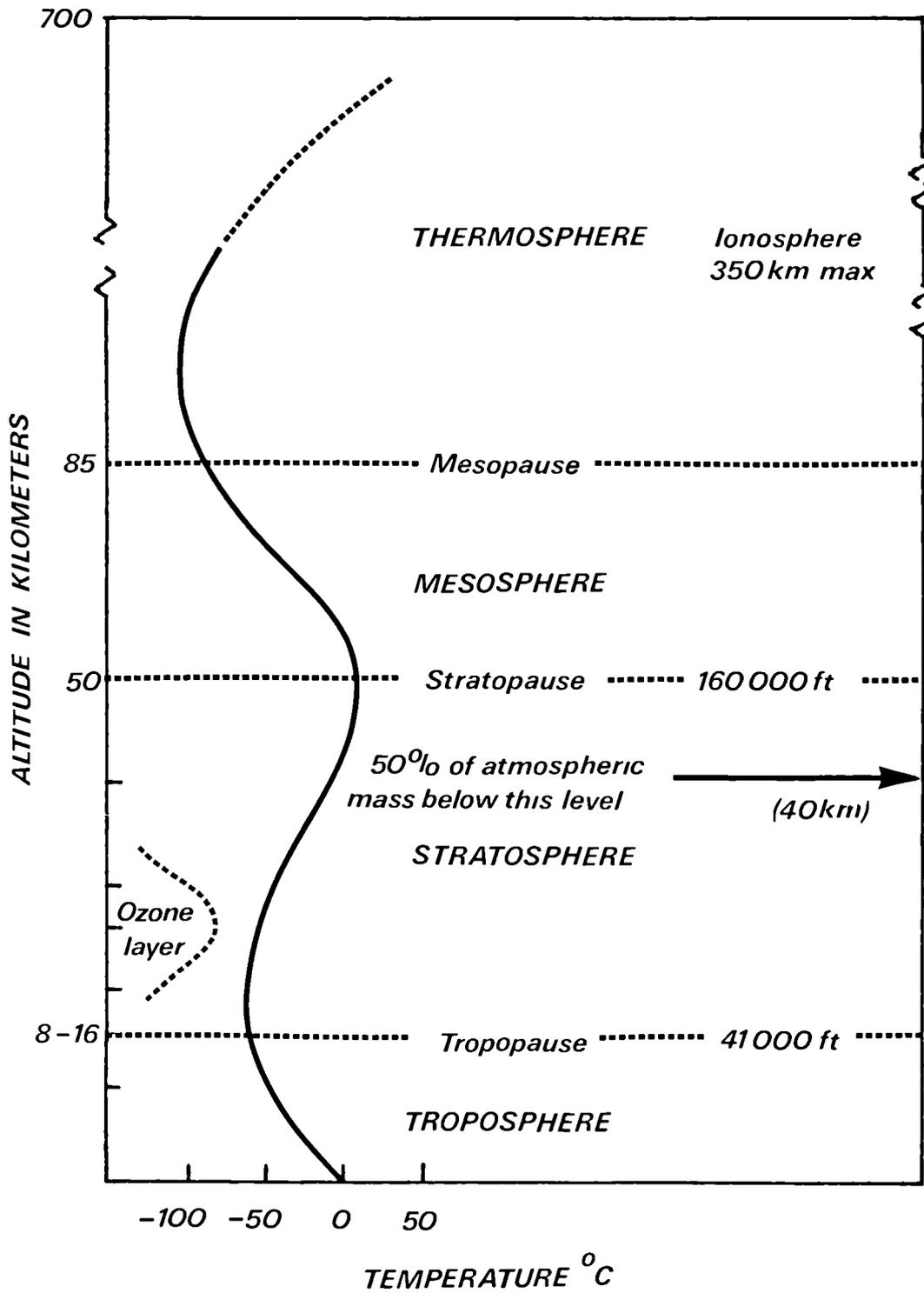


Figure 3

Fig.3 The stratification of the earth's atmosphere. The height of the tropopause varies with latitude (from 8 km at high latitude to 16 km at low latitude), seasons and various atmospheric conditions. The vertical sinuous solid curve is the temperature curve. The ozone layer at mid latitude indicated begins at about 15 km above sea level and reaches a peak 25-30 km above sea level.



changes more rapidly with elevation than the sky component. At noon the two components are approximately equal, but in the early morning and in the late afternoon the sky component is considerably larger. It is this very large sky component which makes it possible to receive a sunburn whilst sitting "in the shade" if one is still exposed to the sky.

#### 4.4 The ozone layer

As seen from space the atmosphere wraps around the earth like a gaseous membrane, thin in comparison to the earth's diameter yet protective of life and constantly in flux because of natural processes and human activities. As depicted in Fig.3, based on temperature variability, the atmosphere can be subdivided into four layers in ascending order from the earth's surface, the troposphere, stratosphere, mesosphere and thermosphere. It is within the stratosphere that the ozone layer, a gaseous "umbrella" lies, at a distance of between 15 and 35 kilometers above the earth's surface filtering out the highly biocidal UV-C. Were this gaseous layer not to exist, life on earth would not be tenable. Ozone is formed primarily by the action of short wavelength radiation upon atmospheric oxygen./

oxygen. An equilibrium is set up between the rate of formation and of decomposition by the longer wavelengths which characterise the absorption spectrum of ozone. The total amount of ozone formed is equivalent to a layer of gas only 3 mm thick at normal temperature and pressure, however, because of its strong absorption its effects are very pronounced. Ozone concentration is greater the higher the latitude. There is also a seasonal variation from a thickness of 2.4-2.6 mm at the equator to 3.1-4.3 mm at 70°N latitude.

#### 5. *PENETRATION OF ULTRAVIOLET LIGHT THROUGH WATER*

During the period 1954-56 Lenoble and her co-workers (see Lenoble 1954, Le Grand et al., 1954, Lenoble, 1956) conducted a series of experiments to measure the penetration of ultraviolet light in the sea. These were designed more specifically to enable the physical characteristics of UV transmission in water, such as vertical and horizontal scattering to be elucidated. Nevertheless her findings provided conclusive evidence that UV transmission within the sea was an environmental factor to be considered in relation to its probable influence upon the complex biological systems operating within the surface layers. Despite these reports and subsequent detailed/

detailed studies on UV transmission through sea water by Jerlov (1968) and Steeman Nielsen (1964) and the observation by Hale & Query (1973) that clear water irrespective of its ionic content was quite transparent to UV-B, aquatic biologists appear to have attached little importance to these findings until the mid-seventies when the work of Calkins (1975) and Calkins et al.(1978) brought the physical and biological aspects of the ultraviolet region in relation to water transmission into perspective. Fortuitously at this time an increasing interest was being shown by US Government agencies into the possible depletion of the ozone layer by aircraft operating in the stratosphere which culminated in a five-year research programme into the subject

The outcome of this work comprised a series of six monographs of which one (CIAP monograph 5) drew attention to the complexities of UV measurement in such a highly complex and constantly changing environment as the aquatic ecosystem. It is pertinent to quote from the introduction and overview to this monograph by Caldwell & Nachway (1975) in relation to UV transmission through water

"As would be expected, attenuation of UV-B radiation in water follows the classic logarithmic decline and is highly dependent on turbidity. For example measurements in some/

some lakes and reservoirs in Kentucky revealed UV-B radiant energy flux to be attenuated to 50 per cent of the surface intensity at less than 10 cm. In contrast penetration in clear waters off the coast of Puerto Rico was remarkably good intensities were attenuated to 50 per cent of surface levels at 4 m. *Certainly the widely held misconception that UV-B radiation does not penetrate into water must be dispelled.*"

Thus the importance of UV-B transmission in surface water was firmly established.

In his studies of light transmission through water Jerlov (1968) subdivided oceanic and coastal waters into varying categories in accordance with their optical clarity. Thus he graded ocean water types I, IA, IB, II and III and coastal waters 1, 3, 5, 7, 9 in descending order of clarity. From Jerlov's data, Zaneveld (1975) computed Table 1, showing the percentage of surface irradiance as a function of depth and water type ( $\lambda = 310 \text{ nm (UV-B)}$ ). From these figures it becomes evident that a considerable disparity exists between oceanic and coastal water types. Oceanic water appears to afford much greater transmission values whereas coastal water, high in 'yellow substance' (*vide infra*) and particulate matter/

matter shows a much greater attenuation of UV-B. It is important, however, to recognise that Jerlov's measurements for coastal waters were conducted when the solar altitude was  $45^{\circ}$  whereas the ocean water measurements were conducted when the sun was at its zenith. The computed measurements for coastal waters thus probably represent a considerable underestimate of true transmission values at maximum UV-B input. Furthermore no data are given on weather conditions at or around the time such measurements were taken. Moderate to high rainfall prior to measurement could have had a significant influence on his findings.

It is appropriate at this point to place into context the adverse effect that such transmission in the UV-B spectrum might have in aquaculture as it is generally acknowledged that a figure of 0.01% UV-B is of biological significance in the induction of cellular change, viz. that amount of light capable of inducing a response within a cell, though not necessarily pathological (Calkins, 1975). From the literature cited earlier it can be seen that all reports of UV induced dermatopathies have been observed in fish held in captivity. Under such conditions, often shallow ponds, tanks or raceways fish have no alternative but to accept levels of ultraviolet radiation transmitted through the water column perhaps far in excess of those to which they would be exposed in nature

In/

TABLE 1 Percent of Surface Irradiance as a Function  
of Depth and Water Type ( $\lambda = 310 \text{ nm (UV-B)}$ )

Source Adapted from Jerlov, 1968

Water Type	I	IA	IB	II	III	1	3	5
Depth	0	100	100	100	100	100	100	100
(m)	1	86	83	80	69	50	16	9
	2	74	69	64	48	25	2.6	0.8
	3	64	57	51	33	13	0.4	0.1
	4	55	47	41	23	6.3	0.1	-
	5	47	39	33	16	3.1	-	-
	6	40	33	26	11	1.6	-	-
	8	30	23	17	5.1	0.4	-	-
	10	22	16	11	2.4	0.1	-	-
	12	16	11	6.9	1.2	-	-	-
	14	12	7.4	4.4	0.6	-	-	-
	16	8.9	5.1	2.8	0.3	-	-	-
	18	6.6	3.5	1.8	0.1	-	-	-
	20	4.9	2.4	1.2	-	-	-	-
	25	2.3	0.9	0.4	-	-	-	-
	30	1.0	0.4	0.1	-	-	-	-
$K_E$ in $\text{m}^{-1}$		0.15	0.19	0.22	0.37	0.69	1.83	2.41
%Tr in 1m		86	83	80	69	50	16	9
%Tr in 10m		22	16	11	2	0.1	-	-
Depth (m) at which radiation is 10% of surface value		15.4	12.1	10.5	6.2	3.3	1.26	0.96

In aquaculture the water column may extend from only a few centimetres to several metres and in the majority of hatcheries and on-growing facilities will almost certainly fall within the 0.5-4 metre range. It is, of course, recognised that the majority of water conditions in aquaculture, as in nature, are not conducive to high transmission of UV, but even allowing for attenuation due either to high turbidity or the presence of 'yellow substance' a composite of humus like compounds (Højerslev, 1982) there are undoubtedly times when climatic and water conditions combine to allow maximal input of UV-B. It is, therefore, this artificial environment which affords greatest interest in terms of the potential damaging effect to fish skin of the ultraviolet spectrum.

SECTION II

MATERIALS AND METHODS

## 6. MATERIALS AND METHODS

### 6.1 Fish

The following fish species were selected for their differing modes of habitat and their significance in commercial aquaculture

Plaice	-	<i>Pleuronectes platessa</i> L.
Turbot	-	<i>Scophthalmus maximus</i> (L.)
Rainbow trout	-	<i>Salmo gairdneri</i> Richardson
Atlantic salmon	-	<i>Salmo salar</i> L.

Plaice and turbot are bottom living fish and consequently tend to occupy the lower levels of the water column seldom rising towards the surface. By contrast salmonids are pelagic, swimming freely throughout the water column.

Plaice of one year plus group, ranging from 5-8 cm in length, were taken by beam trawl from Ardmucknish Bay, near Oban, transferred to the laboratory and held in a flow-through sea water system at ambient temperature. Juvenile turbot were obtained from a local hatchery and held under similar conditions as were rainbow trout and Atlantic salmon fry. For long term simulated 'solar' UV experiments mature salmonids were supplied by Howietown Fish Farm near Stirling.

In/

In addition to the laboratory experiments a limited series of experiments on the direct effects of equatorial sunlight on the skin of rainbow trout fry held in constrained conditions was conducted at Quality Trout Farm, Naro Moru (altitude 2100 m), Kenya, near the equator on the slopes of Mt Kenya.

## 6.2 Irradiation array

The apparatus (Fig.4) consisted of an aluminium framework providing support for two banks of 12, 1200 mm, fluorescent tubes mounted at 7 cm intervals on plywood sheeting and suspended above the irradiation tanks (*vide infra*). Maximum reflectivity of the tubes was attained by sheets of metalized PVC fixed behind the tubes. The duration and 'mix' of irradiance was controlled by linking the tubes in series to automatic time switches. Intensity of irradiation was controlled by raising or lowering the tube bank via a pulley system.

## 6.3 Experimental design

All laboratory studies were conducted indoors under artificial light (*vide infra*) using two quite separate experimental regimes.

*Regime A.* For irradiance experiments with juvenile fish two 120 l capacity rectangular polythene tanks/

tanks were used thus allowing two sets of UV-B experiments to be conducted simultaneously. Dependent upon which species was being irradiated the tanks contained either sea or fresh water. This was continuously aerated and filtered by Eheim mechanical filters.

Into each irradiation tank a holding chamber was placed consisting of 12 circular clear plastic containers (10 cm diameter) fixed in a 2x3 format on to a perspex base (Fig.5). Holes were drilled in the walls of each container to allow an adequate interchange of water. Up to 12 fish were used in each experiment; one fish being placed in each container. To prevent fish swimming free during irradiation the holding chamber was covered with a wide mesh thin filament netting. All experiments on juvenile fish were carried out at a water depth of 40 cm, at a temperature of 10°C.

*Regime B* Irradiation of mature salmonids was conducted in a 1.5 m diameter circular glass fibre tank at a water depth of 1 m with a flow through of approximately 25 l min<sup>-1</sup>. Although the primary seawater supply was drawn via a sub-sand extraction system it was necessary to ensure that constant water clarity be maintained. This was done by devising a filter (Fig.6) which, when coupled to the primary system/

system just prior to entering the tank, had the effect of maintaining optimal water clarity.

#### 6.4 Light sources

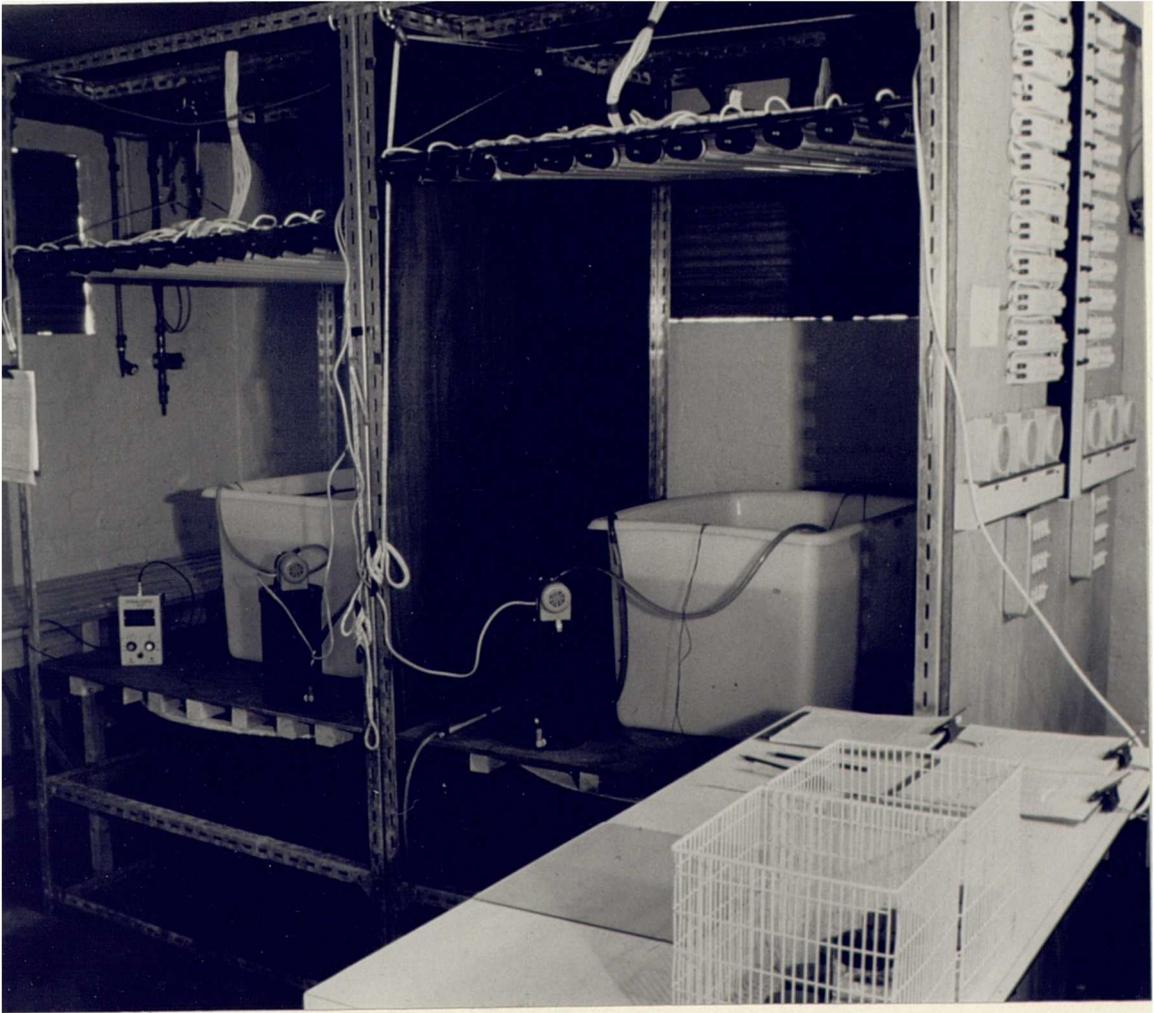
Throughout the study 1200 mm fluorescent tubes were used. Visible light was provided by Thorn 40 W Daylight (Coolwhite) tubes, these being selected because of their minimal output in the UV spectrum and their approximate correlation to natural daylight. The UV-B irradiation source consisted of Phillips TL 40W/12 tubes. For UV-A emission Sylvania 40 W BLB 'Blacklight' tubes were selected. Typical spectral transmission curves of both UV-B and UV-A sources are shown in Fig.7. Before use all tubes were aged for approximately 200 h. in order to reach a relatively flat portion of the energy decay curve supplied by the manufacturer.

#### 6.5 Radiometric measurements

During the initial stages of the study an attempt was made to measure UV tube emission using a Kipp & Zowen solarimeter thermopile coupled to a Hewlett Packard digital voltmeter. This method, however, proved unreliable. There was, for instance, some doubt as to the sensitivity of the detector within the UV-B range of spectral activity; it also proved impractical/

Figure 4

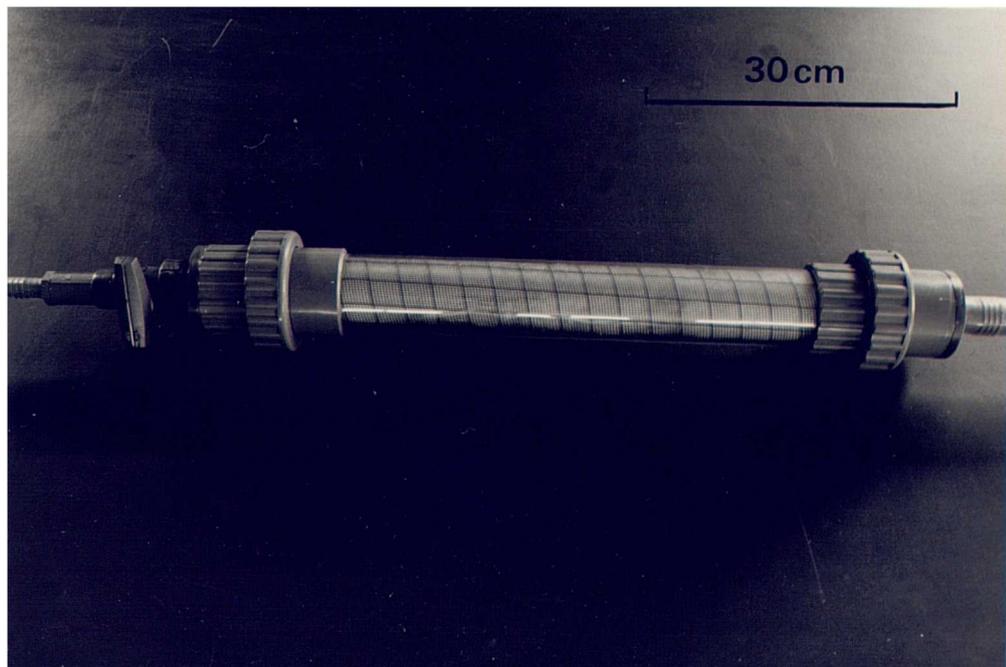
Fig. 4      Irradiation array



Figures 5 and 6

Fig.5 Holding chamber for irradiation experiments

Fig.6 The 'Joyce' filter. Within the clear perspex tube aquarium wool is sandwiched between Netlon in a 'swiss roll' effect. The system not only provides a visual assessment of the degree of fouling but is also readily interchangeable.



impractical to waterproof the device for underwater measurements. Furthermore, no UV-A irradiance values could be obtained. This technique was abandoned therefore in favour of a portable UV radiometer manufactured by Macam Photometrics (Macam Photometrics Limited, Livingstone, Scotland). This instrument provided a digital display through five full scale decades reading from 0-19.99 x 1  $\mu\text{W cm}^{-2}$  through 0-19.99 x 10<sup>4</sup>  $\mu\text{W cm}^{-2}$  with a sensitivity of 0.1  $\mu\text{W cm}^{-2}$ . Two detector heads, one measuring in the UV-B bandwidth, the other UV-A, were supplied to specification. Both incorporated Ga As (P) solid state photodiodes, waterproofed and cosine-corrected for underwater measurements. The peak wavelength response of the UV-B detector centred upon 310 nm with a bandwidth of 34 nm. The UV-A detector peaked at 365 nm with a bandwidth of 37 nm. Calibration checks were carried out annually by the manufacturers. Spectral response curves for both heads are shown in Fig.8.

It is important to recognise the limitations of such instrumentation in relation to radiometric measurements in the ultraviolet. Whilst it provides an accurate assessment of UV output from a matched artificial source, the UV-B detector for example will only record true UV-B irradiance at a monochromatic wavelength of 310 nm, i.e. the peak of its spectral response/

Figure 7

Fig. 7 Typical spectral irradiance curves  
for UV-B and UV-A fluorescent tubes

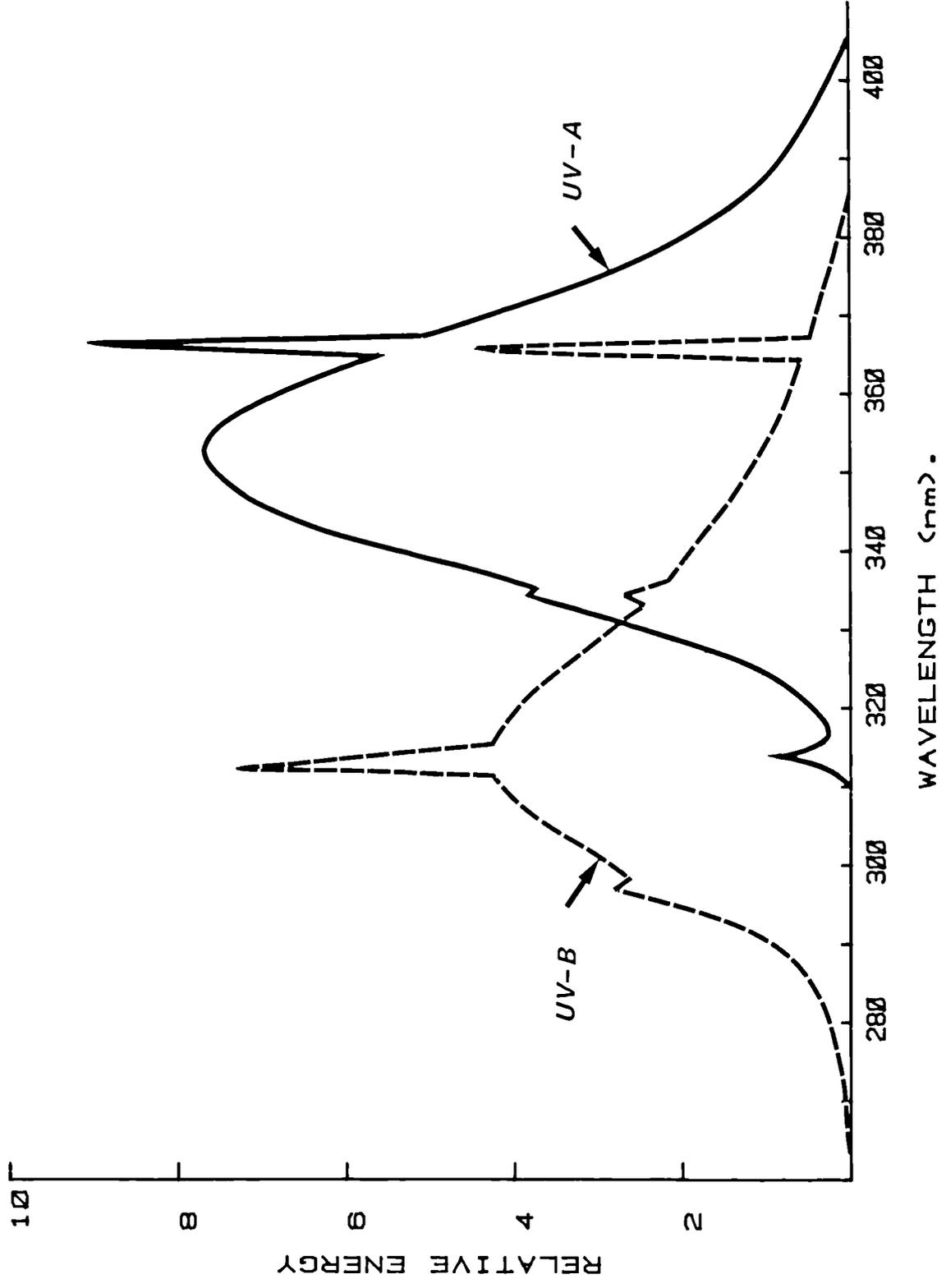
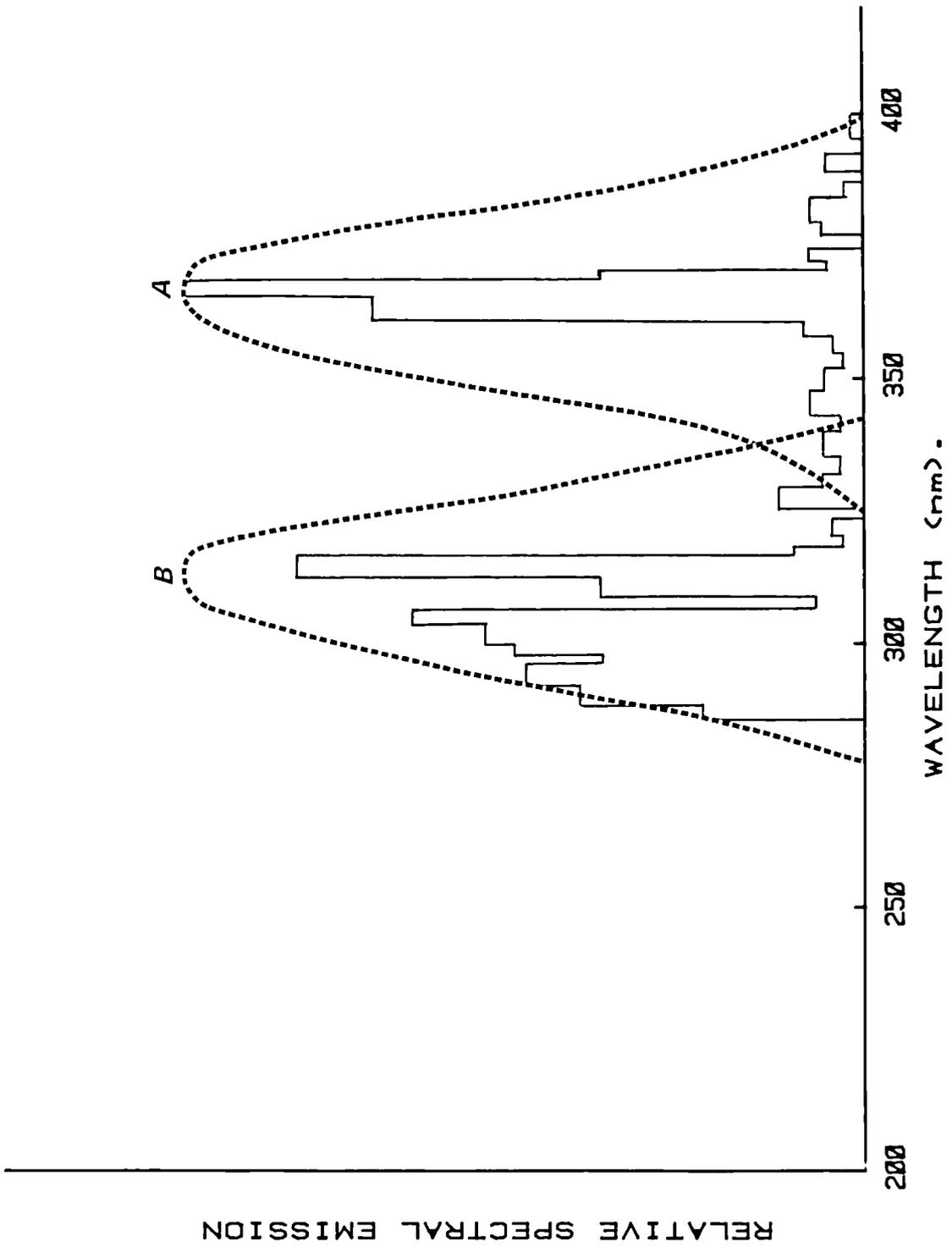


Figure 8

Fig 8 Spectral response curves (broken lines) for UV-B and UV-A detector heads. The solid lines indicate the relative spectral emission over the UV-B and UV-A bandwidths from light sources emitting in the ultraviolet (data by courtesy of Macam Ltd)



response curve. For a UV source emitting any other spectral distribution the detector reading will represent a convolution between the spectral power distribution of the source and the spectral response of the detector.

Thus, whilst it provides an adequate assessment of UV output from the artificial sources used in this study, it is nevertheless desirable to record a biologically-effective UV-B dose termed herein UV-B (bio eff) so that dose measurements at this bandwidth can be compared to those published by other workers. From data provided by Diffey (pers. comm.) and using the biological action spectrum of Caldwell (1971) to represent the relative effectiveness of UV-B to induce a biological response when protein or nucleic acid chromophores are involved, and the values of the diffuse attenuation coefficient of water from Smith & Baker (1979), it was then possible to relate the Macam UV-B detector reading to the UV-B (bio eff) irradiance by the relationship

$$\text{UV-B (bio eff) irradiance } (\mu\text{Wcm}^{-2}) = \text{Macam UV-B detector reading } (\mu\text{W cm}^{-2}) Q$$

where Q is a correction factor which allows for the difference in the biological action spectrum and the spectral response of the detector, and is given by

$$Q/$$

$$Q = \frac{\int I(\lambda) E(\lambda) \exp(-K(\lambda) z) d\lambda}{\int I(\lambda) S(\lambda) \exp(-K(\lambda) z) d\lambda}$$

where  $I(\lambda)$  is the relative intensity of radiation of wavelength  $\lambda$  from the lamp

$E(\lambda)$  is the biological effectiveness of the radiation at wavelength  $\lambda$

$S(\lambda)$  is the spectral response of the detector at wavelength  $\lambda$

$K(\lambda)$  is the diffuse attenuation coefficient ( $m^{-1}$ ) for clear water at wavelength  $\lambda$

and

$z$  is the depth of water (m) at which measurements are made

Values of  $Q$  were calculated for various depths of clear water from 0 to 2 m as follows

<u>Depth of water (m)</u>	<u>Q</u>
0	0.26
0.5	0.25
1.0	0.24
1.5	0.23
2.0	0.22

As can be seen the correction factor  $Q$  is almost invariant of depth thus the UV-B (bio eff) irradiance is/

is equal to one-quarter of the UV-B detector reading.

## 6.6 Filters

The wide-ranging spectral distribution curve from a typical artificial UV-B source (see Fig.7) reveals relative values of the shorter wavelengths which are seldom, if ever, encountered at the earth's surface. In order to investigate the influence of these shorter wavelengths using artificial sources in laboratory studies a cut-off filter, cellulose triacetate (CTA) was incorporated into several of the experiments. In addition an acetate filter (Melinex O), whose cut off characteristics eliminated almost all UV-B, was also used. The transmission data for both are shown in Fig.9. The ineffectiveness of semi-opaque polythene sheet in attenuating UV-B is shown also.

## 6.7 Histology - Juvenile fish.

Irrespective of cumulative dose, radiant intensity or light 'mix' flatfish were sampled over a timescale ranging from immediate post-irradiation up to 72 hours post-irradiation (hpi) and salmonids from immediate post irradiation up to 336 hours (14 days) post-irradiation. From each irradiated batch one fish was/

was removed at random from the holding chamber at a given point in the time scale, anaesthetised then killed. From flatfish blocks of selected areas of the dorsal skin and underlying muscle measuring approximately 1.5 x 1.0 x 0.3 cm were removed and fixed in 10% buffered formalin. At least 24 hours following fixation the samples were trimmed to provide blocks approximately 1 x 0.5 x 0.3 cm. A similar procedure was adopted for salmonids. Due, however, to the smaller surface area exposed to radiation in these species the regions sampled consisted of sequential blocks of tissue along the length of the dorsum. In addition random samples were taken from the upper flank. Mature salmonids from long term exposure trials were sampled in a similar manner.

All tissue blocks were processed for sectioning using the 23 h processing cycle used routinely in this laboratory, (Bullock *et al*, 1976). Sections were cut at 5  $\mu$ m.

Throughout the study the following staining techniques (Culling, 1963) were used routinely

Haematoxylin & Eosin

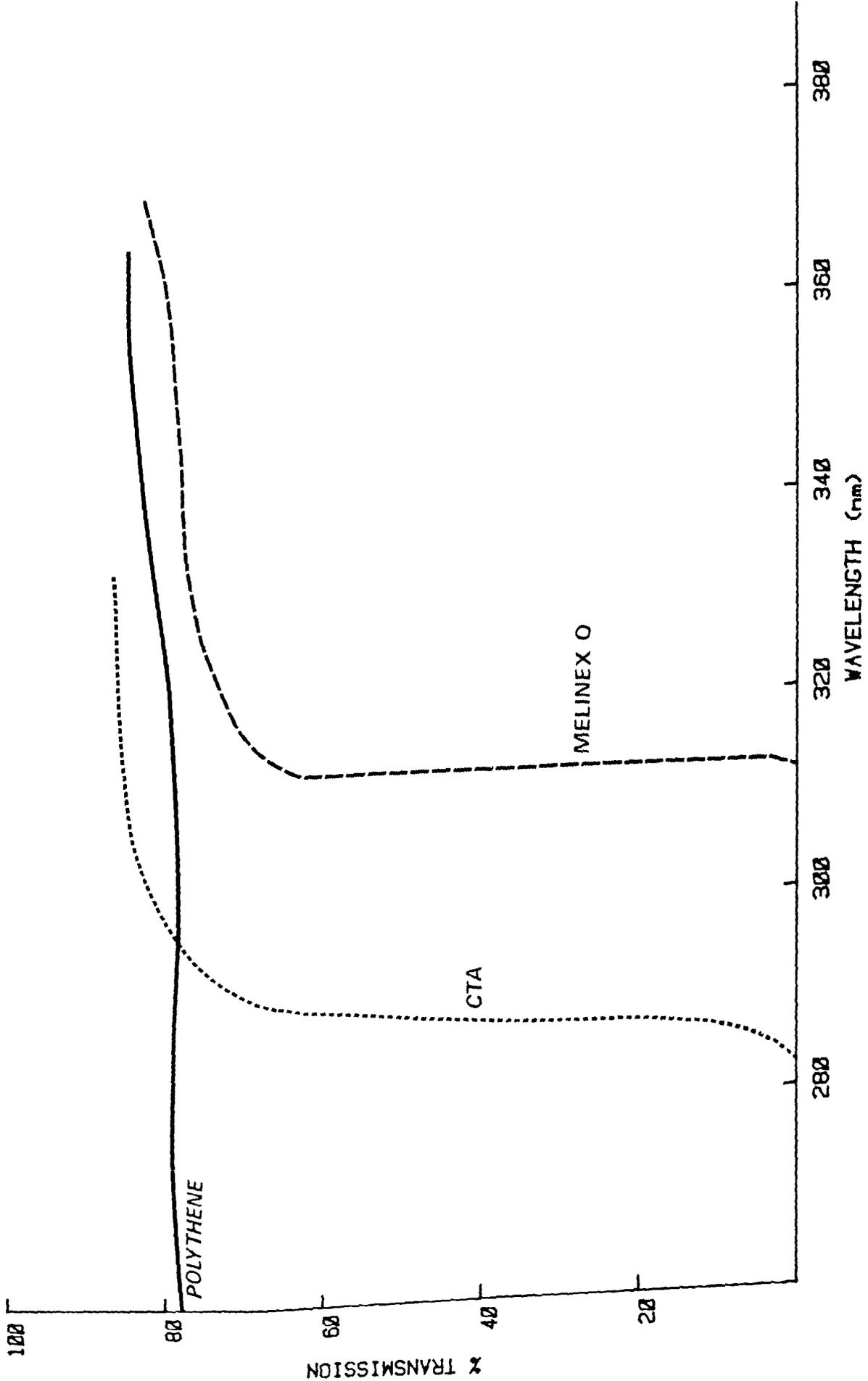
Mallory's trichrome stain

Masson's trichrome stain

Periodic acid-Schiff (PAS) procedure

Figure 9

Fig.9 Spectral transmission curves for cellulose triacetate (CTA), Melinex O and semi-opaque polythene sheet.



## 6.8 Scanning electron microscopy

Following irradiation fish were anaesthetised then killed at 0.5, 1, 2, 4, 8, 24, 32, 48, 56, 72 and 80 hpi. Areas of dorsal skin measuring 1 x 1 x 0.3 cm were removed and fixed in buffered glutaraldehyde (4% formalin + 3% glutaraldehyde in 0.2 m phosphate buffer) for 2 hours, post-fixed for 1 hour in 1% osmium tetroxide to enhance resolution, then rinsed in 70% ethanol. Dehydration was carried out for periods of 3 hours each in 70%, 90%, 95% and absolute ethanol.

Following substitution with acetone and then liquid carbon dioxide, the tissues were critically point dried, mounted on aluminium stubs and gold coated prior to viewing on a Cambridge Stereoscan 600.

SECTION III

EFFECTS OF UV-B UPON THE SKIN  
OF THE PLAICE *Pleuronectes platessa* L. and  
THE TURBOT *Scophthalmus maximus* (L)

### *Introduction*

The skin of teleost fish encompasses a variety of tissue layers of variable morphology, ranging from the outer cuticle to the hypodermis, a zone of loose connective tissue attached to the underlying musculature (Fig.10).

As the actinic response to ultraviolet radiation appears to lie primarily within the epidermis this layer has been the main focus of attention throughout the study. However, as the title implies the skin *per se* has been the subject of investigation and, therefore, where appropriate, attention is drawn to specific aspects of tissue damage within the subjacent layers. Due to the wide variation in susceptibility of these lower layers to the radiation exposures applied no specific scoring value (*vide infra*) has been allocated to them. Where appropriate any abnormality attributed to radiation beneath the epidermis is noted in the description of the sequential radiation response.

Throughout the study, sunburn cells (see Section I) have been identified in all species investigated over a wide range of radiant exposures and sub-divide into two quite distinct categories

*Type A* sunburn cells - irradiated malpighian cells  
whose morphological appearance by light microscopy most closely resembles that described  
for/

for human skin (compare Figs. 11 a and b).

*Type B* sunburn cells - irradiated malpighian cells whose morphology most closely resembles that described by Hunter *et al.* (1979) in eye and brain lesions in larval anchovy and mackerel (Fig. 11 c).

The study of the baseline effects of UV-B on fish skin was carried out on the plaice at radiant intensities of 30 and 60  $\mu\text{W cm}^{-2}$  with duration of each exposure varying from 5 minutes to 120 minutes. This gave series of cumulative dose rates of incident UV-B upon the skin surface of the fish from 9  $\text{mJ cm}^{-2}$  to 432  $\text{mJ cm}^{-2}$  (2.25-108  $\text{mJ cm}^{-2}$  bio eff). A more limited series of experiments was performed on turbot at a radiant intensity of 30  $\mu\text{W cm}^{-2}$  giving a cumulative dose of 72  $\text{mJ cm}^{-2}$ . The white light values at experimentation, important in terms of photoreactivation, ranged from 320  $\mu\text{W cm}^{-2}$  up to 1280  $\mu\text{W cm}^{-2}$  in intensity at the water surface.

Relative values of epidermal degeneration for each cumulative dose were determined by visual assessment at the light microscope level. Following detailed analysis of the responses of the epidermis to varying levels of irradiation it was found that irrespective of individual skin or time scale variation there was a standard sequential development of the lesions characterised by eight specific stages. Thus the sequential pathogenesis of the radiation damage/

Figure 10

Fig. 10      Schematic diagram of the skin structure  
of a typical teleost fish

<i>CUTICLE</i>
<i>EPIDERMIS</i>
<i>BASEMENT MEMBRANE</i>
<i>DERMIS</i> <i>comprising stratum spongiosum</i> <i>and stratum compactum</i>
<i>HYPODERMIS</i> <i>tela subcutanea</i>

*SUBCUTANEOUS MUSCLE*

damage was scored on a scale from 1 to 8, the points on the scale being assessed as follows

- Grade 1* Swelling of mucous cells (and or swelling/movement of acidophilic granule cells) where present
- Grade 2* Irregular surface layer, usually observed in association with perinuclear haloes surrounding the epidermal malpighian cells
- Grade 3* The presence of sunburn cells
- Grade 4* Epidermal oedema and/or hyperplasia
- Grade 5* Epidermal necrosis viz moderate to severe radiation damage
- Grade 6* A focal loss of attachment of malpighian cells usually within the supra basal zone viz. epidermal cleavage
- Grade 7* Initial sloughing of the outer epidermal layer
- Grade 8* Complete sloughing of the epidermis

These various stages are indicated in Fig.12, along with an illustration of the normal structure of flatfish skin.

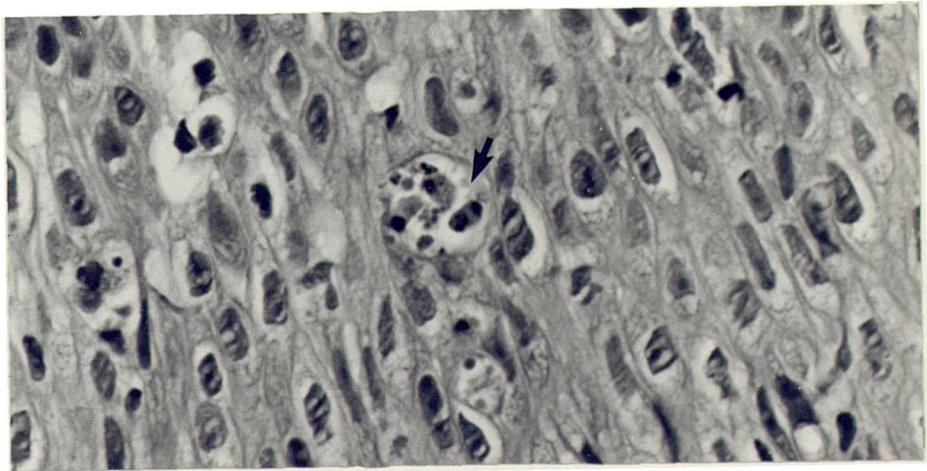
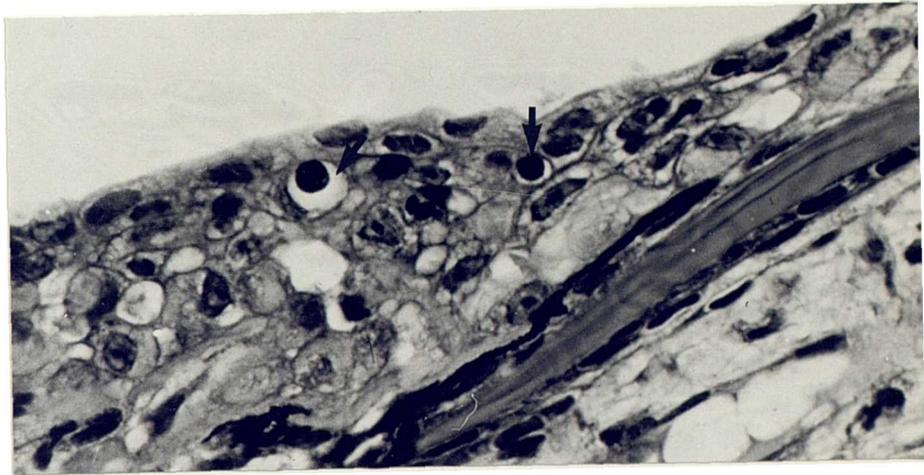
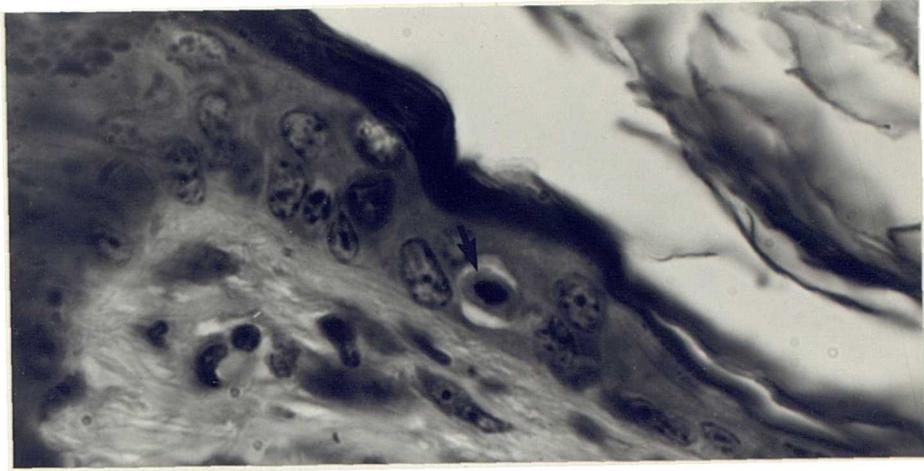
Figures 11a-c

NOTE All photomicrographs, unless otherwise stated, are taken from Haematoxylin & Eosin stained sections. The magnification is x 450 for most prints. Where this changes the appropriate magnification is entered on the figure legend.

Fig. 11a Sunburn cell in human skin  
x 1000

Fig. 11b *Type A* sunburn cells in the skin of the  
Atlantic salmon  
x 1000

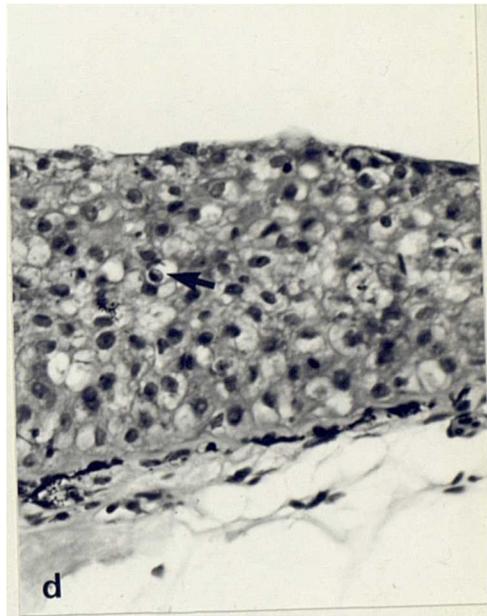
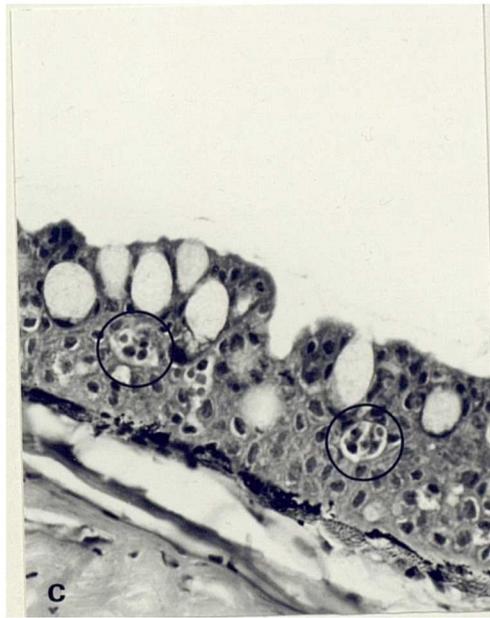
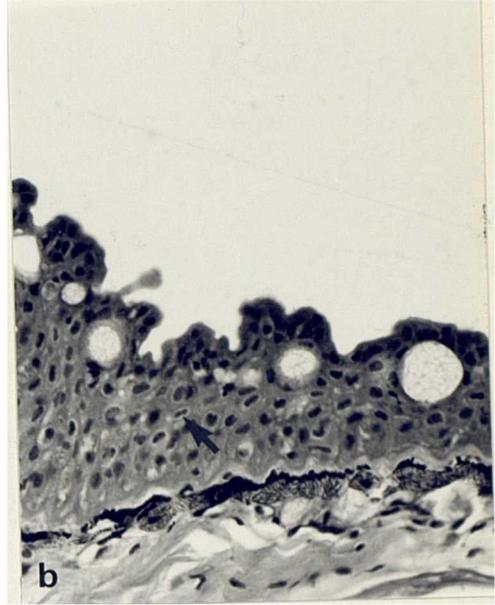
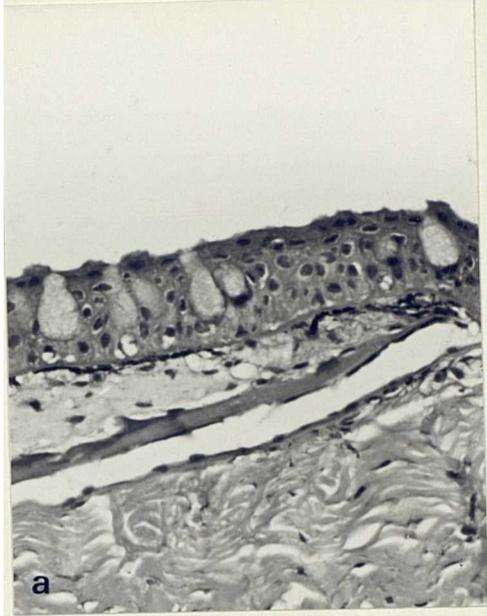
Fig. 11c *Type B* sunburn cell in the skin of the  
rainbow trout  
x 1000



Figures 12a-d

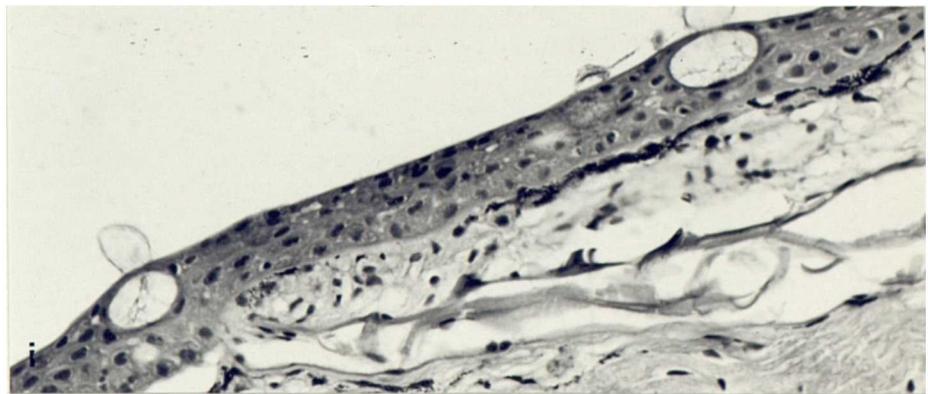
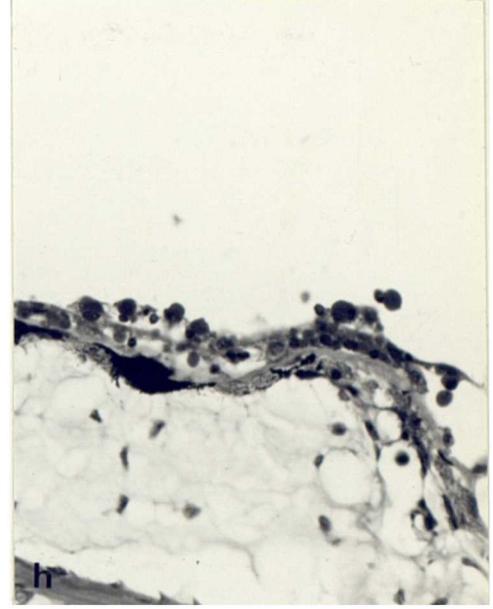
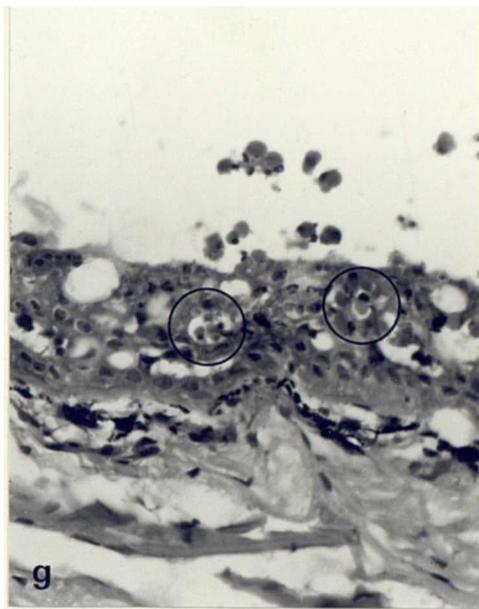
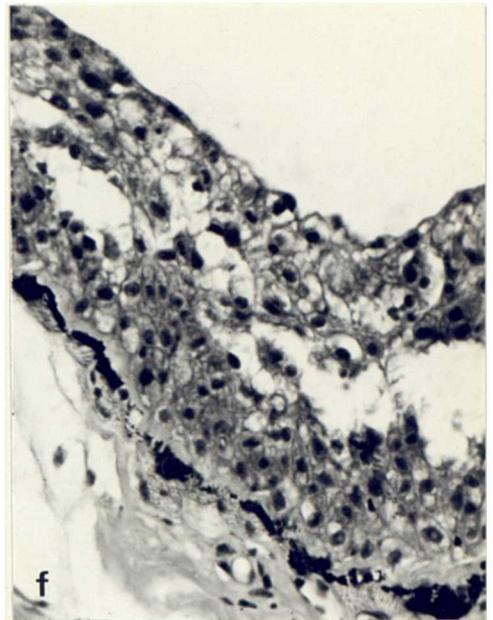
Fig.12 Photographs a-h illustrate the various stages in the sequential response of plaice skin to UV-B used in assessing the relative values of epidermal damage. x 425

- a Mucous cells exhibit a limited increase in size and the acidophilic granule cells are swollen and devoid of content
- b Irregular surface in association with the development of perinuclear haloes (arrowed)
- c *Type B* sunburn cells within the mid-zone
- d The epidermis exhibits hyperplasia and oedema. One *Type A* sunburn cell is present (arrowed)



Figures 12e-1

- e A generalised necrosis is now underway  
many nuclei appearing pyknotic. Numerous  
nuclear droplets are prevalent throughout
- f Epidermal cleavage is now a major feature  
of the middle layer
- g Sloughing of the outer layer has commenced.  
Below, *Types A* and *B* sunburn cells (circled)  
can be seen
- h Destruction of the epidermis is complete.  
Only a few necrotic cells about the basement  
membrane prior to sloughing
- i Normal, non-irradiated plaice skin



7.1 The effect of cumulative doses of UV-B ranging from 9 to 216 mJ cm<sup>-2</sup> upon the skin of plaice

*RESULTS*

Fig.13 shows the effect of UV-B upon plaice skin at cumulative doses of 9, 18, 27, 36 and 54 mJ cm<sup>-2</sup>. At 9 mJ the response to radiation was limited throughout the timescale to a moderate thickening of the epidermis by 56 hpi (Fig.14). Surface irregularity was first seen at 4 hpi and sunburn cells at 24 hpi. By contrast a cumulative dose of 18 mJ cm<sup>-2</sup> produced surface irregularity as early as 0.5 hpi with sunburn cells present at 2 hpi (Fig.15). By 4 hpi at this higher level of radiation there was a substantial increase in sunburn cells and the surface layer was extremely convoluted (Fig.16) leading by 24 hpi to epidermal oedema and finally a moderate sloughing of the outer layer by 56 hpi.

At a dose of 27 mJ cm<sup>-2</sup> the general response to radiation during the initial stages followed a pattern similar to that observed at 18 mJ cm<sup>-2</sup>. By 4 hpi, however, the melanocytes situated within the *stratum spongiosum* of the dermal layer exhibited nuclear swelling with a concomitant outward dispersal of the melanosomes from the immediate perinuclear zone (Fig./

(Fig.17). This feature was not observed at the lower doses. Epidermal oedema was present by 24 hpi leading to initial sloughing of the outer layer by 48 hpi compared to 56 hpi at  $18 \text{ mJ cm}^{-2}$ . Radiation damage did not proceed beyond this point.

During the initial stages of skin damage at 36 and  $54 \text{ mJ cm}^{-2}$  there was a delay in the appearance of sunburn cells until 4 hpi as opposed to 2 hpi at lower doses. At  $36 \text{ mJ cm}^{-2}$  radiation damage did not proceed beyond stage 7. By contrast fish irradiated at  $54 \text{ mJ cm}^{-2}$  exhibited cleavage of the epidermis at the supra-basal level by 24 hpi in conjunction with a generalised necrosis throughout the upper zone (Fig.18). At the termination of the experiment epidermal destruction was complete with the underlying dermis also exhibiting radiation damage (Fig.19).

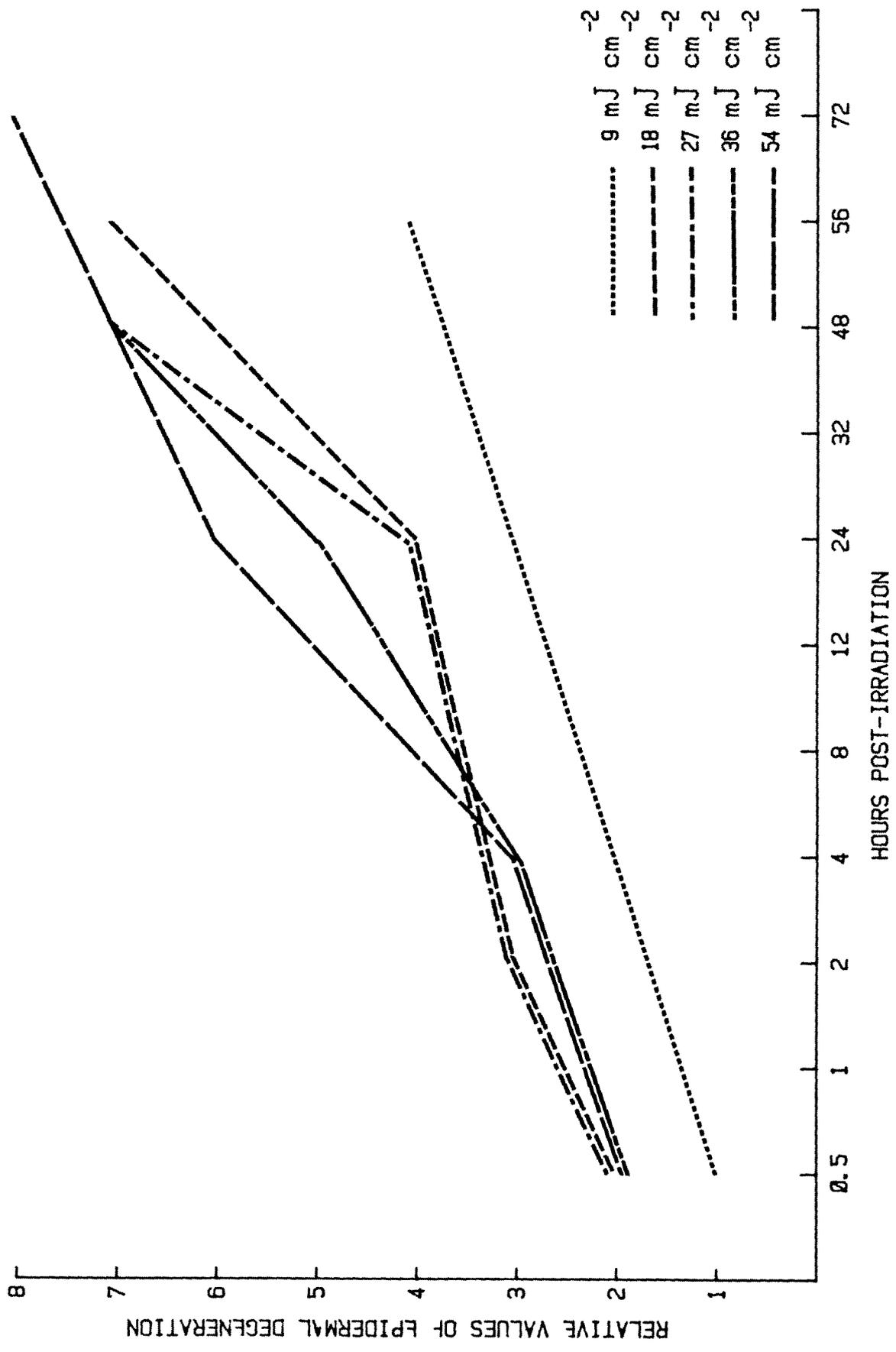
Cumulative doses of 70, 90, 108, 162 and  $216 \text{ mJ cm}^{-2}$  (Fig.20) showed a similar sequence of radiation damage but with a significant decrease over the time-scale required to induce complete sloughing of the epidermal layer; a feature common to all irradiance values within this grouping.

The appearance of sunburn cells varied from between 2-4 h following irradiation. For example at 72, 90 and  $108 \text{ mJ cm}^{-2}$  they first occurred at 4 hpi by comparison with 2 hpi at the higher dose levels of/

Figure 13

Fig. 13

The effect of UV-B given at a radiant intensity of  $30 \mu\text{W cm}^{-2}$  is shown for cumulative doses of 9, 18, 27, 36 and  $54 \text{ mJ cm}^{-2}$  respectively. Four tubes of 'white light' ( $640 \mu\text{W cm}^{-2}$ ) were incorporated in all five experiments



of 162 and 216  $\text{mJ cm}^{-2}$ . Although this might first appear to indicate a dose relationship it is necessary to note that sunburn cells also appeared at 2 hpi at the much lower dose levels of 18 and 27  $\text{mJ cm}^{-2}$ .

By 24 hpi a generalised necrosis extending throughout the entire epidermis was present in those fish receiving 72, 90 and 108  $\text{mJ cm}^{-2}$ . A typical response is shown in Fig.21. Prominent also at this point in the timescale, but limited to fish receiving 90  $\text{mJ cm}^{-2}$  (see Fig.21 also) was the appearance of isolated surface cells, rounding off prior to dehiscing. This observation contrasted sharply with the response at 32 hpi, subsequent to a dose of 108  $\text{mJ cm}^{-2}$  (Fig.22) where the outer layer was seen to be composed entirely of necrotic malpighian cells quite devoid of any mucous cell component. This process of sloughing from the underlying mid-zone of the epidermis was more representative of the response associated with stage 7 in the pathogenesis of the lesions.

At 162 and 216  $\text{mJ cm}^{-2}$  there was a general increase in the susceptibility of the skin to radiation over a shorter timescale. Sunburn cells were first seen at 2 hpi and by 12 hpi the epidermis swiftly assumed a necrotic appearance. By 24 hpi at a dose of 162  $\text{mJ cm}^{-2}$  the degree of epidermal damage was/

was markedly increased (Fig.23) when compared to that seen, for example, at 32 hpi following radiation of  $108 \text{ mJ cm}^{-2}$  (see Fig.22). Throughout the epidermis the nuclei of the malpighian cells appeared pyknotic and their associated cytoplasmic component most often adopted a spherical form, staining strongly eosinophilic. The presence of large vacuoles was noted at all levels and although those morphologically resembled mucous cells, selective staining for mucopolysaccharides failed to demonstrate the presence of a mucoid component. It was, therefore, considered that they represented a focal loss of attachment between the surrounding malpighian cells.

At  $216 \text{ mJ cm}^{-2}$  epidermal erosion was complete by 24 hpi and examination of later stages of the lesion, for example, 48 hpi (Fig.24) showed a massive dilatation of the underlying blood vessels with a concomitant breakdown of the collagenous matrix of the *stratum compactum*

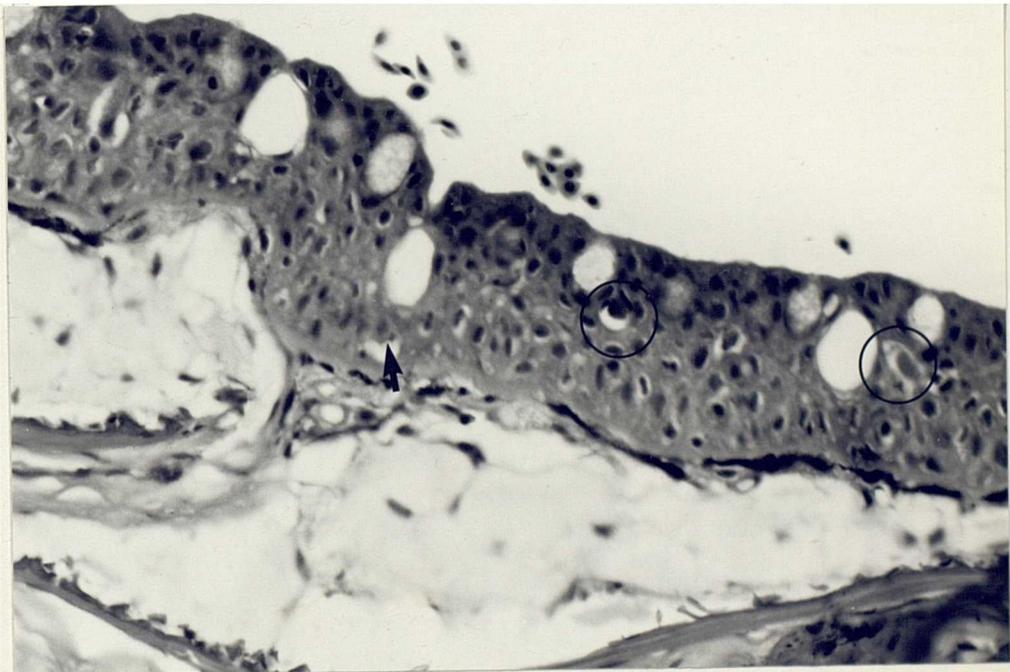
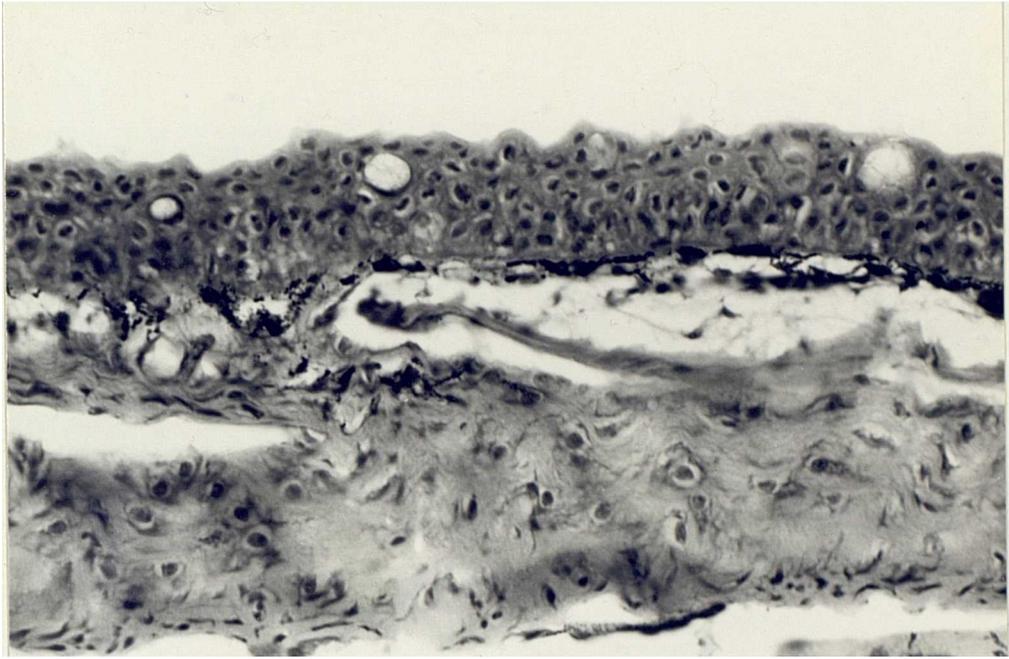
## 7.2 The effect upon plaice skin of doubling the radiant intensity of UV-B

In order to determine whether a significant difference in the skin response to radiation might be induced by doubling the output of UV-B from  $30 \text{ } \mu\text{W cm}^{-2}$  to  $60 \text{ } \mu\text{W cm}^{-2}$  a series of experiments was/

Figures 14 and 15

Fig.14 9 mJ cm<sup>-2</sup>, 56 hpi. The epidermis exhibits perinuclear haloes and slight surface irregularity with a concomitant hyperplasia of the epidermal layer.

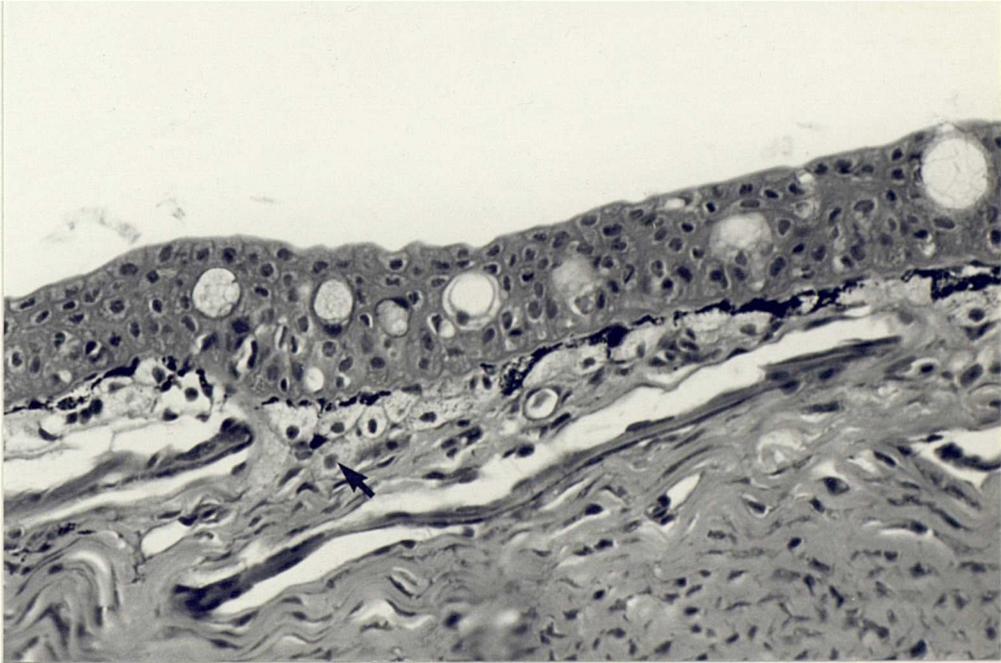
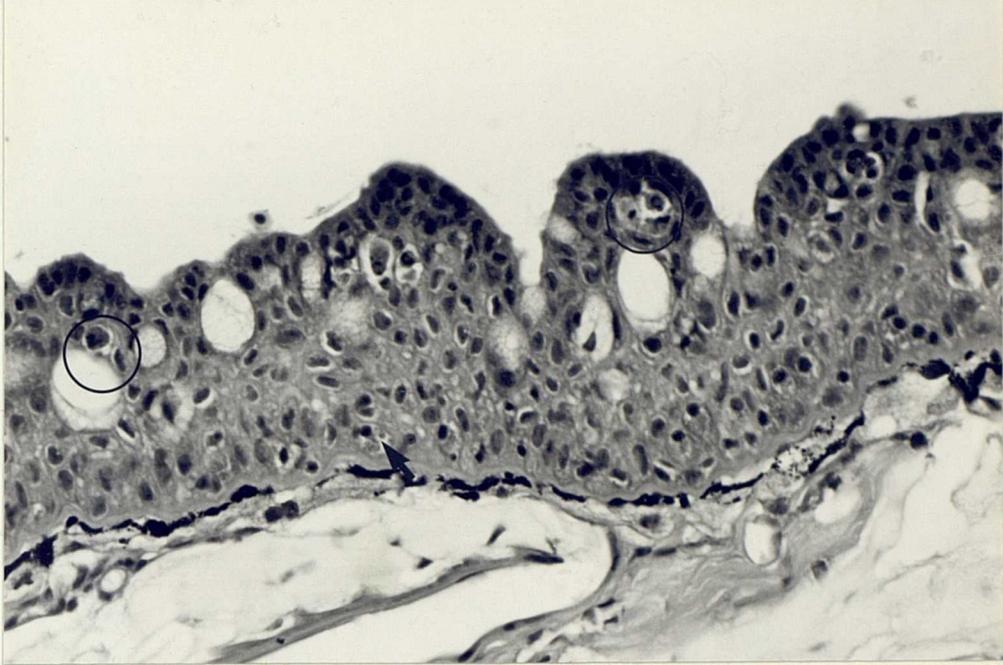
Fig.15 18 mJ cm<sup>-2</sup>, 2 hpi. Perinuclear haloes are more prominent and *Type A* sunburn cells (circled) are present within the mid layer. Note the vacuolated acidophilic granule cell within the basal zone (arrowed).



Figures 16 and 17

Fig.16 18 mJ cm<sup>-2</sup>, 4 hpi. The surface topography shows extreme convolution. *Types A and B* sunburn cells are present (circled) within the upper layer. Acidophilic granule cell activity (arrowed) is evident within the basal zone.

Fig.17 27 mJ cm<sup>-2</sup>, 4 hpi. Perinuclear haloes are prominent throughout the epidermis. Note the marked swelling of the melanocyte nuclei (arrowed) within the *stratum spongiosum*



Figures 18 and 19

Fig.18 54 mJ cm<sup>-2</sup>, 24 hpi. A generalised necrosis is evident throughout the epidermis, with a selective cleavage of the epidermis occurring within the supra-basal layer. Note also the dehiscing surface cells.

Fig.19 54 mJ cm<sup>-2</sup>, 72 hpi. Complete sloughing of the epidermis has occurred with the underlying dermis showing numerous necrotic fibroblasts (F) and densely aggregated melanocytes (arrowed) within the *stratum spongiosum*

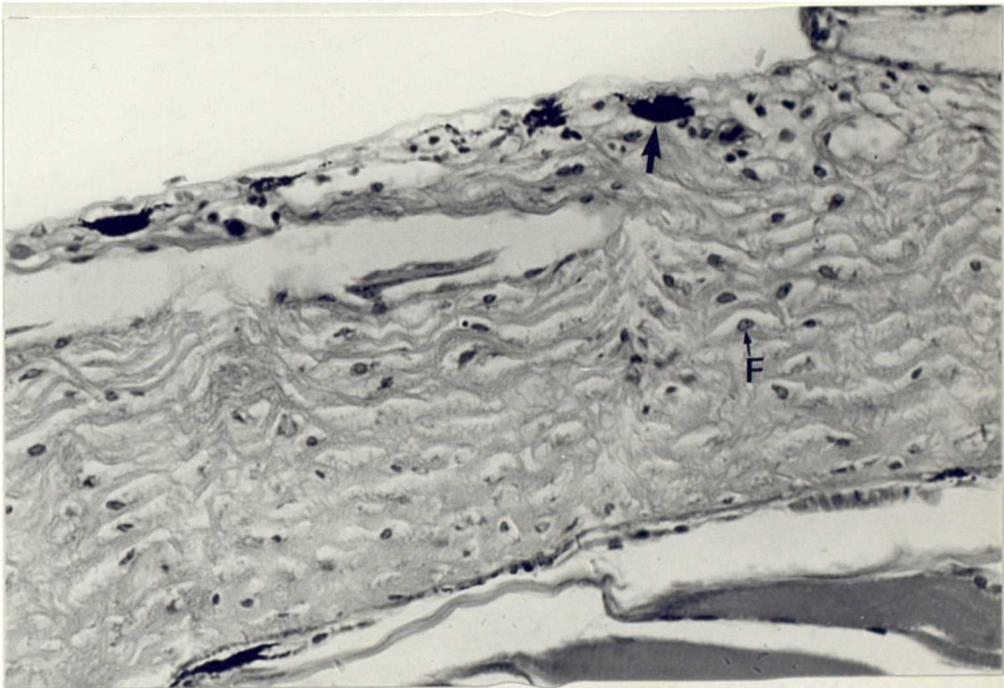
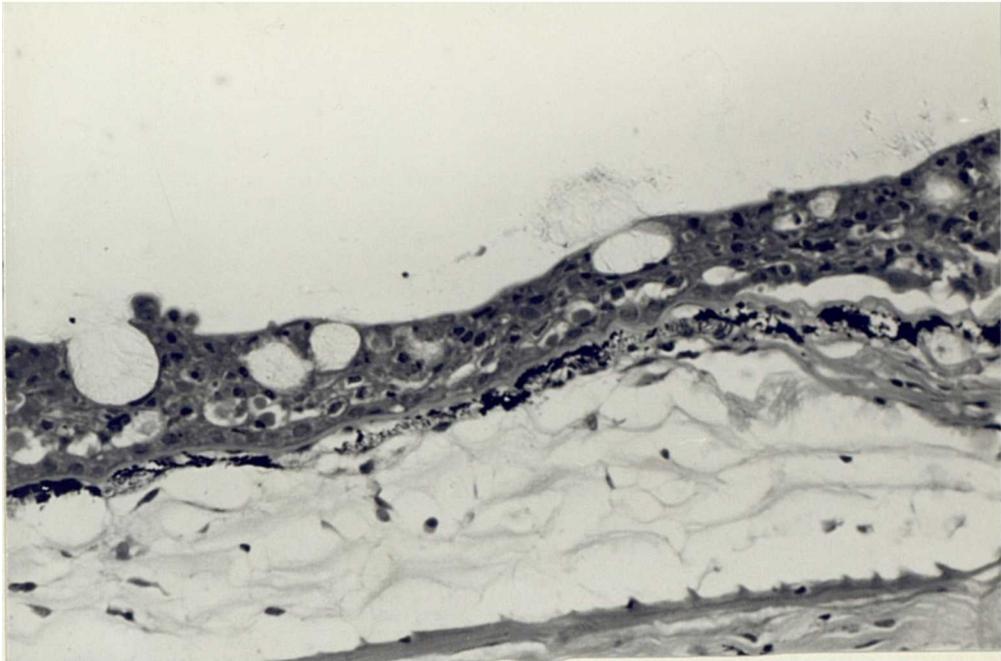
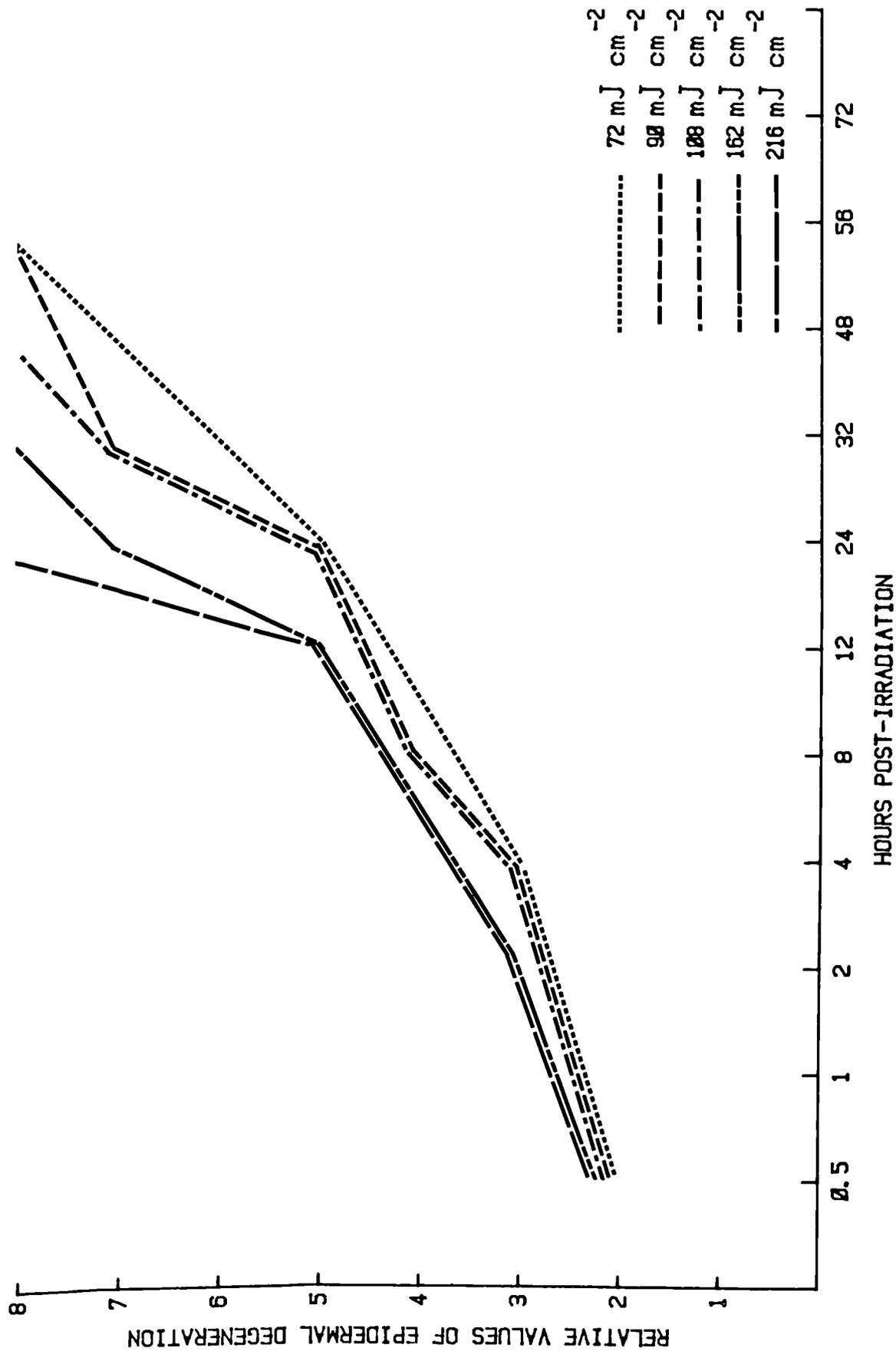


Figure 20

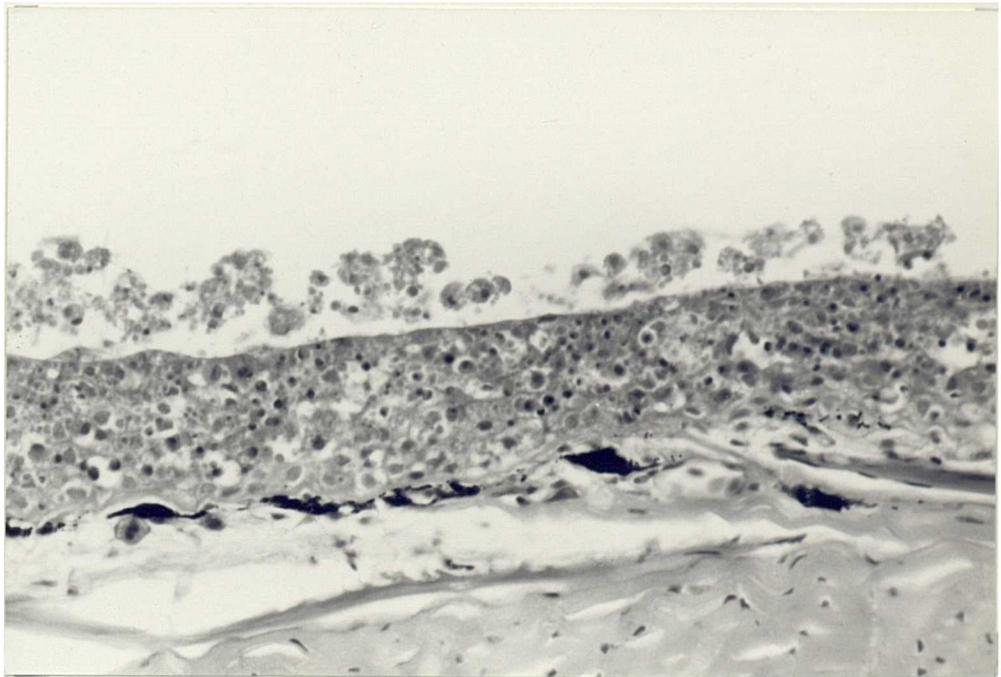
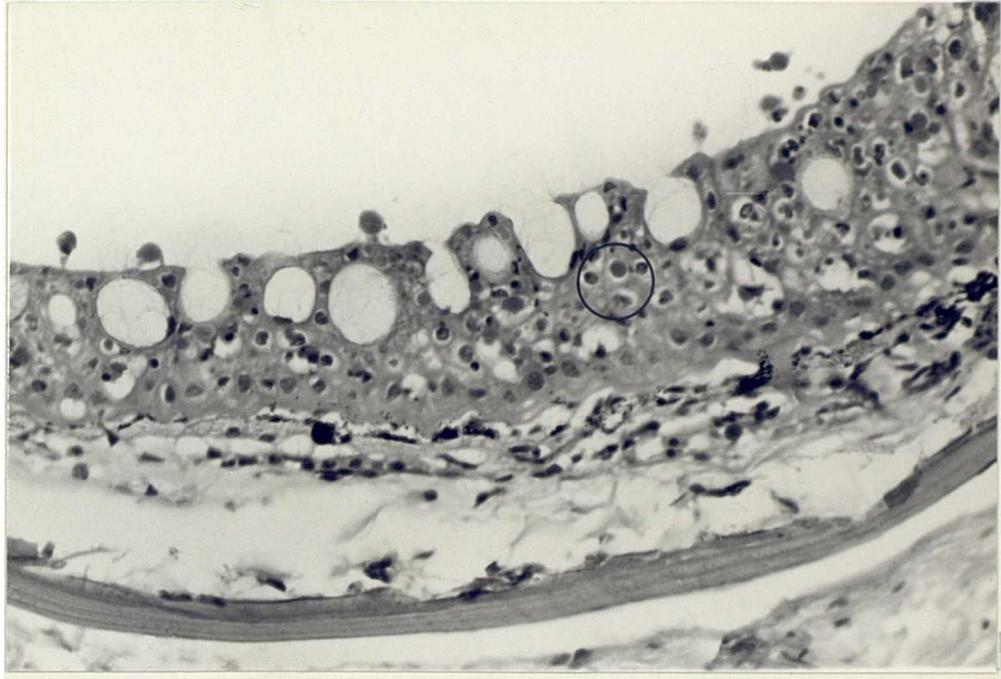
Fig.20 The effect of UV-B given at a radiant intensity of  $30 \mu\text{W cm}^{-2}$  is shown for cumulative doses of 72, 90, 108, 162 and  $216 \text{ mJ cm}^{-2}$  respectively. Four tubes of white light ( $640 \mu\text{W cm}^{-2}$ ) were incorporated in each experiment.



Figures 21 and 22

Fig.21 90 mJ cm<sup>-2</sup>, 24 hpi. Advanced necrosis of the epidermis exhibiting numerous sunburn cells, primarily *Type A* (circled). Loss of attachment of surface cells is also evident as is the copious mucus secretion over the surface layer.

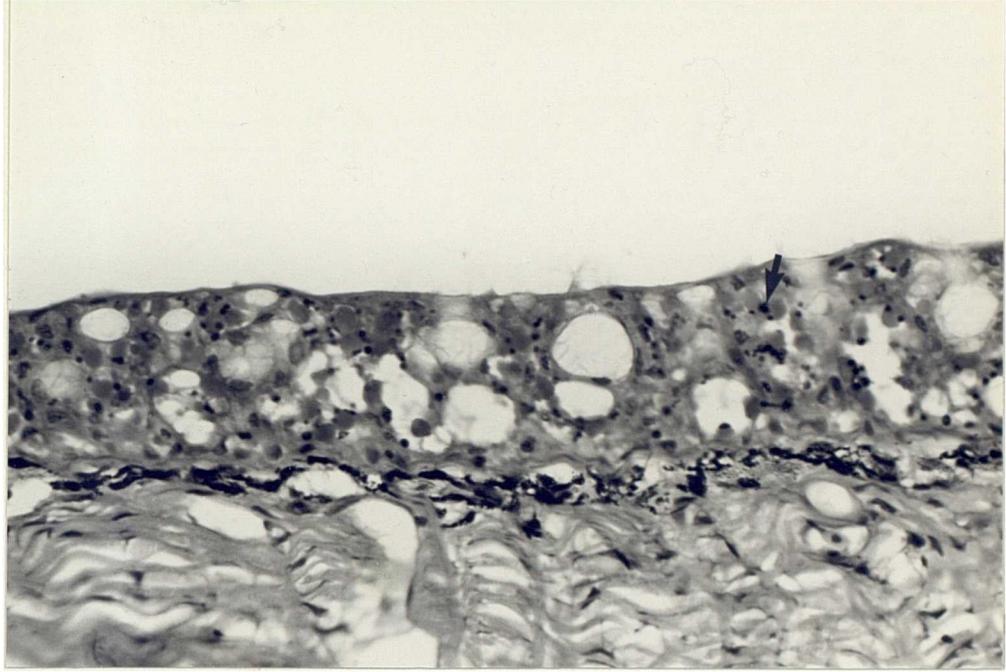
Fig.22 108 mJ cm<sup>-2</sup>, 32 hpi. A characteristic UV induced response. The upper epidermal layer has sloughed and beneath lies a necrotic zone of malpighian cells.



Figures 23 and 24

Fig.23 162 mJ cm<sup>-2</sup>, 24 hpi. The epidermis exhibits numerous vacuoles throughout. These represent the focal loss of attachment between surrounding malpighian cells. The nuclei stain densely and are pyknotic (arrowed).

Fig.24 216 mJ cm<sup>-2</sup>, 48 hpi. Epidermal sloughing is almost complete, leaving only a few isolated cells abutting the basement membrane (arrowed). Note the massive vasodilatation within the *stratum spongiosum* and the dense aggregation of dermal melanocytes suggesting a loss of function.



was performed at an intensity of  $60 \mu\text{W cm}^{-2}$  over the same timescale and using identical exposure times to those used in the lower irradiance studies. The results are summarised in Fig.25

The sequential response to  $18 \text{ mJ cm}^{-2}$  delivered at  $60 \mu\text{W cm}^{-2}$  closely followed that seen at  $30 \mu\text{W cm}^{-2}$ . However, hyperplasia was first observed at 12 hpi as opposed to 24 hpi at the lower intensity. By contrast the endpoint of the response at  $60 \mu\text{W cm}^{-2}$  did not progress beyond epidermal cleavage whereas at  $30 \mu\text{W cm}^{-2}$  partial sloughing of the outer layer was observed at 56 hpi.

At a cellular level the dissimilarity between the two irradiance values became more noticeable.  $18 \text{ mJ cm}^{-2}$  delivered at  $60 \mu\text{W cm}^{-2}$  produced by 2 hpi prominent perinuclear haloes surrounding the epidermal malpighian cells (Fig.26) the nuclei often adopting bizarre forms becoming either elongated or horseshoe shaped. The prominence of the perinuclear halo effect increased by 4 hpi with the nuclei appearing more spherical and densely staining (Fig.27). Surface irregularity was minimal in the early stages when compared to that seen at the lower irradiance level.

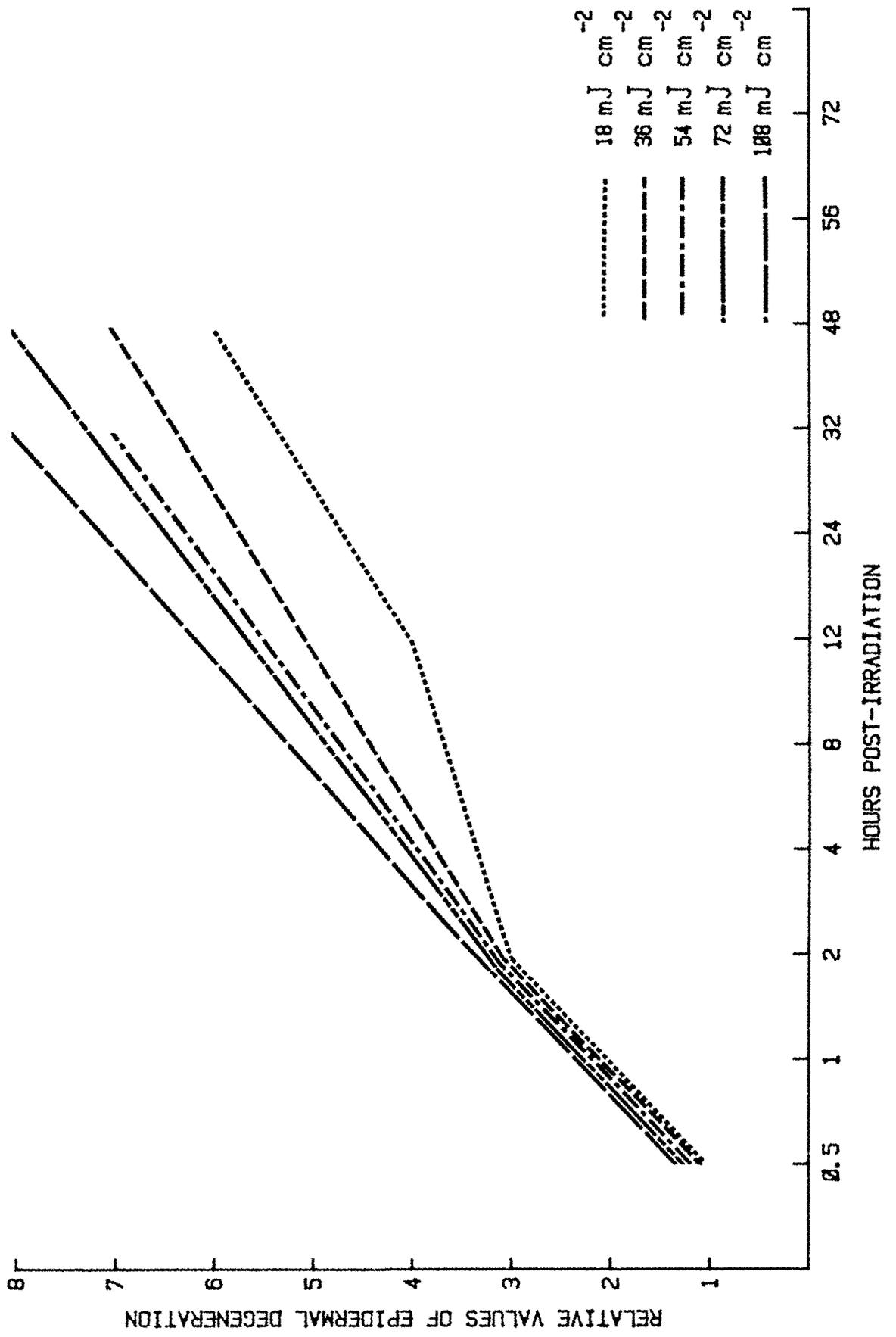
By 12 hpi the general appearance of the epidermis had altered considerably. The malpighian cells no longer/

longer exhibited darkly staining nuclei nor perinuclear haloes but appeared ill-defined and pale staining giving an overall foamy appearance to the epidermis, usually accompanied by slight oedema (Fig.28). At this point the underlying musculature also exhibited a similar foamy appearance (Fig.29). By 32 hpi the epidermis showed little cellular organisation with numerous vacuoles appearing within the supra-basal zone. By 48 hpi this had progressed to a point where a major separation of the epidermis immediately above the basal cell layer occurred (Fig.30) many of the cells within the upper zone appearing necrotic.

Cumulative doses of 36, 54, 72 and 108 mJ cm<sup>-2</sup> showed little variation in the early stages of the response with sunburn cells being noted at 2 hpi (Fig.31). Beyond this point, however, there was an increasing tendency for the higher doses to induce radiation damage at earlier points on the timescale. At 36 mJ cm<sup>-2</sup> for example, the endpoint of the response did not exceed sloughing of the outer layer, the underlying juvenile mucous cells being stimulated into voiding mucus over the remaining cells within the basal zone (Fig.32). At 56 hpi a dose of 72 mJ cm<sup>-2</sup> produced clear evidence of cleavage of the lower epidermal zone (Fig.33) prior to complete sloughing by 72 hpi. A higher dose of 108 mJ cm<sup>-2</sup> produced initial/

Figure 25

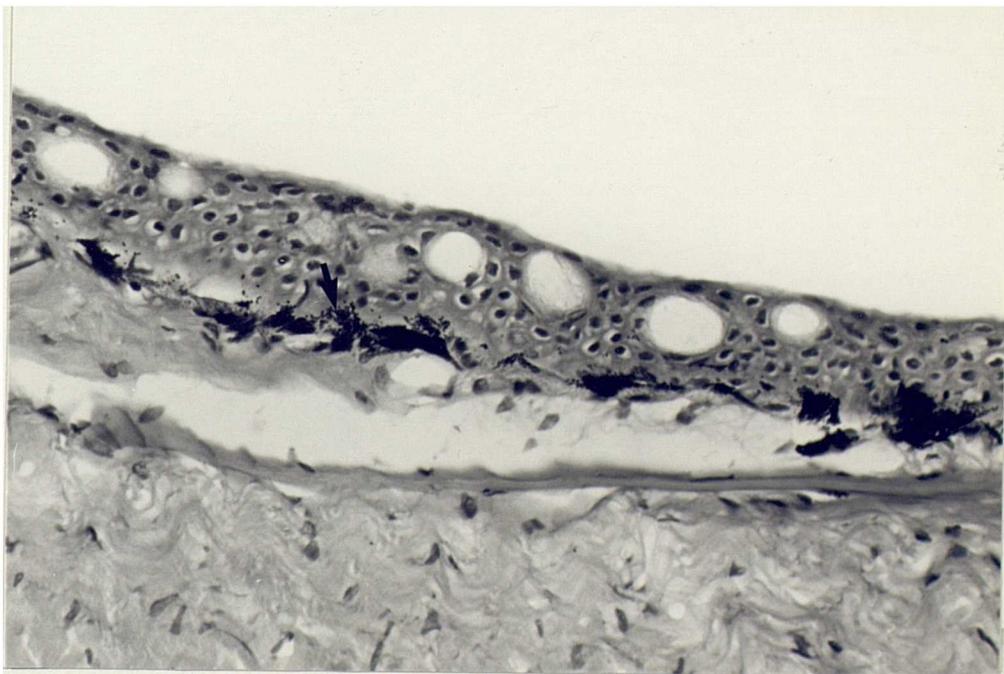
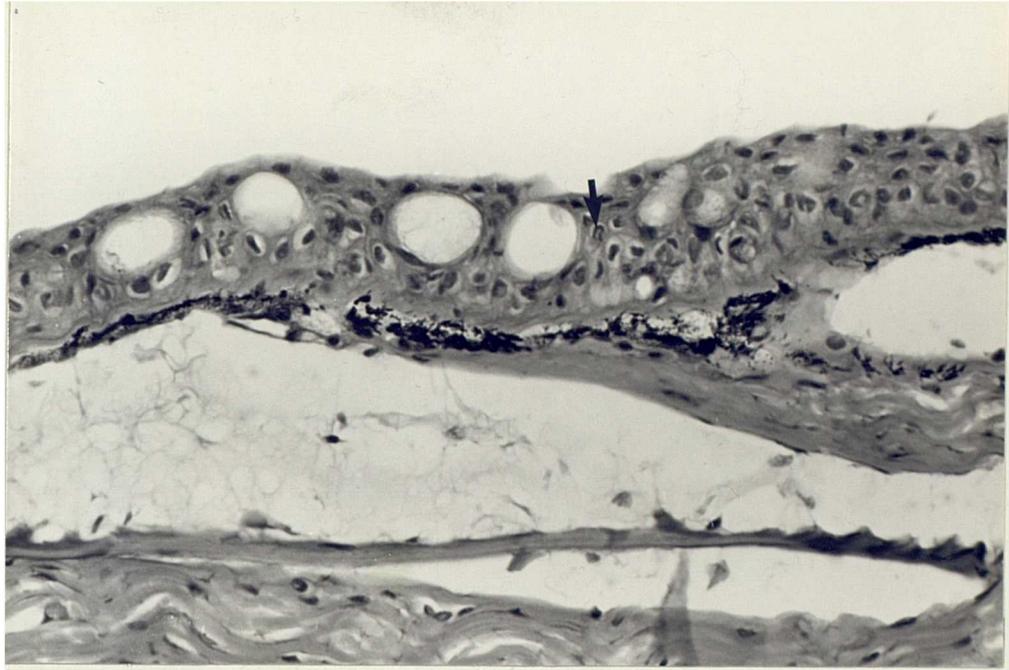
Fig.25 The effect of UV-B at an increased radiant intensity of  $60 \mu\text{W cm}^{-2}$  resulting in cumulative doses of 18, 36, 54, 72 and 108  $\text{mJ cm}^{-2}$ .



Figures 26 and 27

Fig.26 18 mJ cm<sup>-2</sup>, 2 hpi. Perinuclear haloes are very prominent and the pale staining nuclei often adopt bizarre forms (arrowed).

Fig 27 18 mJ cm<sup>-2</sup>, 4 hpi. The nuclei stain densely when compared to 2 hpi at the same dose level. Also the perinuclear haloes are markedly more prominent. The dermal melanocytes show a significant response. Individual melanosome granules are easily discerned (arrowed).



initial sloughing by 24 hpi and complete erosion by 48 hpi

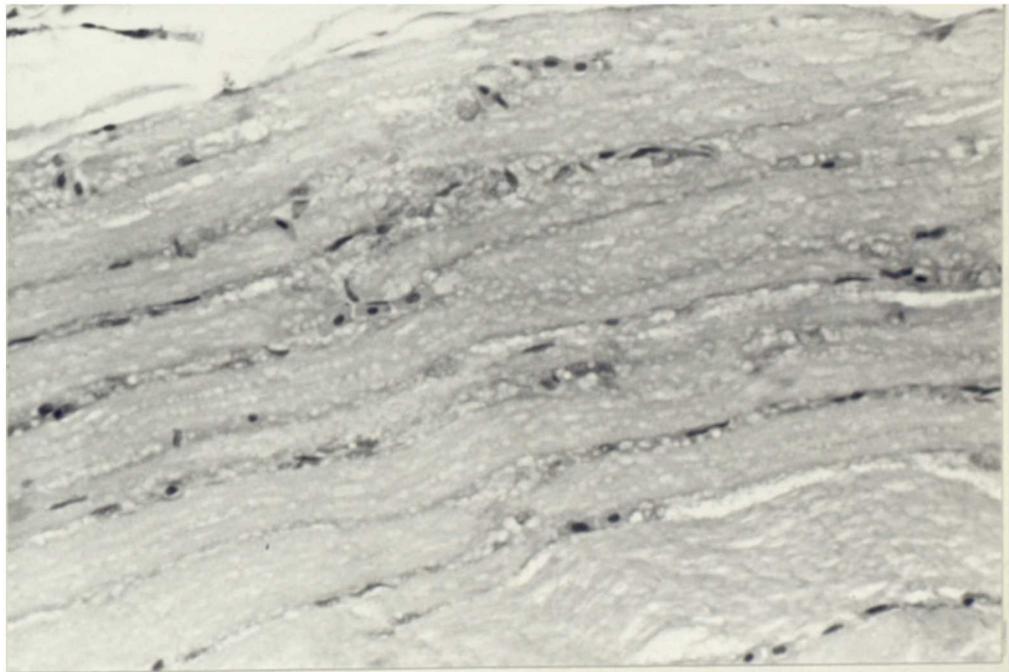
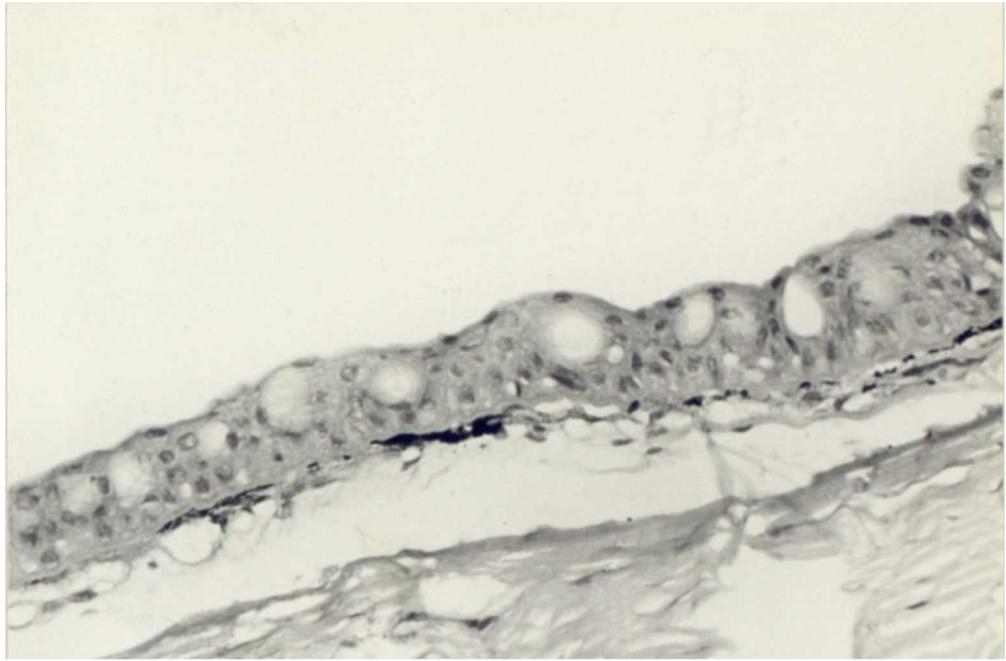
The effect of cumulative doses of 144, 180, 216, 324 and 432  $\text{mJ cm}^{-2}$  delivered at a radiant intensity of 60  $\mu\text{W}$  is shown in Fig.34. At these higher values the overall response tended to be more uniform in pattern resulting in complete destruction of the epidermal layer by 32 hpi at 144 and 180  $\text{mJ cm}^{-2}$  and by 24 hpi at 216, 324 and 432  $\text{mJ cm}^{-2}$ .

Typical examples of the damage inflicted at these increased doses are shown in Figs.35 and 36. By 1 hpi, for instance, at a cumulative dose of 180  $\text{mJ cm}^{-2}$  sunburn cells were a common feature throughout the epidermis. Over a much shorter timescale a higher dose of 216  $\text{mJ cm}^{-2}$  showed that epidermal necrosis was complete by 24 hpi leaving only discrete groups of dead malpighian cells dehiscing from the basement membrane.

Figures 28 and 29

Fig.28 18 mJ cm<sup>-2</sup>, 12 hpi. At this stage the staining propensity of the epidermis alters considerably and its general morphology appears ill-defined and oedematous.

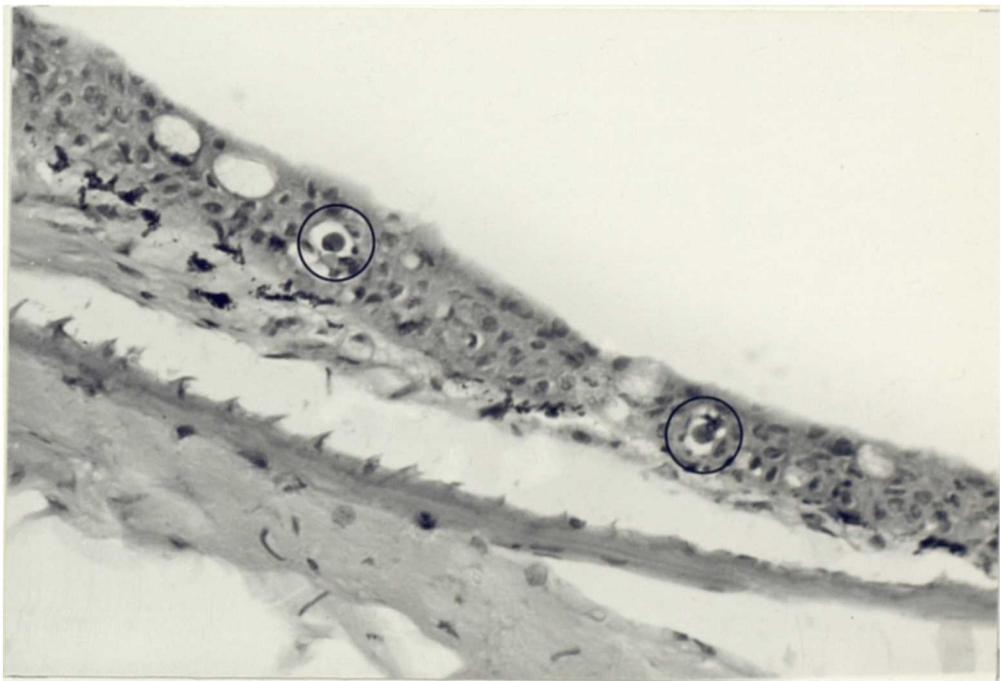
Fig.29 18 mJ cm<sup>-2</sup>, 12 hpi. The underlying musculature also exhibits a foamy pale staining appearance.



Figures 30 and 31

Fig.30 18 mJ cm<sup>-2</sup>, 48 hpi. A focal separation of malpighian cells (viz epidermal cleavage) can be seen with the supra-basal zone. Surrounding malpighian cells exhibit large pale staining nuclei whilst smaller numbers within the uppermost layer are pyknotic (arrowed).

Fig.31 54 mJ cm<sup>-2</sup>, 2 hpi. Characteristic *Type A* sunburn cells (circled) are present within the epidermis.



Figures 32 and 33

Fig.32 36 mJ cm<sup>-2</sup>, 48 hpi. Following an initial sloughing of the outer layer the juvenile mucous cells (arrowed) within the supra-basal zone increase considerably in size and void their mucus over the remaining epidermis. At this exposure sloughing did not proceed beyond this point.

Fig 33 72 mJ cm<sup>-2</sup>, 56 hpi. Typical UV induced separation within the supra-basal zone. The appearance of the epidermis immediately prior to separation can be seen on either side of the lesion (arrowed) where it exhibits a pronounced intercellular oedema.

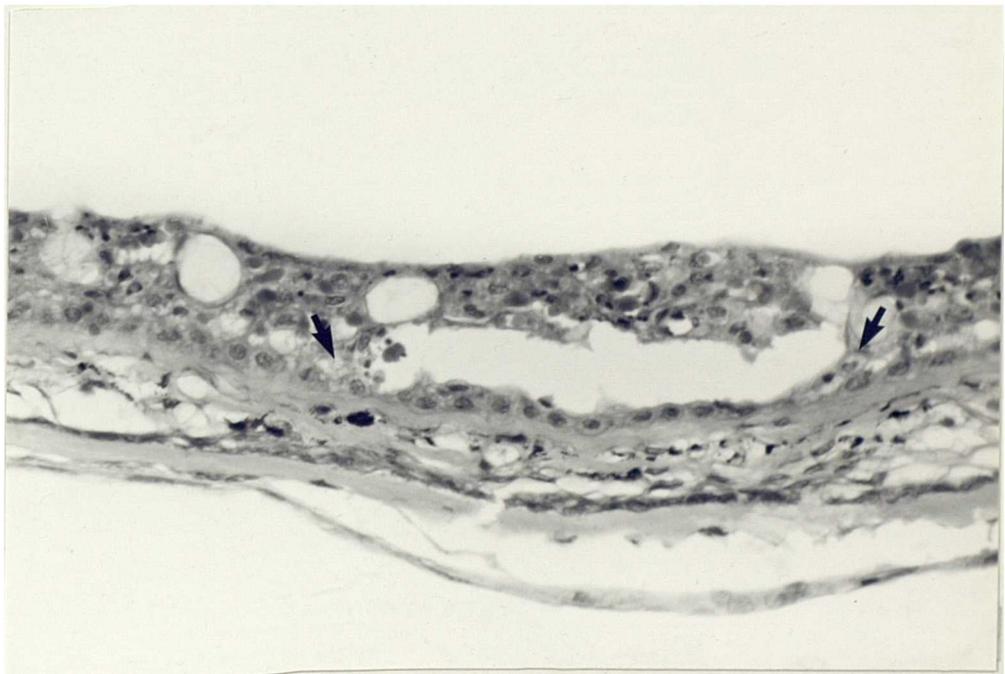
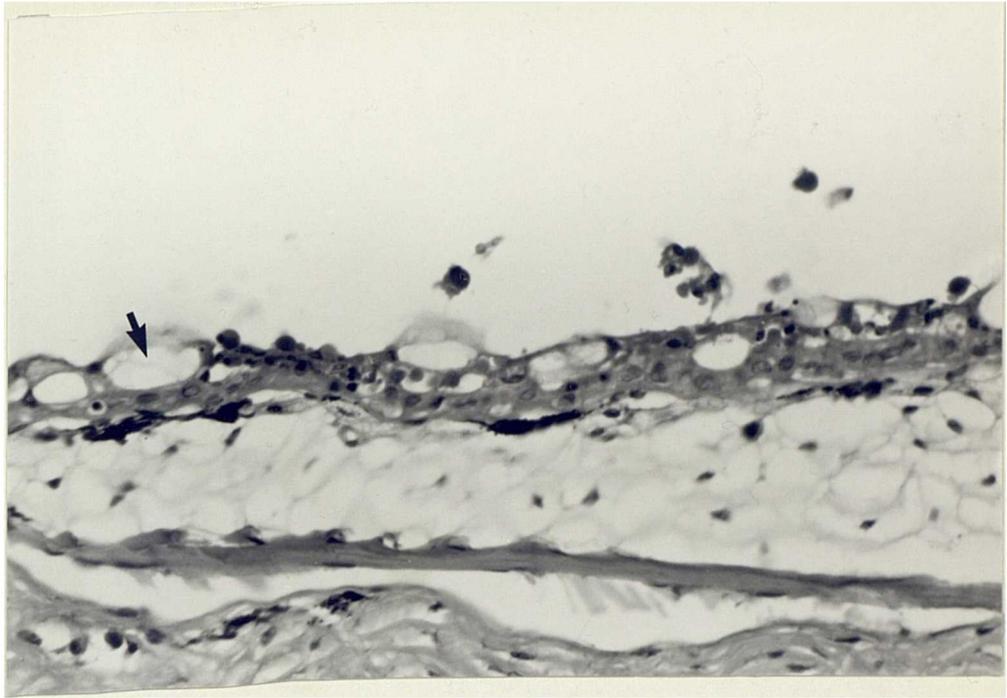
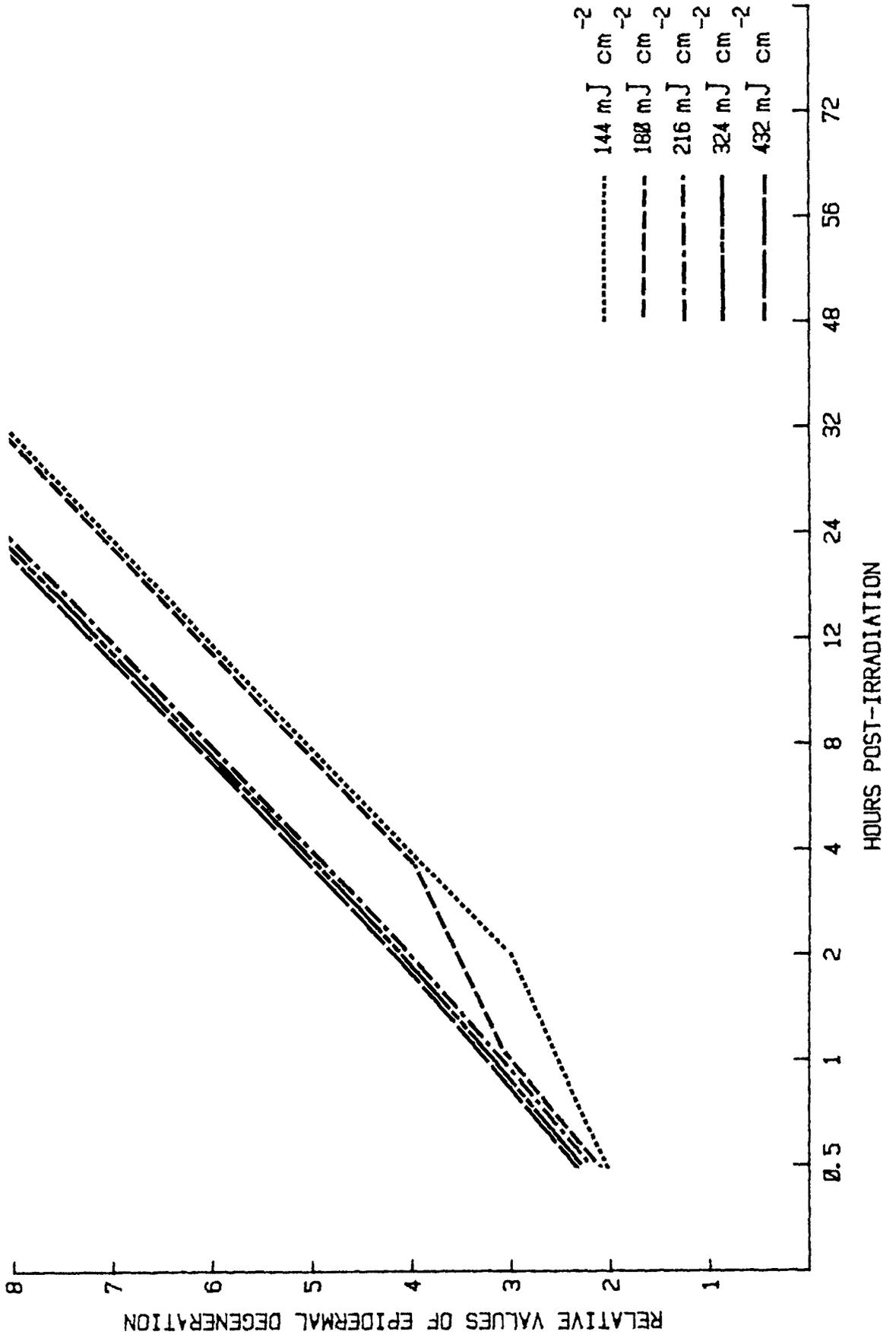


Figure 34

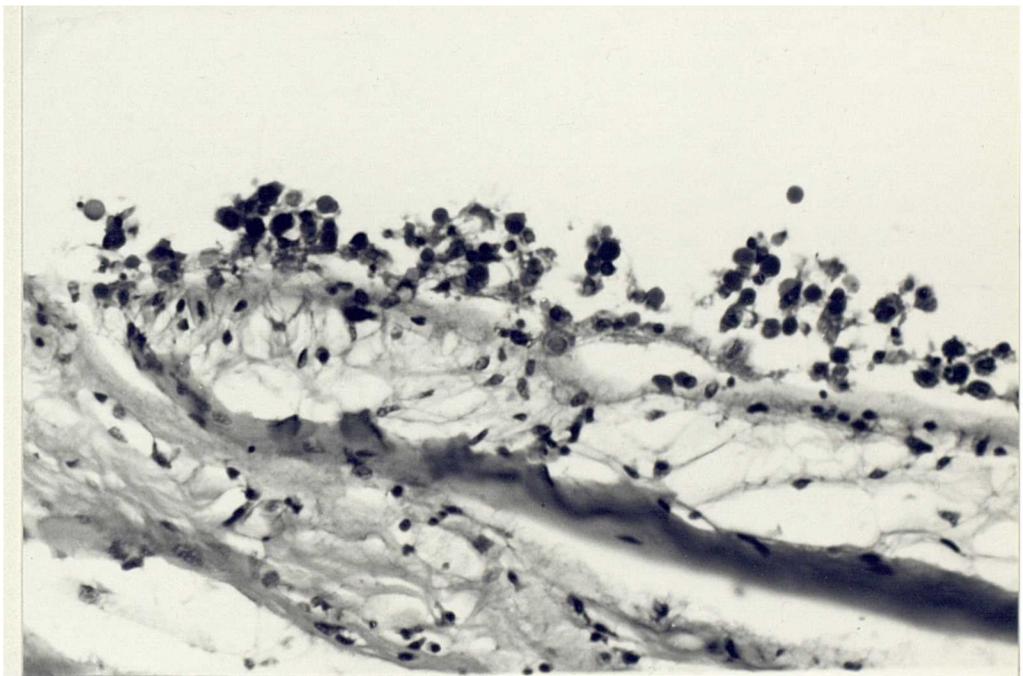
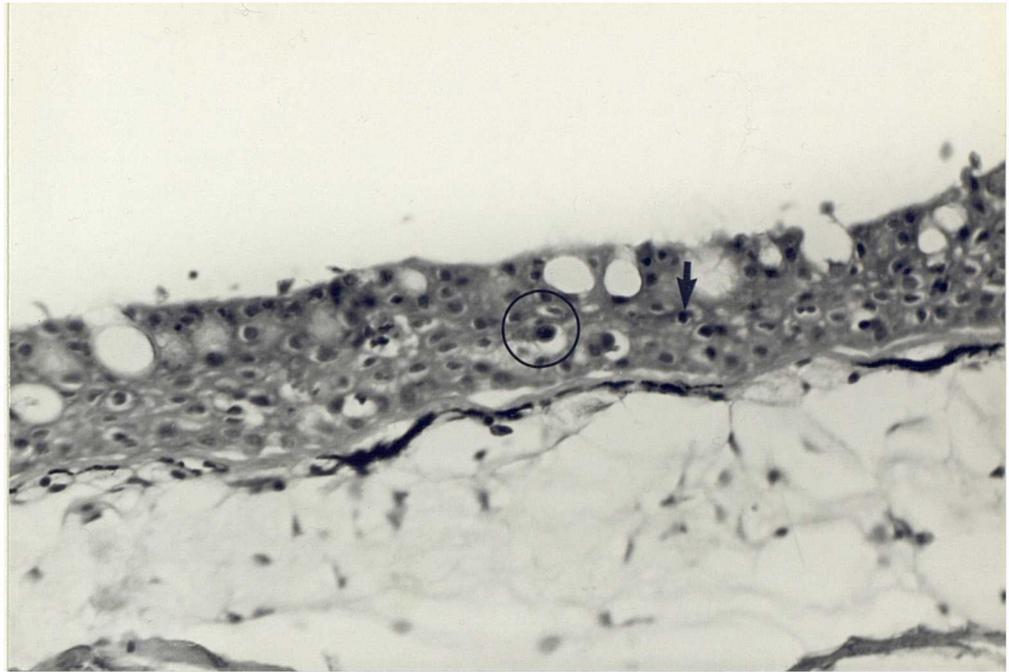
Fig.34 The effect of UV-B at a radiant intensity of  $60 \mu\text{W cm}^{-2}$  is shown for cumulative doses of 144, 180, 216, 324 and 432  $\text{mJ cm}^{-2}$  repectively. The visible light input at all exposures consisted of  $640 \mu\text{W cm}^{-2}$  'white light'.



Figures 35 and 36

Fig.35 180 mJ cm<sup>-2</sup>, 1 hpi. The epidermis contains moderate numbers of *Type A* sunburn cells (circled). In addition numerous pyknotic nuclei can be seen (arrowed) and also prominent perinuclear haloes.

Fig.36 216 mJ cm<sup>-2</sup>, 24 hpi. Epidermal sloughing is almost complete only isolated groups of necrotic malpighian cells upon the basement membrane.



7.3 The skin response of plaice to UV-B following the incorporation of a cellulose triacetate (CTA) 'cut-off' filter during irradiation

The spectral power distribution curve from a typical UV-B source used in photobiological studies covers a portion of the ultra-violet spectrum ranging from 265-320 nm (see Fig.7). It is generally acknowledged, however, that from natural sunlight only a minute proportion of the wavelengths below 295 nm reach the earth's surface due to their attenuation by the ozone layer. Studies on human skin have shown that the relative importance of these shorter wavelengths in the erythema response (viz. that amount of UV-B required to induce a reddening of the skin) to be the subject of some controversy.

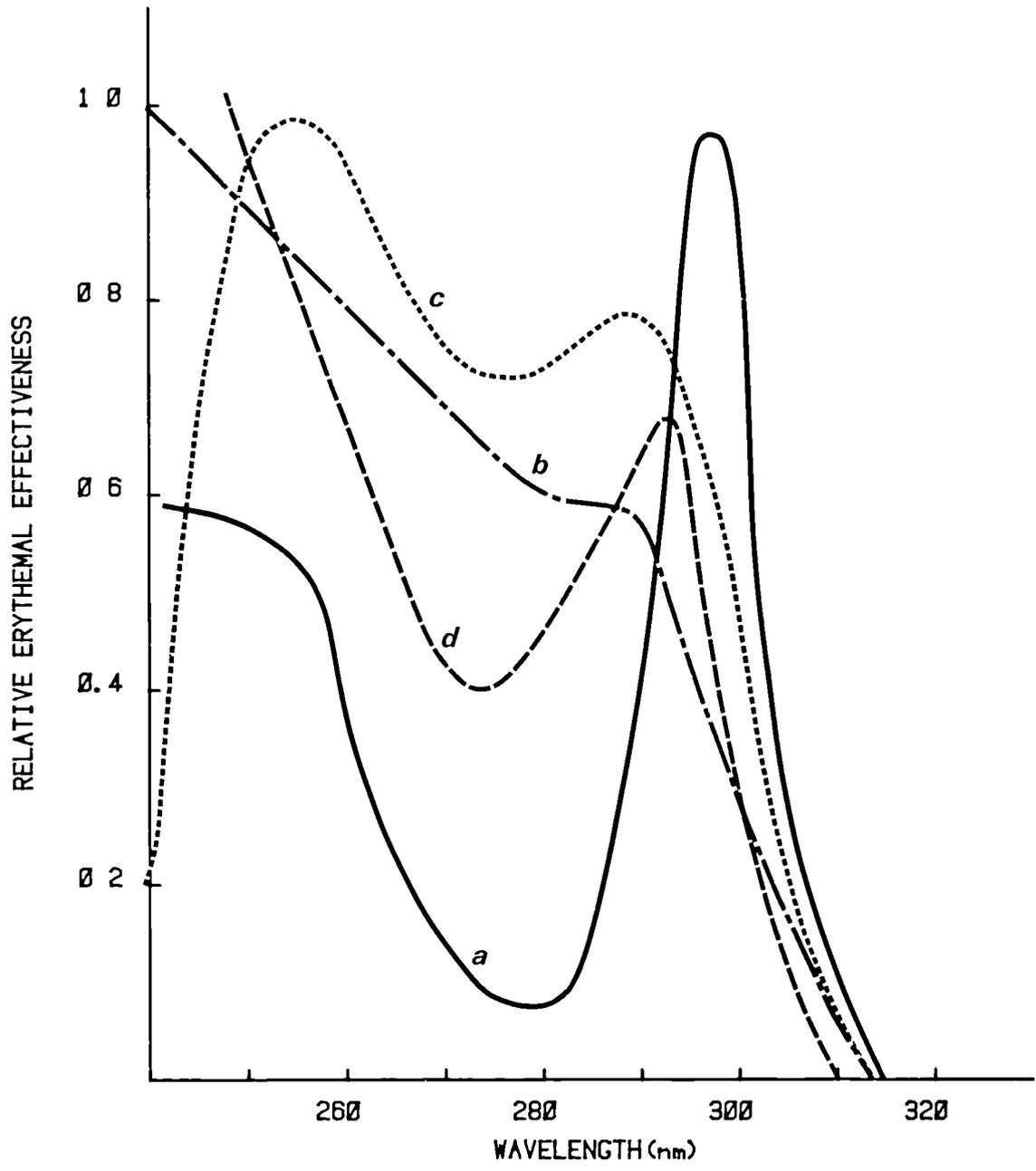
The erythema response in human skin is highly dependent upon the wavelength of the radiation and is expressed by the action spectrum, this being a plot of the reciprocal of the dose required for a given effect against wavelength. The first precise determination of the action spectrum was determined in 1922 and exhibited a major peak of activity at 297 nm, a minimum at 280 nm. Later studies showed close agreement with these figures and a standard erythemal dose for human skin, based upon these figures, was/

was adopted by the International Commission on Illumination (ICI) in 1935. More recent studies, however, show curves with increasing amplitude, relative to shorter wavelengths with a 'shoulder' at around 300-280 nm followed by a continuation of the amplitude relative to the erythema effectiveness down to at least 260 nm. A comparison of the standard erythema curve with the erythema action spectra determined by various workers is shown in Fig.37.

From the more recent data presented it is clear that the shorter wavelengths emitted by an artificial source must have a considerable bearing on the overall erythema action spectrum in human skin. The results of the baseline study upon the response by plaice skin to UV-B show a high susceptibility to radiation even at the lower doses of  $9-54 \text{ mJ cm}^{-2}$  ( $2.3-18 \text{ mJ cm}^{-2}$  bio eff) when compared with that amount required to induce a minimal erythema in human skin ( $72-144 \text{ mJ cm}^{-2}$ ). It was, therefore, considered desirable to investigate the effect of wavelengths shorter than 285 nm on plaice skin. This was done by repeating the high dose experiments of 144, 180, 216, 324 and  $432 \text{ mJ cm}^{-2}$  at a radiant intensity of  $60 \text{ } \mu\text{W cm}^{-2}$  used in the baseline study but with the addition of a CTA filter (Bexfilm T).  
By/

Figure 37

Fig.37 A comparison of the standard erythemal curve  
(ICI 1935) with the erythema action spectra  
shown by  
ICI (1935) *a*  
Everett et al (1965) *b*  
Freeman et al (1966) *c*  
Cripps & Ramsay (1970) *d*



By placing this filter over the tanks at a point midway between the radiation source and the fish all wavelengths below 285 nm were removed. The spectral response curve for CTA is shown in Fig.9.

The results of these experiments are summarised in Fig.38. Irrespective of cumulative dose it can be seen that there was an overall diminution of radiation damage when plotted against the timescale in all groups receiving filtered UV-B. For example, at a dose of  $144 \text{ mJ cm}^{-2}$  unfiltered UV-B produced complete destruction of the epidermis by 32 hpi; by contrast filtered UV-B at the same cumulative dose induced a response that did not progress beyond an initial sloughing of the outer zone.

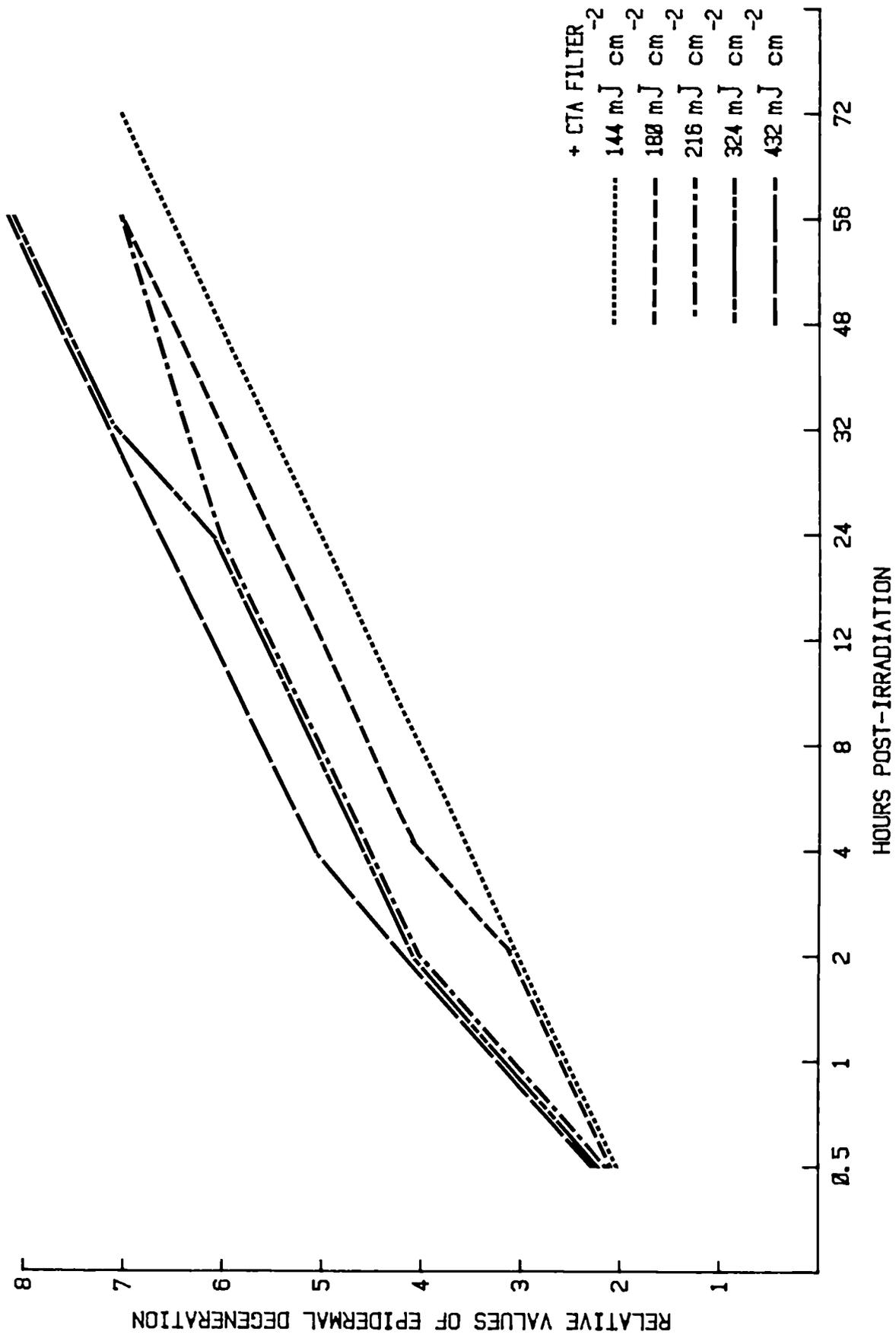
Amongst the more prominent features of the filtered UV-B reaction was a delay in the hyperplastic response by around 6 h at a dose of  $144 \text{ mJ cm}^{-2}$ . Whilst this stage was apparent by 2 hpi with unfiltered UV-B a comparable response was not observed until 8 hpi within the filtered group (Fig.39). Furthermore, at  $144 \text{ mJ cm}^{-2}$  (unfiltered) epidermal destruction was complete by 32 hpi whereas sections examined from the filtered group at the same dose indicated that the epidermis remained intact throughout the timescale of the experiment.

By/

Figure 38

.

Fig.38 The effect of UV-B filtered through cellulose triacetate (CTA) upon the skin of plaice at cumulative doses of 144, 180, 216, 324 and 432  $\text{mJ cm}^{-2}$  respectively. 'White light' input consisted of 4 x 40 watt fluorescent tubes.



By 72 hpi sunburn cells were numerous and the remaining malpighian cells exhibited large pale staining nuclei often with prominent nucleoli. In addition the morphological appearance of the dermal melanocytes differed considerably from those usually seen in skin during the final stages of irradiation damage. They exhibited a conspicuous dispersal of their melanosomes within the *stratum spongiosum*, the centrally placed nuclei in many instances having been destroyed and appearing in sections as large vacuoles devoid of any nuclear debris (Fig.40).

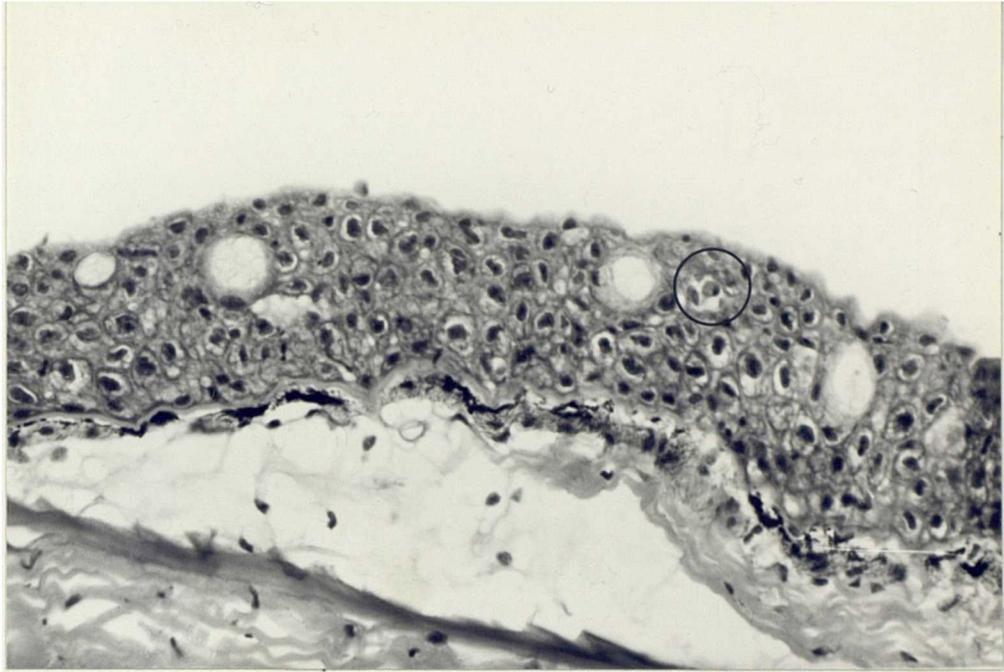
Filtered doses of 180 and 216  $\text{mJ cm}^{-2}$  showed a similar picture whereby the lesion did not proceed to complete necrosis within the timescale although sunburn cells were observed at earlier stages relative to increased dose.

By 24 hpi unfiltered UV-B at doses of 216, 324 and 432  $\text{mJ cm}^{-2}$  had produced a complete sloughing of the epidermis whilst those fish protected by the CTA filter at these high irradiance levels had a significantly reduced response over the timescale. In the earlier stages of the lesion a quite distinctive feature of the response at the highest cumulative dose of 432  $\text{mJ cm}^{-2}$  was the extreme convolution observed at the skin surface by around 1 hpi (Fig.41). Although exhibited to a lesser degree in the lower doses/

Figures 39 and 40

Fig.39 144 mJ cm<sup>-2</sup> + CTA filter 8 hpi. The epidermis exhibits prominent perinuclear haloes in association with moderate oedema. One *Type A* sunburn cell is present within the upper layer (circled).

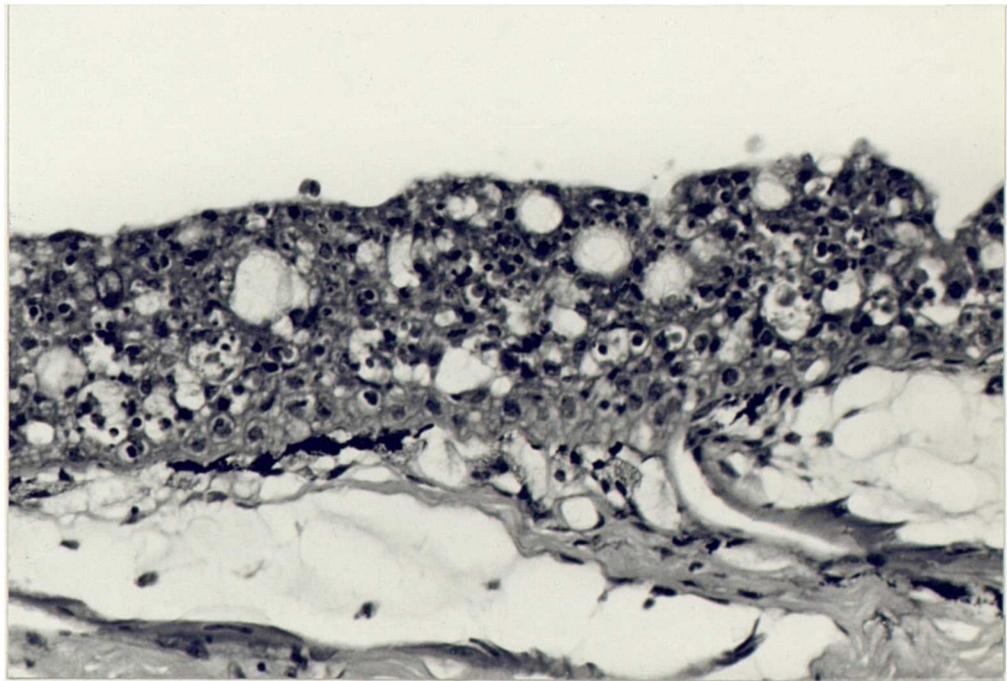
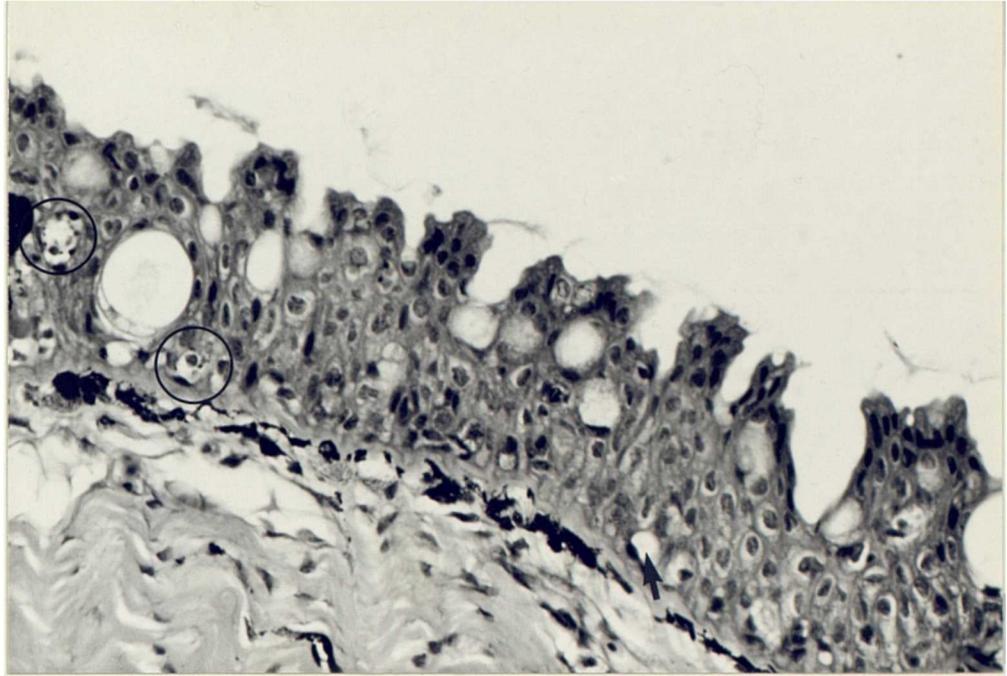
Fig.40 144 mJ cm<sup>-2</sup> + CTA filter 72 hpi. Several sunburn cells are present throughout the epidermis (circled). In addition the nuclei of the remaining malpighian cells are generally large and pale staining. Note the vacuolated central core of each melanocyte (arrowed) within the *stratum spongiosum*.



Figures 41 and 42

Fig.41 432 mJ cm<sup>-2</sup> + CTA filter 1 hpi. The skin surface appears extremely convoluted and *Type B* sunburn cells (circled) are present within the mid and basal layers. Acidophilic granule cell vacuolation is also evident (arrowed).

Fig.42 432 mJ cm<sup>-2</sup> + CTA filter 4 hpi. The epidermis appears generally necrotic throughout, the majority of malpighian cells exhibiting densely staining, spherical, pyknotic nuclei.



doses it appeared most prominent with the highest dose. By 4 hpi, however (Fig 42), the surface irregularity had subsided to a minimal response with the epidermis exhibiting an overall necrotic appearance

7.4 The influence of the visible spectrum and its intensity upon the skin of plaice when irradiated with UV-B through a cellulose triacetate (CTA) filter

The importance of the visible (white light) spectrum in the photorepair of mammalian skin has been described in Section I. In order to test the validity of such a response in relation to fish skin a group of experiments was carried out on plaice at a radiant intensity of  $30 \mu\text{W cm}^{-2}$  UV-B with exposure times of 10 and 30 minutes resulting in cumulative doses of 18 and  $54 \text{ mJ cm}^{-2}$  respectively. A CTA filter was employed throughout all irradiations, filtering both the UV-B and visible wavelengths.

The purpose of the  $18 \text{ mJ cm}^{-2}$  dose was to provide a baseline control at a radiant exposure known to induce a response within the skin but not sufficient to destroy completely the epidermis. In addition, four separate experiments, each at a cumulative dose of  $54 \text{ mJ cm}^{-2}$  were conducted with a/

a variable light input ranging from complete darkness up to 8 x 40 watt fluorescent tubes (Thorn-Daylight (Coolwhite)). The results were plotted using the same scoring values as in previous experiments and are presented in Fig.43.

At  $18 \text{ mJ cm}^{-2}$ , filtered UV-B with the addition of two tubes of white light (approximately  $320 \text{ } \mu\text{W cm}^{-2}$  at the water surface) produced an initial response in the epidermis at 0.5 hpi identical to that seen in the unfiltered experiment incorporating an identical white light value. Sunburn cells were not observed, however, until 4 hpi in the filtered group as opposed to 2 hpi in the unfiltered group. Thereafter the response was diminished in the filtered group so that by 72 hpi the epidermis showed only slight evidence of epidermal necrosis. In contrast the unfiltered  $18 \text{ mJ}$  dose showed the same response as early as 32 hpi followed by an initial sloughing at 56 hpi.

The comparison of a cumulative dose of  $54 \text{ mJ cm}^{-2}$  of filtered UV-B at variable light intensities demonstrated the importance of the visible spectrum in the photorepair of plaice skin.

If irradiation was carried out in complete darkness the appearance of sunburn cells occurred by 1 hpi followed by a rapid deterioration in epidermal/

Figure 43

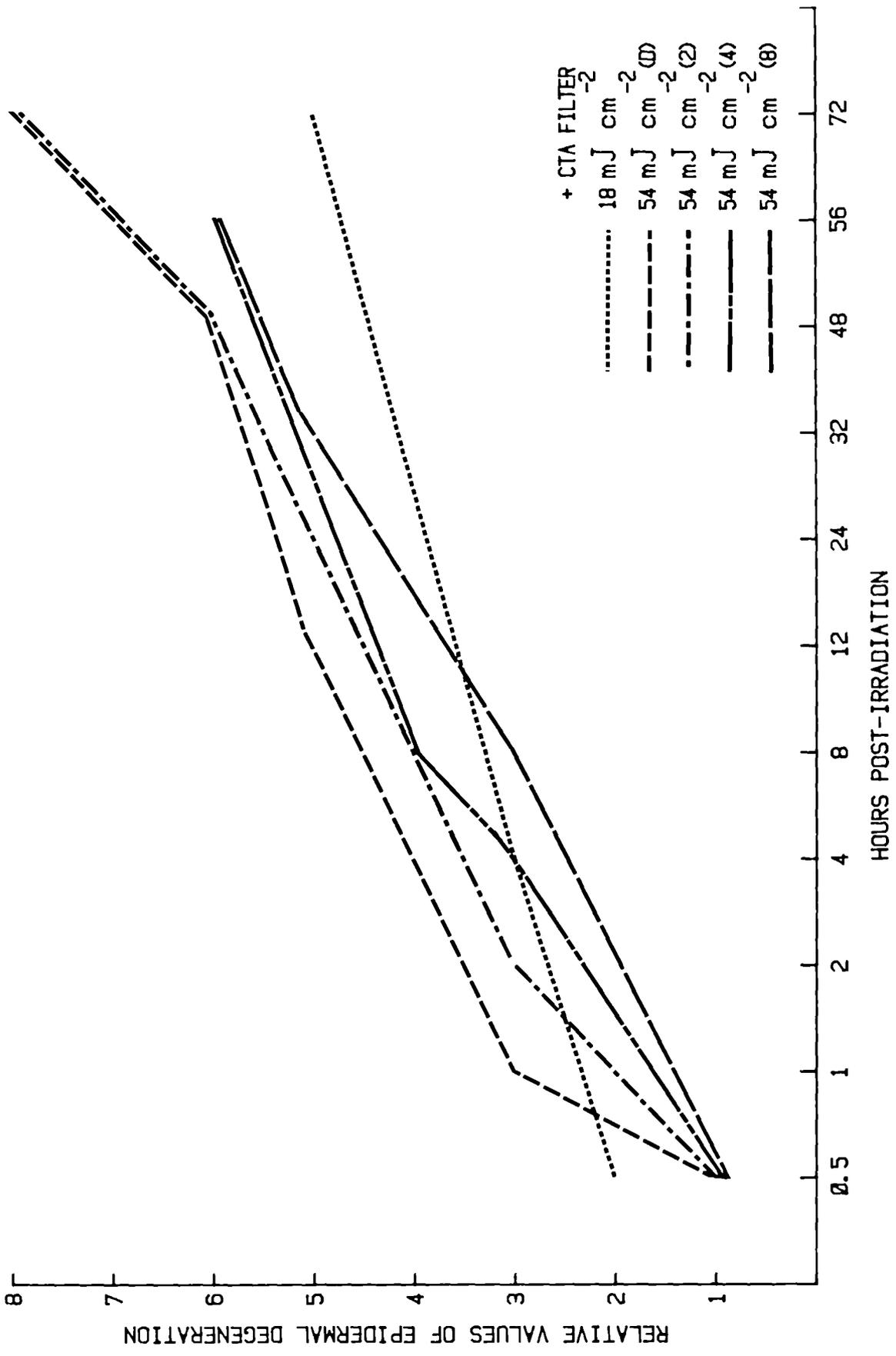
Fig 43 The impact of variable white light intensity upon plaice skin when irradiated with UV-B filtered through cellulose triacetate at cumulative doses of 18 and 54  $\text{mJ cm}^{-2}$

(D) = darkness

(2) = 2 tubes white light ( $320 \mu\text{W cm}^{-2}$ )

(4) = 4 tubes white light ( $640 \mu\text{W cm}^{-2}$ )

(8) = 8 tubes white light ( $1280 \mu\text{W cm}^{-2}$ )



epidermal integrity by 12 hpi. Cleavage within the supra-basal zone was apparent by 48 hpi and complete sloughing by 72 hpi. A similar sequential pattern emerged when the visible spectrum was limited to two tubes although the initial response was retarded by comparison with the experiment conducted in total darkness.

Increasing white light irradiance by doubling the intensity in each subsequent experiment showed that  $640 \mu\text{W cm}^{-2}$  (four tubes) and up to  $1280 \mu\text{W cm}^{-2}$  (eight tubes) of white light reduced the incidence of radiation damage proportionally. The incorporation of  $640 \mu\text{W cm}^{-2}$  (four tubes) delayed the response so that sunburn cells were not present until 4 hpi followed by oedema at 8 hpi. At this value the response did not go beyond a focal separation of cells within the supra-basal zone. Using eight tubes of white light a similar trend was observed, the response being attenuated even further in the initial stages with sunburn cells not becoming evident until 8 hpi. Subsequent examination of the latter stages of the response to radiation within this group showed that it also did not produce complete sloughing.

### 7.5 The skin response of plaice following irradiation through a Melinex type 'O' cut-off filter

The spectral transmission curve for Melinex type 'O', a plastic 'cut-off' filter, is shown in Fig.9. From this curve it can be seen that this filter effectively reduces transmission of the UV-B spectrum to a tiny fraction of the total bandwidth with an almost total absorption of wavelengths below 320 nm.

The high susceptibility of plaice skin to UV-B has been demonstrated in previous experiments so a further two sets of exposures were conducted, one in total darkness, the other incorporating visible light in order to determine whether any significant radiation response might perhaps be induced by the small 'tail' of shorter wavelengths transmitted under variable light conditions. In each experiment Melinex type 'O' was incorporated in place of the CTA filter and each group of fish was subjected to an identical irradiation regime incorporating UV-B at an intensity of  $30 \mu\text{W cm}^{-2}$  for an exposure time of 40 minutes resulting in a cumulative dose of  $72 \text{ mJ cm}^{-2}$ .

From Fig.44 it can be seen that in total darkness a progressive deterioration of the skin was/

was evident. This commenced at around 8 hpi when a slight swelling of the mucous cells was noted. The response escalated to a point whereby the epidermis at 48 hpi was markedly necrotic (Fig.45). By 72 hpi initial sloughing had occurred (Fig.46) although the lower half of the epidermis retained its morphology.

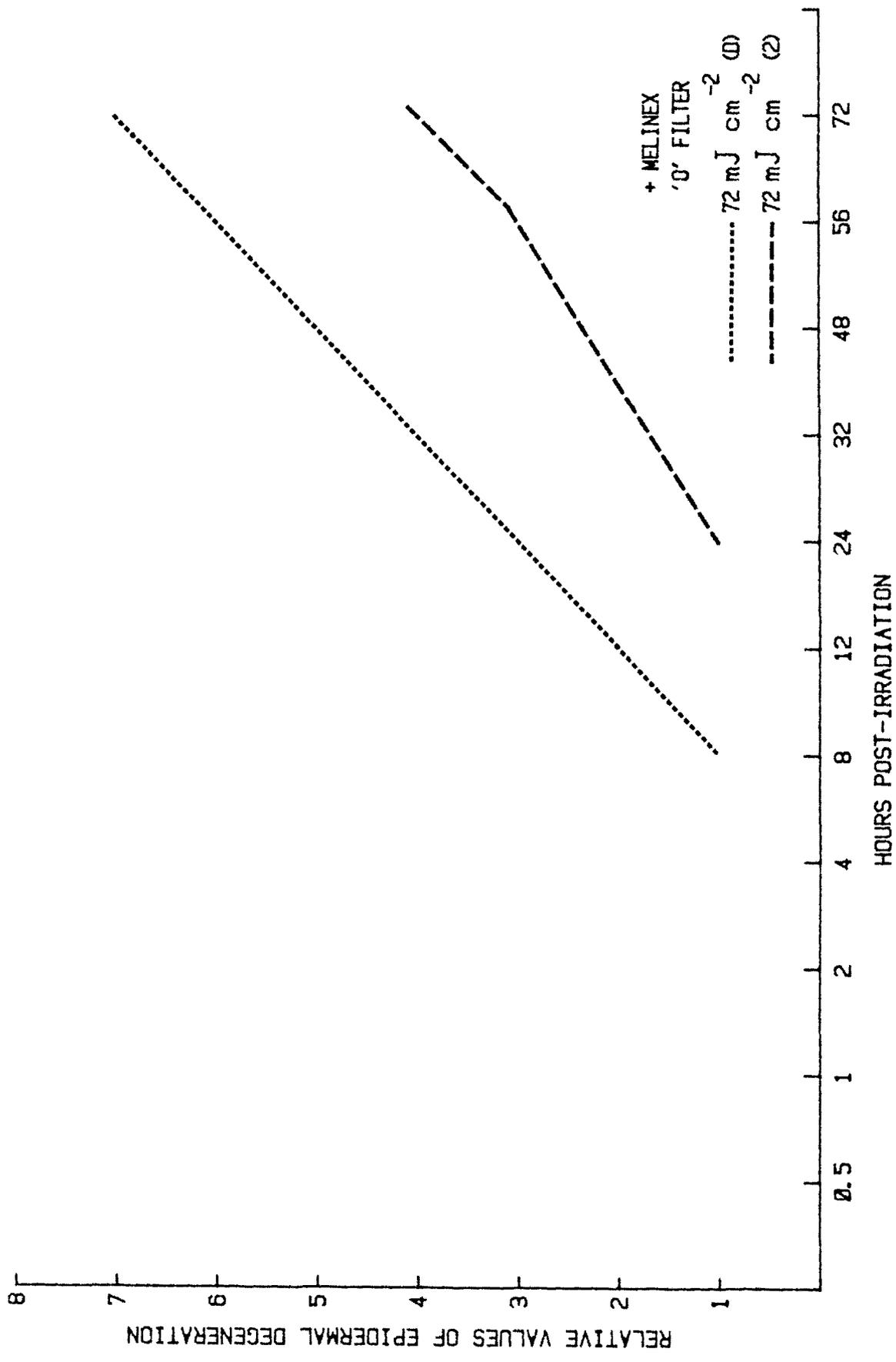
Under visible light the response was considerably less noxious. By 24 hpi the skin did show surface irregularity with some movement of the acidophilic granule cells into the supra-basal zone (Fig.47). At the termination of the experiment (72 hpi) the epidermis had retained its integrity although a number of features associated with radiation damage were present; these included slight oedema, perinuclear haloes and occasional sunburn cells. More prominently a massive vasodilation of the blood vessels within the *stratum spongiosum* and also the collagenous matrix of the *stratum compactum* (Fig.48) had occurred.

#### 7.6 The sequential development of the UV-B induced lesion in plaice skin as revealed by scanning electron microscopy (SEM)

In section, the sequential histopathology of the/  
the/

Figure 44

Fig 44 The skin response of plaice to UV-B  
following irradiation through a Melinex  
type '0' 'cut-off' filter at a cumulative  
dose of  $72 \text{ mJ cm}^{-2}$   
(D) = darkness  
(2) = 2 tubes white light ( $320 \mu\text{W cm}^{-2}$ )



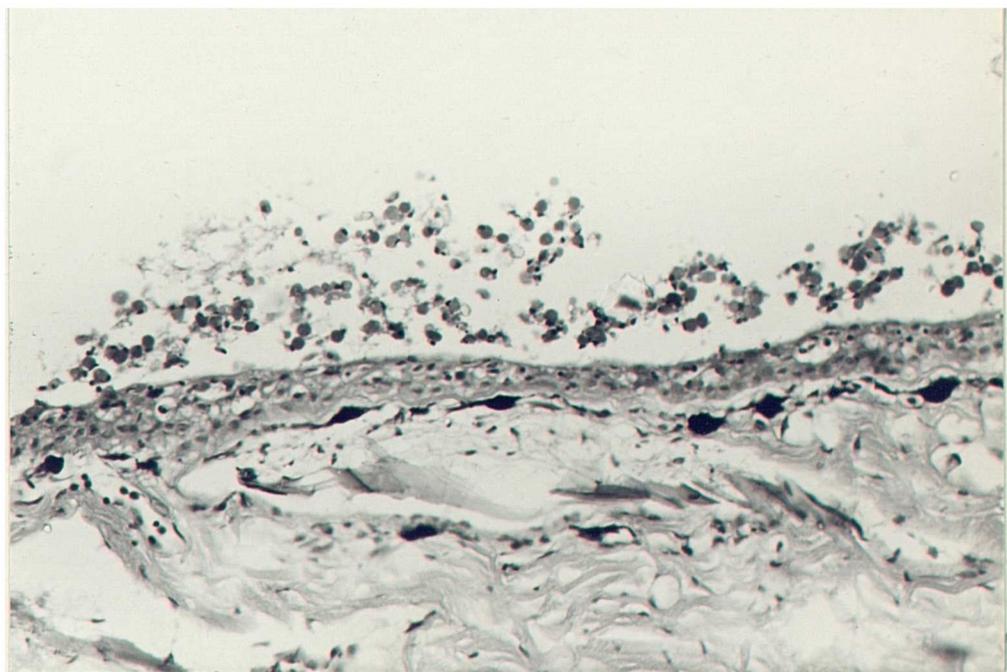
Figures 45 and 46

Fig.45 72 mJ cm<sup>-2</sup>, 48 hpi. Following irradiation in total darkness the epidermis exhibits an overall necrosis at all levels. The malpighian cell nuclei appear pyknotic throughout.

x 320

Fig.46 72 mJ cm<sup>-2</sup>, 72 hpi. Sloughing is limited to the outer layer, the underlying epidermis retaining its morphology.

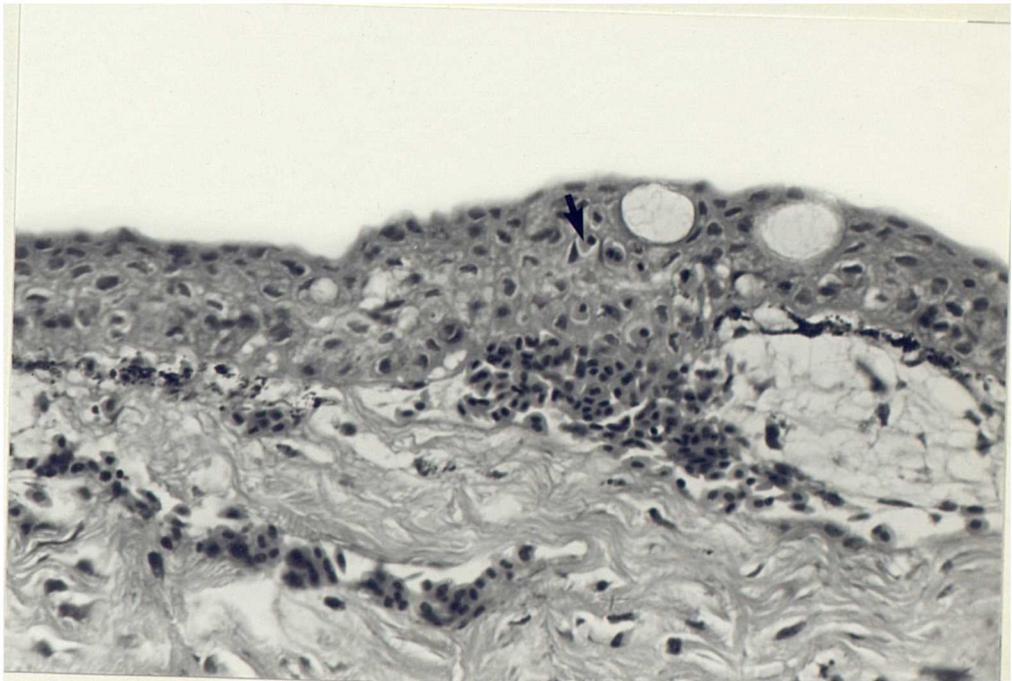
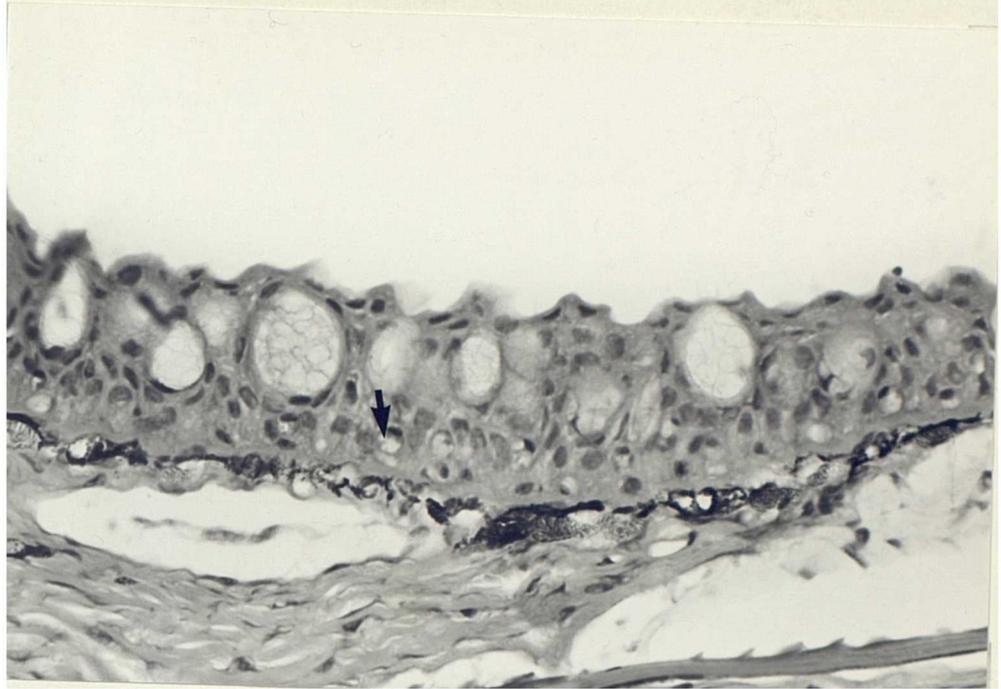
x 320



Figures 47 and 48

Fig.47 72 mJ cm<sup>-2</sup>, 24 hpi. Following irradiation but including visible light the epidermal changes are seen to be minimal. Malpighian cell nuclei do not exhibit the perinuclear haloes normally associated with radiation trauma. A limited response by the acidophilic granule cells is noted (arrowed).

Fig.48 72 mJ cm<sup>-2</sup>, 72 hpi. Extensive vasodilatation is present within the *stratum spongiosum* leading to rupture of the basement membrane and subsequent infiltration of blood cells (arrowed) into the epidermal layer.



the radiation lesion in plaice skin showed a diverse series of changes in surface topography as the lesion developed. These ranged from an almost immediate convolution of the epidermal surface to complete erosion of the epithelial layer.

The cellular events and major changes in surface appearance that occurred between these two extremes were so wide ranging that an investigation of the lesion by SEM was undertaken in order to provide a more detailed assessment of the surface of the developing lesion, particularly in relation to the interaction between the degenerating epithelium and its surrounding aqueous environment.

Following a cumulative dose of  $54 \text{ mJ cm}^{-2}$  given at a radiant intensity of  $30 \text{ } \mu\text{W cm}^{-2}$  skin samples were removed at 0.5 h, 1, 2, 4, 8, 24, 32, 48, 56, 72 and 80 hpi then fixed and prepared for SEM as described in Section II. In addition a further sample of non-irradiated skin was taken for control purposes.

Fig.49 shows the surface topography of normal plaice skin as viewed by SEM and reveals two major features. Of these the most prominent being the concentric microplicate patterning associated with the outermost surface of the malpighian cells. Overlying this delicate patterning a layer of particulate/

culate fibrillar material can be seen in close association with these ridges. This is the cuticle, an amorphous secretion emanating from the uppermost malpighian cells. Its extreme susceptibility to mechanical abrasion during processing seldom allows this layer to be demonstrated by light microscopy although its presence has frequently been demonstrated by transmission EM.

By 0.5 hpi a major change in the distribution and appearance of the cuticle had occurred. Although present, it no longer formed a confluent layer covering the surface of the microridges but was limited to the surface of specific cells (Fig.50) and showed a slight thickening in these areas, tending to mask the microridge patterning apparent in the control skin. The majority of cells no longer exhibited any evidence of a cuticular component. Two hours following irradiation the overall appearance of the skin surface was similar to that seen at 0.5 h but with a substantial increase in the thickness of the cuticle where present (Fig.51) indicating an increase in cuticular secretion from within these cells. By 4 hpi the surface topography continued to exhibit the selectivity of cuticular secretion seen at the earlier irradiation times and also revealed a small number of cells which had reached an advanced state of/

of necrosis. These appeared as shrunken, skeletal cellular membranes in the process of sloughing from the surface (Fig.52). Detailed examination of the microvillar ridges at this stage revealed also a minor change in their appearance. When compared to the control skin it was evident that the ridges were undergoing a subtle morphological change. No longer did they exhibit an integral uniformity throughout their length but had developed an irregular form, the ridges becoming ill-defined with local swelling at various points. By 8 hpi this feature had developed to a point whereby parallel ridges were occasionally bridged (Fig.53).

Twenty-four hours following irradiation the surface architecture showed a quite dramatic alteration in its appearance with an almost complete loss of microridge patterning although in a few areas the last vestiges of the microridges could be seen (Fig.54). As the lesion progressed to 32 hpi the surface exhibited large numbers of necrotic cells. These varied considerably in outward appearance, both in size and form, ranging from a relatively smooth sphere to a bizarre crenellated structure (Fig.55) accompanied by numerous intermediate stages. By 48 hpi the outward surface consisted primarily of a delicate reticulum of fibres (Fig.56) forming a complex/

complex mesh entrapping large numbers of cells undergoing necrosis. This fibrous matrix appeared to represent a transient phase as by 56 hpi the fibrillar network was no longer apparent and had given way to an immensely variable substrate of dying cells (Fig.57).

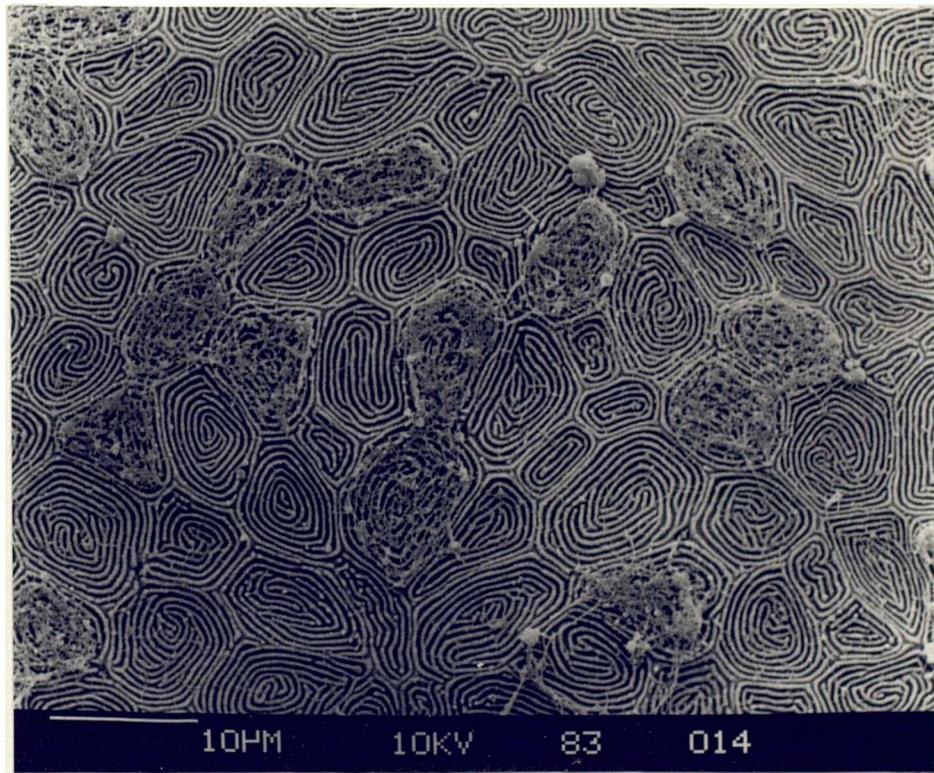
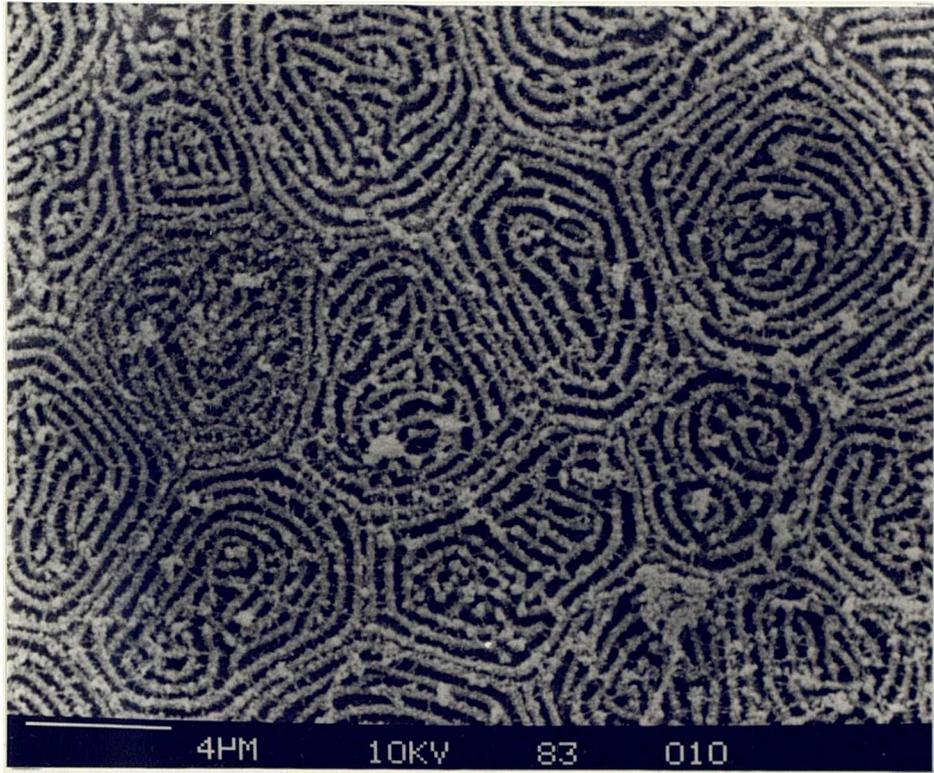
By 72 hpi the first clear evidence of the radiation lesion providing a suitable base for opportunistic bacteria from the surrounding environment was apparent when moderate numbers of bacteria could be seen attached to the surface of the underlying malpighian cells (Fig.58) and also invading the interface between the sloughing cells and the underlying tissue (Fig.59).

The 80 hour lesion revealed a highly convoluted substratum upon which the remaining malpighian cells mainly formed dense aggregations (Fig.60). However, small discrete patches of basal epithelia were observed which appeared to retain their morphology although not exhibiting microridge patterning. The peripheral attachment of these patches to the underlying tissue was limited to strands of material, putatively cytoplasmic in origin. Below, the substratum was thrown up into numerous undulations which appeared to follow the contour of the underlying scales.

Figures 49 and 50

Fig.49 Scanning electron micrograph of normal plaice skin. Note the cuticular secretion attached to the micro ridges.

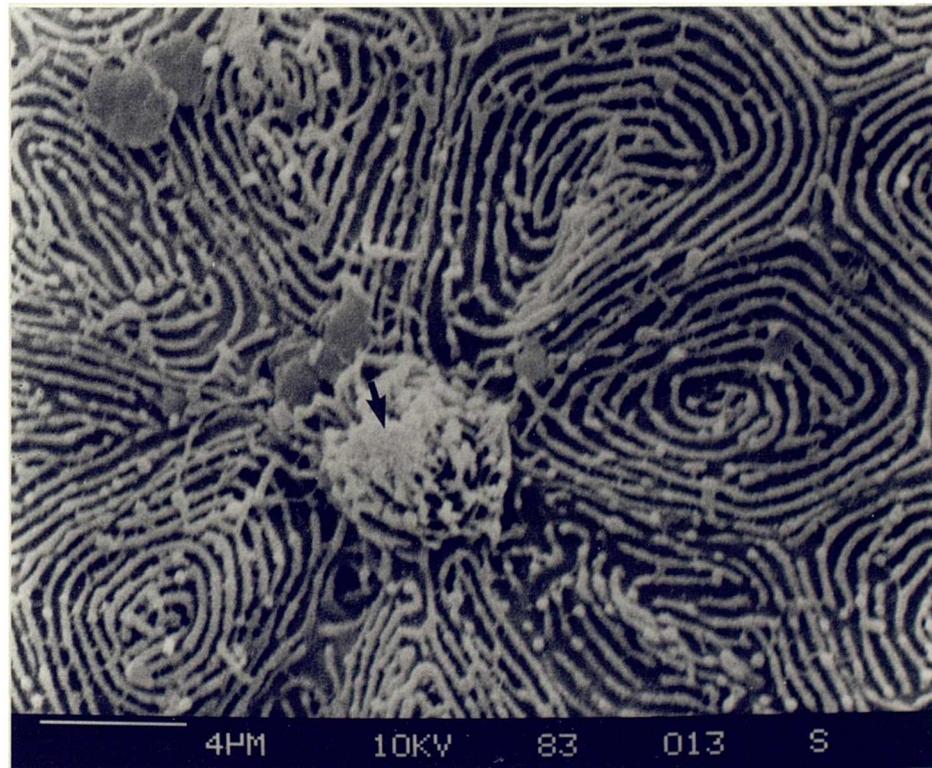
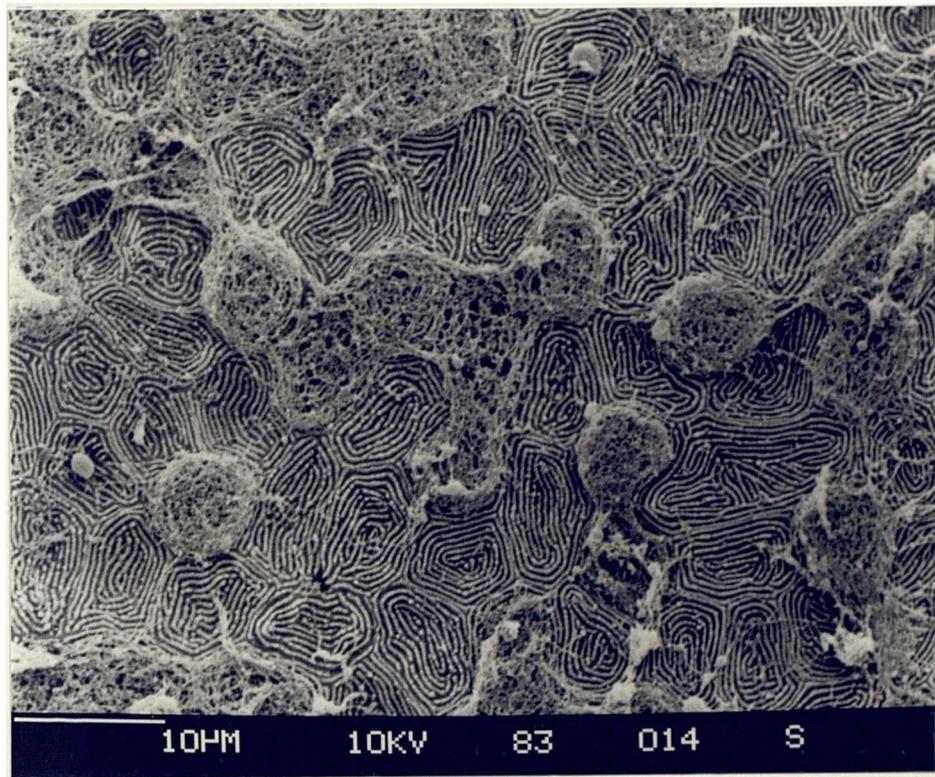
Fig.50 0 5 hpi following irradiation. The generalised distribution of the cuticle is no longer apparent whilst the remainder is cell specific and has increased in thickness, almost obscuring the underlying malpighian cells.



Figures 51 and 52

Fig.51 2 hpi. By this stage the cuticular secretion has increased considerably and is sharply delineated from the adjacent malpighian cells.

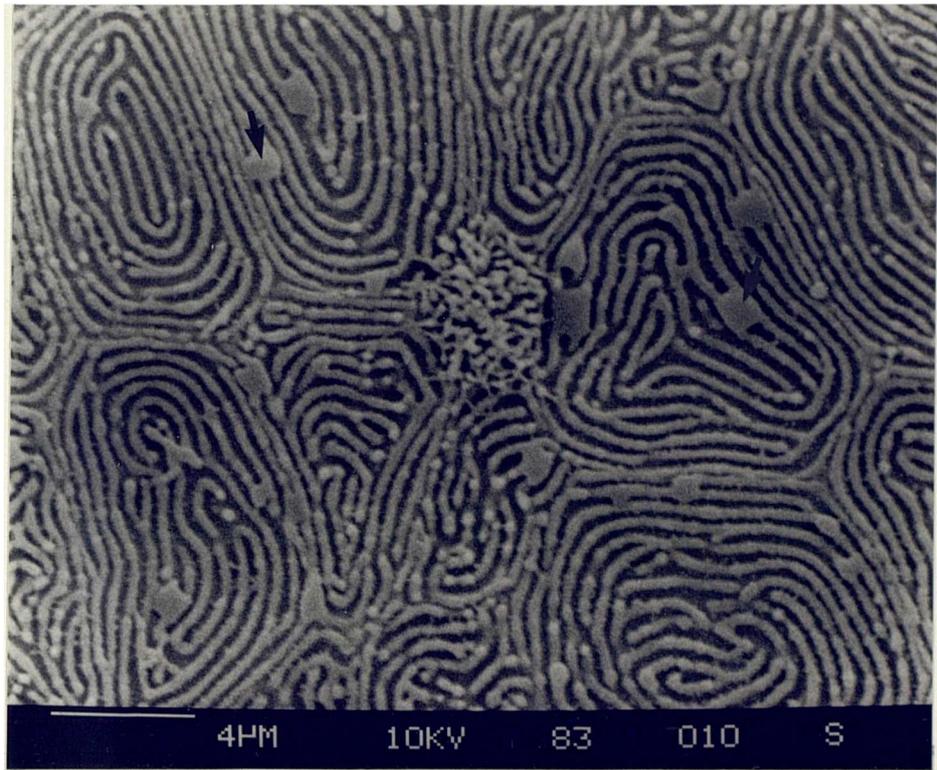
Fig.52 4 hpi. A necrotic malpighian cell (arrowed) is present upon the surface. Note also that the microridges no longer exhibit a structural uniformity but appear distinctly irregular in form when compared with earlier stages.



Figures 53 and 54

Fig.53 8 hpi. The microridges continue to show a progressive breakdown in uniformity sometimes exhibiting discrete 'blebs' along their length. More prominent, however, are the bridges (arrowed) formed by the loss of tonicity between adjacent ridges.

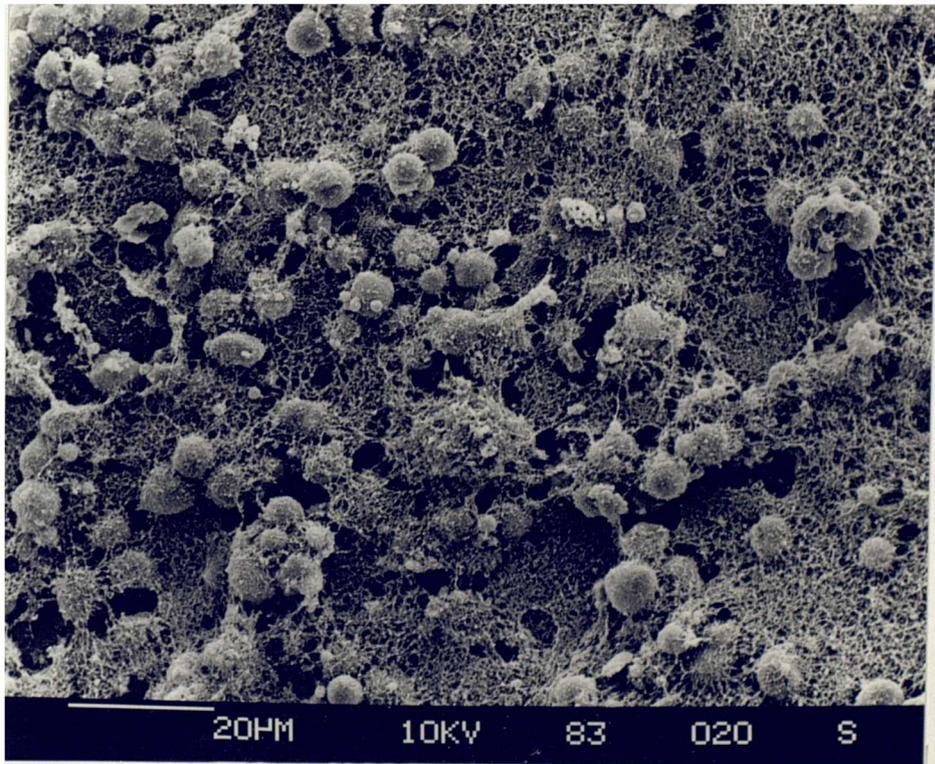
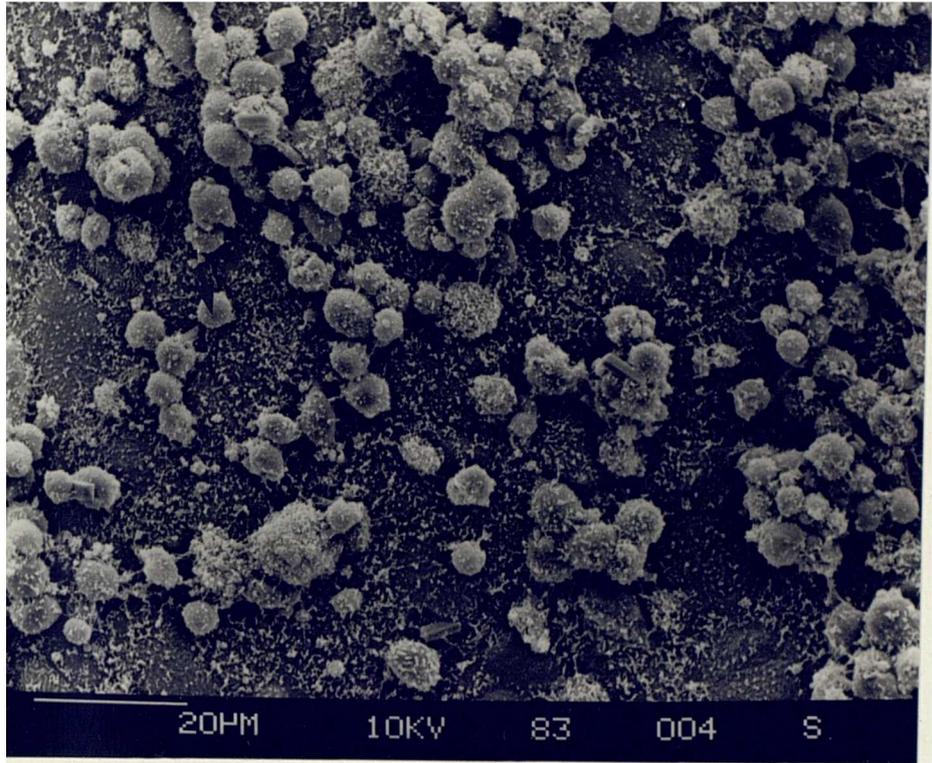
Fig.54 24 hpi. At this point the microridges have all but disappeared although some cells continue to exhibit the remains of the characteristic patterning. Note the faint patterning upon the surface of the adjacent cells (arrowed).



Figures 55 and 56

Fig.55 32 hpi. Numerous cells can be seen undergoing necrosis. They vary in form from relatively smooth spheres (arrowed) to markedly crenellated structures.

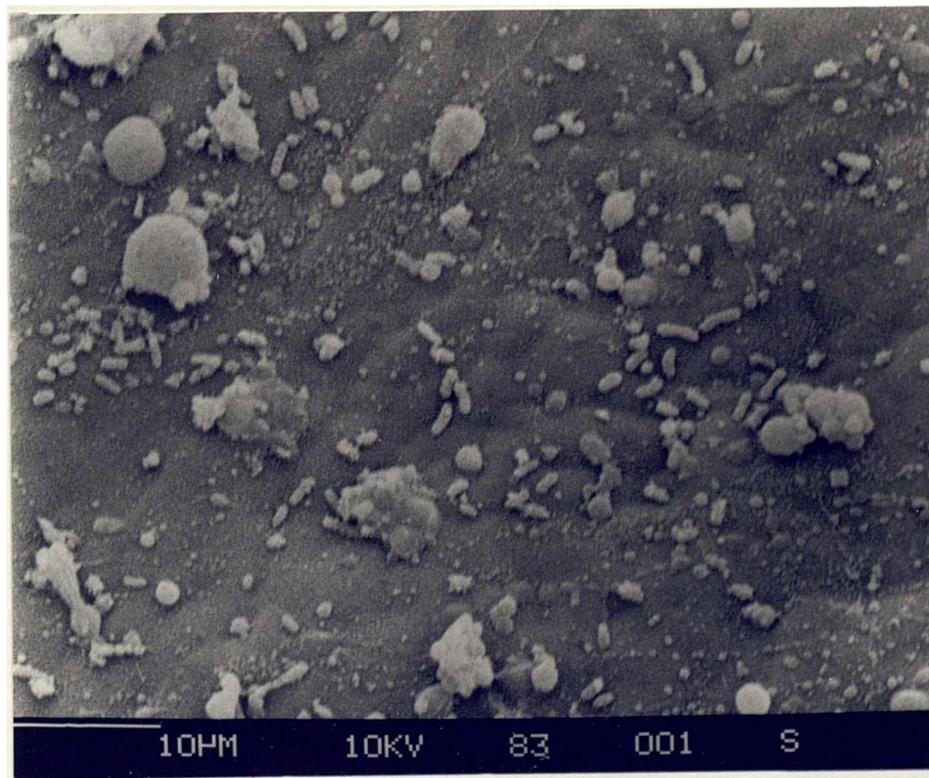
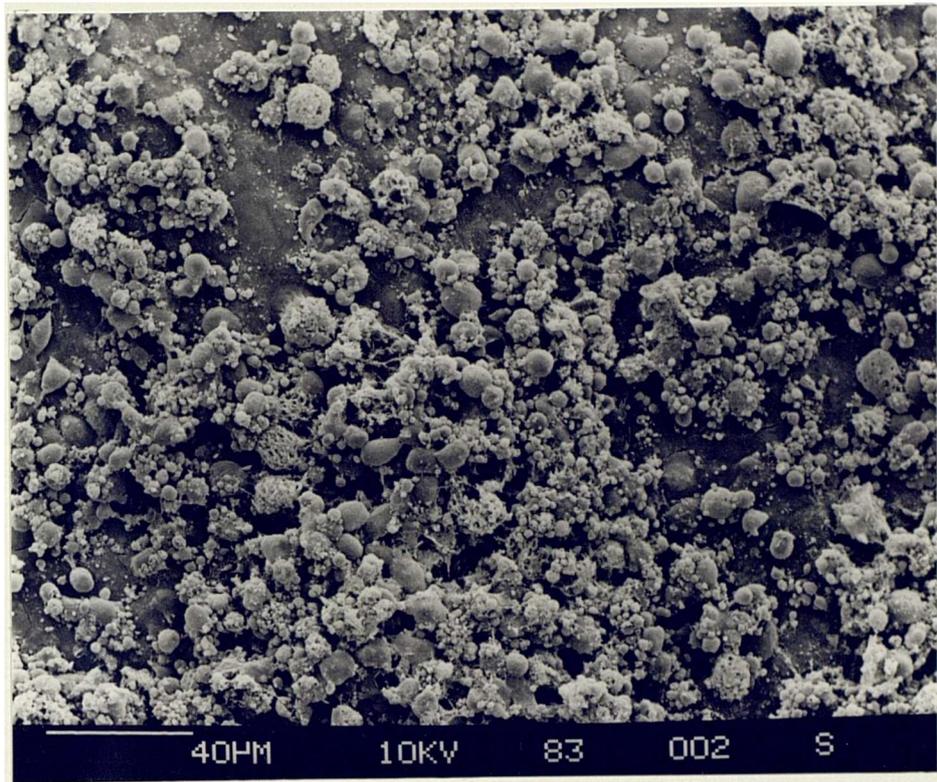
Fig.56 48 hpi. The most prominent feature at this stage is the dense fibrous matrix encompassing the entire surface and entrapping the sloughing cells.



Figures 57 and 58

Fig.57 56 hpi. The surface of the lesion now exhibits a mass of cellular debris in association with numerous cells at various stages of necrosis.

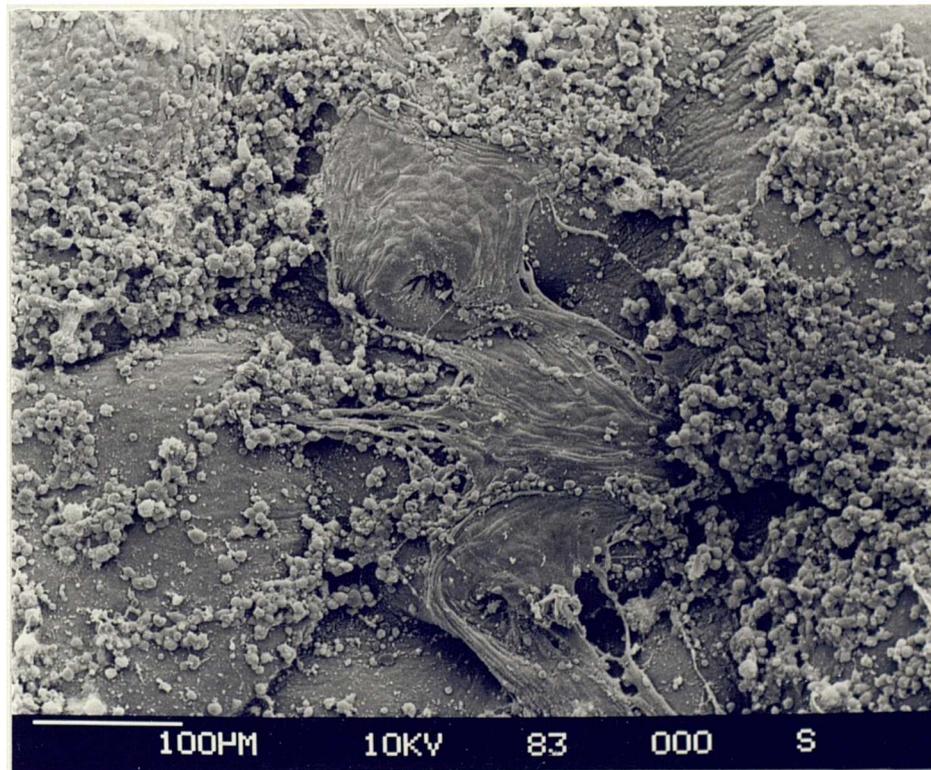
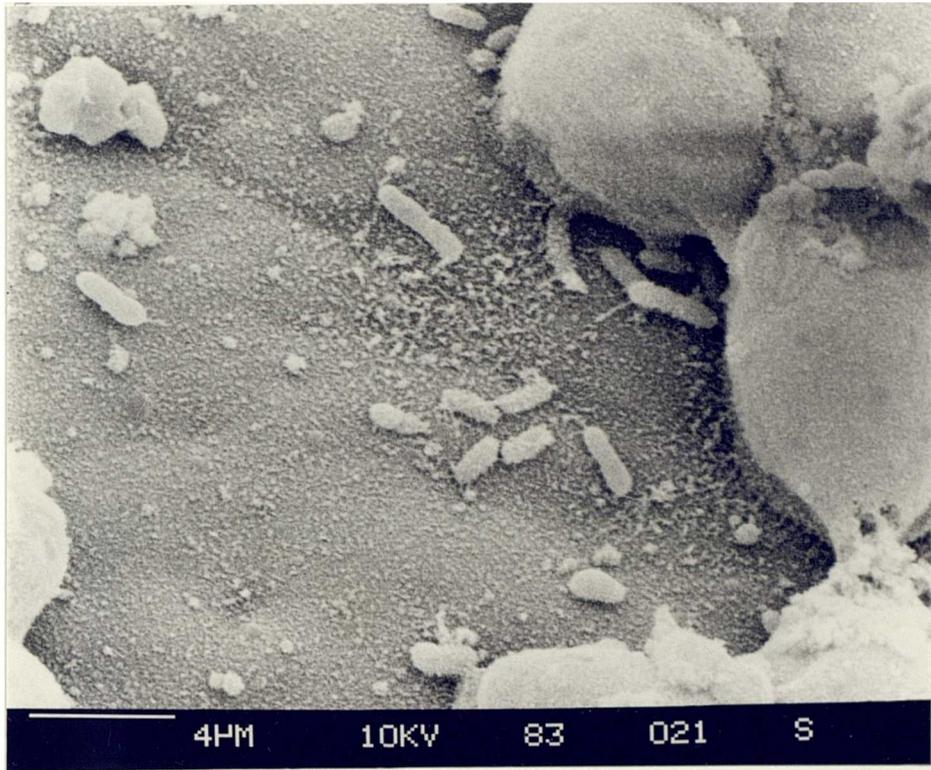
Fig.58 72 hpi. Bacteria can be seen attached to the surface layer.



Figures 59 and 60

Fig.59 72 hpi. Bacteria can be observed penetrating the interface between sloughing malpighian cells and the underlying substrate.

Fig.60 80 hpi. The surface topography of the lesion exhibits dense aggregations of cellular debris. The basal structure is thrown up into a series of undulations which probably represent the contours of the scales below.



### 7.7 The skin response of turbot to UV-B

So that a comparison might be made between the skin response of species occupying a similar ecological niche, a series of experiments was conducted on turbot using the experimental regime applied to plaice. A cumulative dose of  $72 \text{ mJ cm}^{-2}$  given at a radiant intensity of  $30 \text{ } \mu\text{W cm}^{-2}$  was selected for two reasons. Firstly, it had been shown during the plaice irradiation experiments that such doses of unfiltered UV-B were sufficient to produce complete sloughing of the epidermis within 56 hpi, well within the timescale allocated to each experiment. Secondly, a cumulative dose of this magnitude represents the minimal erythema dose (MED) viz. the amount of ultraviolet light required to induce a minimal reddening of previously non-irradiated human skin at this latitude ( $55^{\circ}$ ). By exposing turbot to this level of radiation some measure could be made of the variation in susceptibility of two similar species of fish.

From Fig.61 it can be seen that irrespective of white light intensity, darkness, or the incorporation of a CTA filter during irradiation, turbot skin did not show the same susceptibility to UV-B when compared with plaice skin. Over the 72 h timescale/

timescale there was a tendency for the pathological events encountered at each of the scoring values to be delayed by up to 4 hours during the initial stages of the irradiation response in turbot skin, although by 24 hpi it was identical for both species. By 56 hpi, however, plaice skin had shown complete epidermal erosion, whereas it took until 72 hpi for a similar response to be elicited in turbot skin.

Histologically turbot skin followed a similar sequential pathology to plaice skin but exhibited significant differences in its response to radiation. The initial swelling of mucous cells and irregular surface observed in plaice by 0.5 hpi did not occur in turbot. The initial reaction was seen to be the development of perinuclear haloes surrounding the malpighian cells with a delay of 30 minutes in their appearance when compared to plaice. Sunburn cells of both A and B type were observed, *type A* being most prominent in the CTA filter experiment (Fig.62) by 8 hpi whereas *type B* were more common throughout the epidermis in those fish receiving unfiltered UV-B (Fig.63).

In darkness, experiments using unfiltered UV-B revealed a transient response in turbot skin whereby the epidermis showed a marked hyperplasia as early as 4 hpi, prior to the appearance of sunburn cells. This/

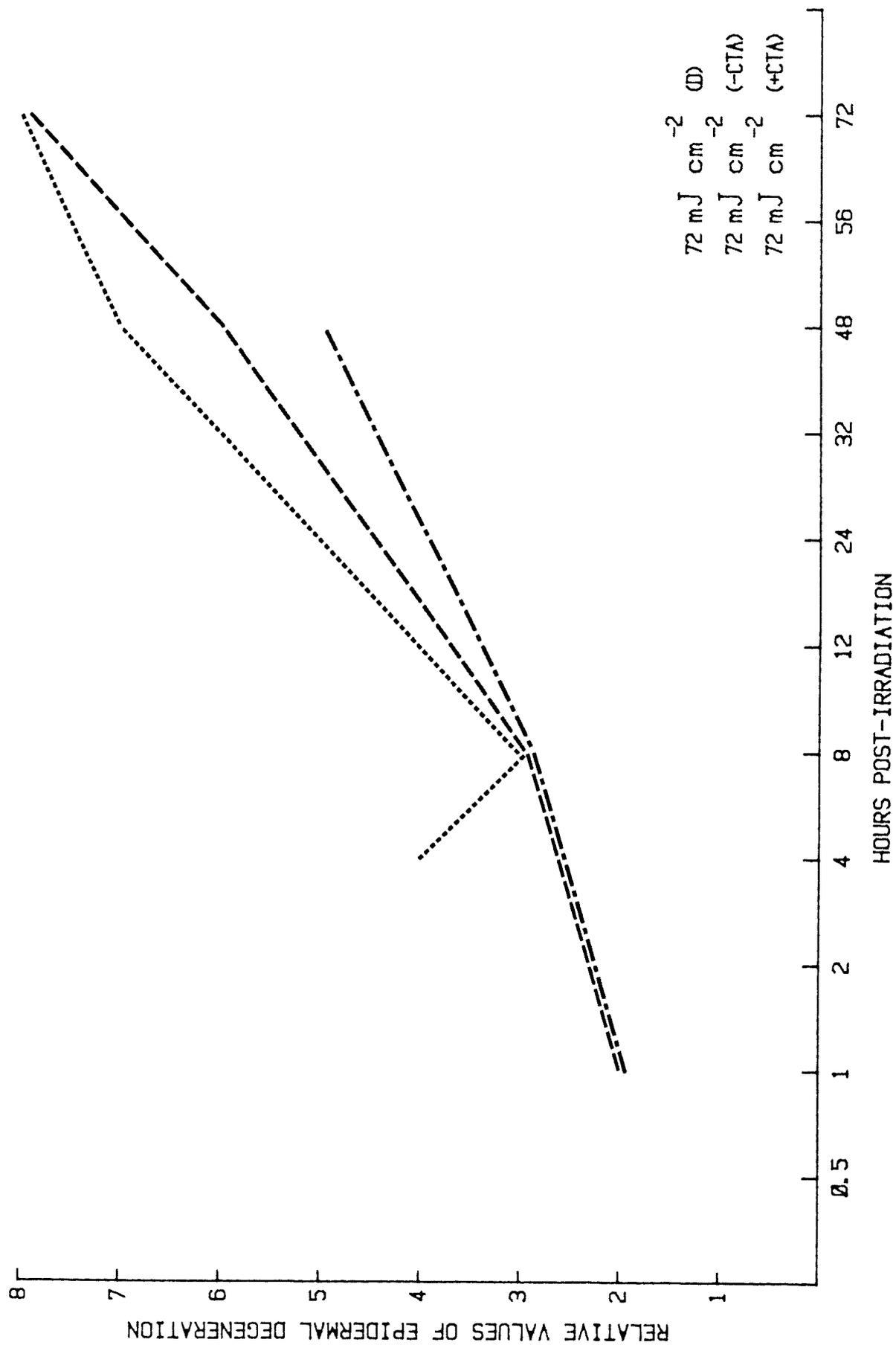
Figure 61

Fig 61 The effect of UV-B (filtered and unfiltered) at a cumulative dose of  $72 \text{ mJ cm}^{-2}$  upon the skin of turbot under variable light conditions

(D) = darkness

(-CTA) = without cellulose triacetate filter

(+CTA) = with cellulose triacetate filter



This feature usually diminished by 8-12 hpi then reappeared at around 24 hpi when a more generalised necrosis was apparent. This was followed by an initial sloughing at 48 hpi (Fig.64) and almost complete epidermal erosion by 72 hpi (Fig 65).

In conjunction with visible light, filtered UV-B showed that radiation damage was effectively blocked by 48 hpi (Fig.66) by which time the lesion exhibited only moderate numbers of necrotic malpighian cells. Histological examination of the skin at subsequent intervals of 56 and 72 hpi confirmed that the lesion did not develop beyond this point.

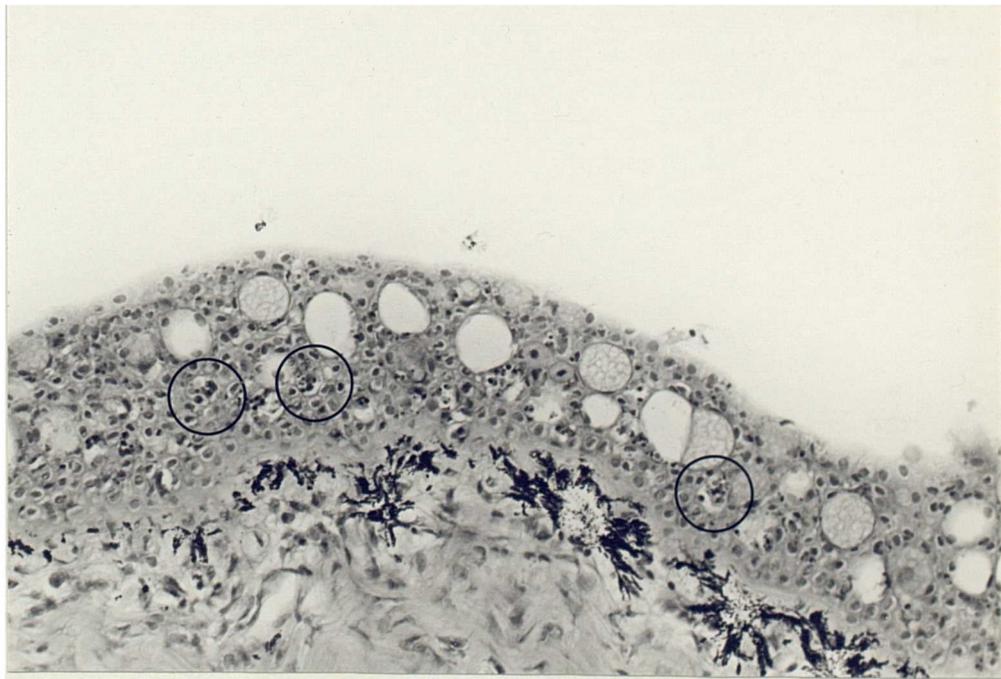
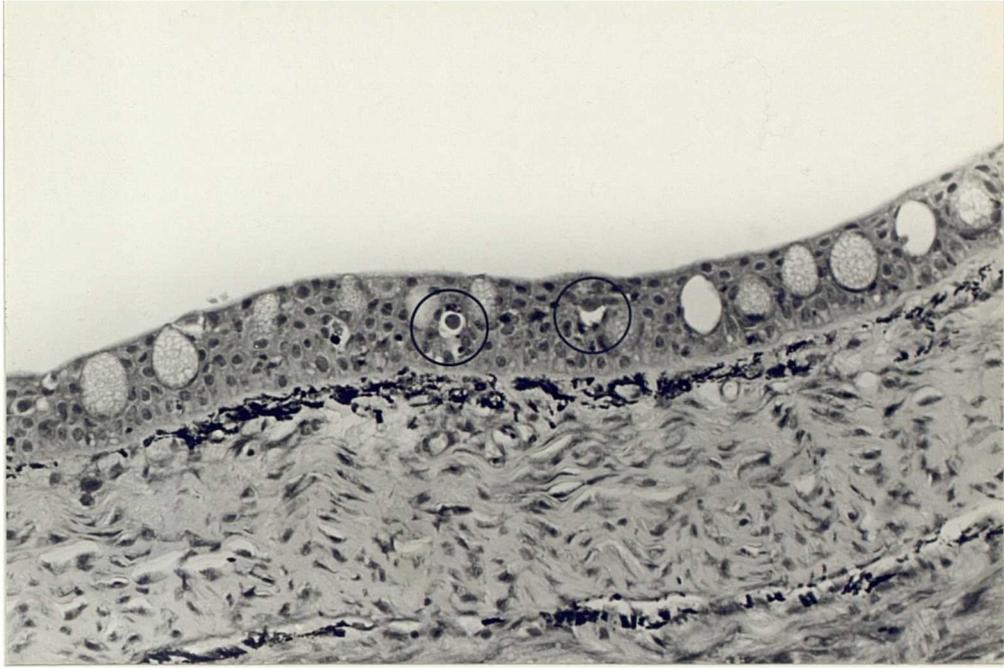
Figures 62 and 63

Fig.62 72 mJ cm<sup>-2</sup>, 8 hpi (CTA filtered UV-B + white light). *Type A* sunburn cells (circled) can be seen within the mid-zone of the epidermis.

x 320

Fig 63 72 mJ cm<sup>-2</sup>, 12 hpi (unfiltered UV-B + white light). Numerous *Type B* sunburn cells (circled) are present in association with a general thickening of the epidermal layer.

x 320



Figures 64 and 65

Fig.64 72 mJ cm<sup>-2</sup>, 48 hpi (dark - no white light).  
The epidermis exhibits advanced necrosis at all levels. The dense focal aggregations of melanin represent the epidermal melanocytes prevalent within this species.

x 320

Fig 65 72 mJ cm<sup>-2</sup>, 72 hpi (dark - no white light). The epidermis is almost completely destroyed leaving only a thin, non-viable layer of dead cells dehiscent from the basement membrane (arrowed).

x 320

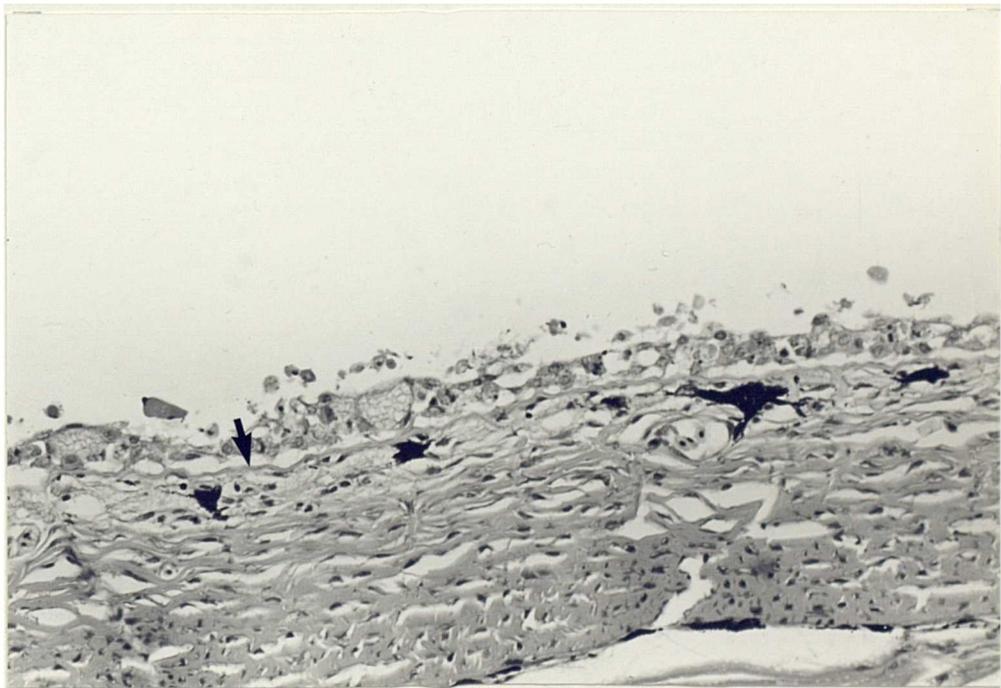


Figure 66

Fig.66 72 mJ cm<sup>-2</sup>, 48 hpi (CTA filtered UV-B + white light). The epidermis, although lacking organisation, shows only moderate numbers of necrotic cells and limited oedema within the basal layer.

x 320



SECTION IV

THE EFFECT OF UV-B UPON THE SKIN  
OF THE RAINBOW TROUT *Salmo gairdneri* RICHARDSON  
AND THE ATLANTIC SALMON *Salmo salar* L.

### *Introduction*

Intensive cultivation of rainbow trout and Atlantic salmon forms the basis of an economically significant industry in the UK. The husbandry problems associated with the development of this highly complex commercial enterprise have, as a consequence, provided much of the clinical material upon which fundamental research into fish diseases has been conducted over recent years.

Neither species, under natural conditions, would be exposed to the immensely variable environmental parameters associated with fish farming. The skin, a highly delicate organ, is intimately associated with this environment so it is therefore not unexpected to find that a variety of skin conditions occur in cultivated fishes. Many of these are associated with subliminal viral or bacterial infections, culminating in a breakdown of the *milieu intèrieure* of the skin. Others may be related to ectoparasitic infestations or physical trauma. Nevertheless clinical cases are presented on occasion which appear to have some correlation to sunlight and such instances have been reviewed in Section I. More recently, however, clinical cases (see Section VI) of lesions specific to the dorsum have been reported usually following prolonged outbreaks of uninterrupted sunlight.

The/

The vulnerability of UV-B of plaice and turbot, both species of a benthic habitat, has been demonstrated. Therefore, it was considered desirable to investigate the potential susceptibility of the two most common artificially reared pelagic species under similar circumstances.

### 8.1 Results

Initial experiments on rainbow trout and Atlantic salmon were conducted using the standard irradiation technique previously applied to plaice and turbot at a radiant intensity of  $60 \mu\text{W cm}^{-2}$ . Exposure times ranged from 30 minutes to 3 hours resulting in cumulative doses ranging from  $68 \text{ mJ cm}^{-2}$  to  $324 \text{ mJ cm}^{-2}$ .

In sharp contrast to the response observed in flatfish, detailed microscopical examination of the dorsal skin of both salmonid species revealed a substantial decrease in overall sensitivity to UV-B. Histologically the skin response at a cumulative dose of  $68 \text{ mJ cm}^{-2}$  of unfiltered UV-B was limited primarily to a moderate but progressive stimulation of the mucous cell component, a feature amply demonstrated by the Periodic Acid-Schiff reaction. By using this histochemical technique it was possible to demonstrate the subtle changes in the response to radiation over the 72 h timescale. For example, at 0.5 hpi (Fig.67) the mucous cells present at all levels within the epidermis/

epidermis exhibited a strongly PAS + v.e. reaction with their secretion being strictly limited to within the confines of the cell membrane. By 1 hpi, however, a gradual diffusion of the mucus through the cell wall was detectable (Fig.68) the secretion assuming a foamy, vacuolated appearance within the cell.

Examination of sections from skin sampled at 4 hpi showed a substantial increase in mucous cell numbers, mostly within the basal and supra-basal layers. Their staining propensity had also markedly increased (Fig 69). At this point the perinuclear haloes, an integral feature of the malpighian cell response to radiation, became more prominent. Comparison of the response at 1 hpi with that at 4 hpi showed this to be most dramatic within the lower half of the epidermal layer with the malpighian cell nuclei staining densely at all levels.

No other changes were noted over the timescale although by 24 hpi the mucous cells within the upper zone were actively secreting outwards and generally gave a pale staining response when compared with those below (Fig.70). Within the supra-basal zone an occasional malpighian cell nucleus appeared pyknotic. By 72 hpi the response was identical to that observed at 24 hpi. Similar findings were observed at doses of/

of 108 and 216 mJ cm<sup>-2</sup> (Fig.71).

The apparent lack of response to unfiltered UV-B over the 72 h timescale lead to a further series of experiments using the same radiant intensity of 60 μW cm<sup>-2</sup> but in fish which were sampled over a much increased timescale. Following a dose of 324 mJ cm<sup>-2</sup> rainbow trout and Atlantic salmon were sampled at daily intervals over a 14-day period. The results are plotted in Fig.72. From the linear response it can be seen that an exceedingly slow but progressive necrosis does occur in salmonids but over a significantly longer period than that in flatfish.

In section, during the initial stages, the response at 324 mJ cm<sup>-2</sup> was marginally more severe than that seen at 68, 108 and 216 mJ cm<sup>-2</sup>, with a moderate hyperplasia in association with a breakdown in the organisation of the malpighian cells within the epidermis being evident by 48 hpi (Fig.73). This feature prevailed with no evidence of morphological change until day 5, when a moderate focal separation between the cells of the basal and supra-basal zone was noted (Fig.74). This appeared to be a transient feature, perhaps due to the photoreactivating properties of the skin, allowing repair of the damaged tissue, as by day 9 it was no longer evident in section. There was by this time, however, a/

a general acantholysis throughout the entire epidermis within the body and head regions exposed to radiation (Figs.75 and 76), perinuclear haloes being a prominent feature also.

A gradual but progressive deterioration in the integrity of the epidermis was observed in subsequent samples so that by day 13 (Fig.77) the head epidermis exhibited a marked acantholysis in association with prominent perinuclear haloes, mucous cells were relatively few in number. The body skin exhibited a marginally less severe reaction but was also noticeably lacking in mucous cells (Fig.78). Numerous necrotic cells were present although none could strictly be defined as a sunburn cell. Despite these degenerative changes there was no evidence of a total breakdown of the epidermis as observed in the plaice and, to a lesser degree, the turbot.

In an attempt to determine the level of radiation required to induce complete sloughing of the epidermis, fish were subjected to increasing doses of UV-B but with the insertion of a CTA filter in order to attenuate the shorter wavelengths and so more closely simulate natural solar UV.

Experiments incorporating doses of 1296, 4320, 5832 and 6048 mJ cm<sup>-2</sup> with exposure times ranging from a total of 24 h (8 h per day for 3 days) up to

56/

56 h (8 h per day for 7 days) were conducted. During all irradiations the visible (white light) input was approximately  $640 \mu\text{W cm}^{-2}$  providing optimal conditions for photoreactivation. First sampling was carried out on the day immediately following the final irradiation with further samples taken at daily intervals thereafter over a 14-day period. The results for both species are plotted in Fig.79.

At a dose of  $1296 \text{ mJ cm}^{-2}$  the lesion in both species progressed through the various stages described earlier for  $324 \text{ mJ cm}^{-2}$ , culminating by day 14 in a separation of the entire epidermal layer from the basement membrane. This finding appeared to be more specific to the head (Fig.80) than the body epidermis (Fig.81). In both areas there was evidence of leucocyte activity within the supra-basal zone.

Increasing the dose to  $4320 \text{ mJ cm}^{-2}$  induced a hitherto unobserved response in the rainbow trout epidermis by around day 3. At the lower doses the reaction had been typified by the characteristic perinuclear halo effect and densely staining nuclei, a feature common to all species examined. In contrast the malpighian cells now exhibited large pale staining nuclei and by day 5 (Fig.82) the body epidermis formed an ill-defined stratum with numerous leucocytes present/

present within the supra-basal zone. Examination of the head epidermis at day 6 revealed a similar response, each cell exhibiting a foamy vacuolation within the confines of the cell membrane (Fig.83). Leucocyte infiltration was not evident. By day 9 the malpighian cells tended to resume their characteristic perinuclear halo morphology, although the nuclei remained pale staining and ill-defined (Fig.84). A similar reaction was noted in the dorsal and pectoral fins (Figs.85 and 86) with *Types A* and *B* sunburn cells present towards the tip of the fins in association with nuclear droplet formation.

By day 11 leucocyte activity was a prominent feature within the dorsal body epidermis (Fig.87) but with relatively fewer numbers of leucocytes present within the head skin. Examination of pectoral fin tissue at day 11 revealed a massive hyperplasia in association with a generalised breakdown of the epidermal layer. Histologically (Fig.88) the appearance of the pectoral fin at this point was morphologically similar to that reported in salmon fry affected by solar radiation under natural conditions. Towards the base of the fin the reaction throughout the epidermis was less severe being limited to a separation of the epidermal layer from the underlying basement membrane (Fig.89). By day 14 the body skin exhibited a/

a similar response (Fig.90).

Detailed examination of irradiated salmon skin receiving the same dose showed a similar progressive necrosis over a 14-day period. In the earlier samplings the malpighian cells did not, however, exhibit the foamy vacuolation observed in trout. Although pale staining the cells remained well-defined and nucleoli were often prominent (Fig.91) as were *Type A* sunburn cells (Figs.92 and 93). By day 5, however, the lesion had progressed to a point where the malpighian cells appeared no different from those in trout skin at the same point on the timescale (Fig.94). Similar findings were apparent in subsequent samplings of the base of the dorsal fin at day 6, exhibiting a classic *Type A* sunburn cell reaction (Fig.95) identical to that seen under natural conditions (see Fig.122; case histories). Towards the fin tip a major hyperplastic response was observed (Fig.96).

An interesting feature by day 8 was the presence of numerous *Type B* sunburn cells (Fig.97) not previously observed in any great numbers in trout skin. These were particularly prominent within the dorsal body skin where their distribution was confined primarily to the middle and upper layers. The noxious effect of UV-B upon the upper surface of the pectoral fin was amply demonstrated by day 10 (Fig.98) where the skin/

skin surface exposed to the direct rays exhibited a focal hyperplasia with numerous pale staining necrotic cells and sporadic groups of nuclear drop-lets within the central zone. Beneath, the epidermis, ostensibly protected from UV-B, appeared normal.

Higher doses of CTA filtered UV-B at 5832 and 6048  $\text{mJ cm}^{-2}$  showed a similar trend in both species with initial sloughing of the epidermis occurring by days 9 and 7 respectively. By day 12 the head skin in both species showed advanced necrosis (Fig.99) with considerable oedema and sloughing of the outer layer. The final sampling on day 14 showed an almost complete erosion of the head epidermis (Fig.100) in Atlantic salmon.

Figures 67 and 68

Fig.67 68 mJ cm<sup>-2</sup>, 0.5 hpi. The epidermis presents a normal appearance with numerous mucous cells apparent throughout.

Periodic acid-Schiff

Fig.68 68 mJ cm<sup>-2</sup>, 1 hpi. The mucous cell secretion exhibits a foamy vacuolated appearance. In addition the malpighian cells are generally pale staining.

Periodic acid-Schiff



Figures 69 and 70

Fig.69 68 mJ cm<sup>-2</sup>, 4 hpi. In contrast to the previous samples the mucous cell component now shows a considerable increase in numbers and staining propensity. Note also the prominent perinuclear haloes surrounding the malpighian cell nuclei.

Periodic acid-Schiff

Fig.70 68 mJ cm<sup>-2</sup>, 24 hpi. Surface mucous cells are pale staining and actively secrete their contents onto the surface. Within the supra-basal layer several pyknotic cells can be seen.

Periodic acid-Schiff

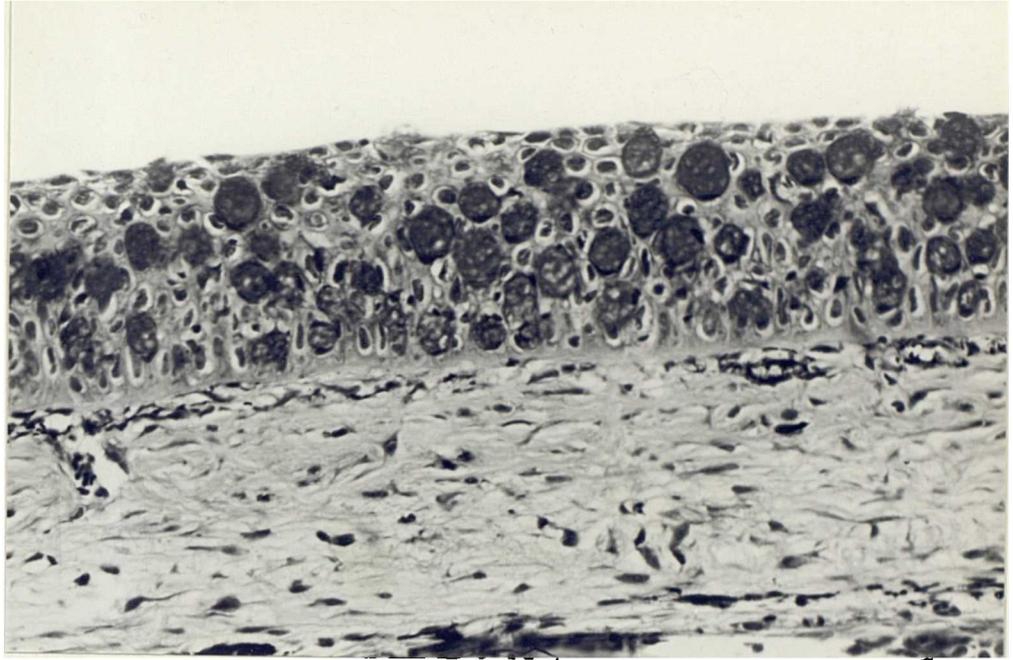


Figure 71

Fig 71 The effect of cumulative doses of  
68, 108 and 216 mJ cm<sup>-2</sup> UV-B are shown  
for rainbow trout and Atlantic salmon  
The results are identical for both  
species  
(RT + AS) = Rainbow trout + Atlantic  
salmon

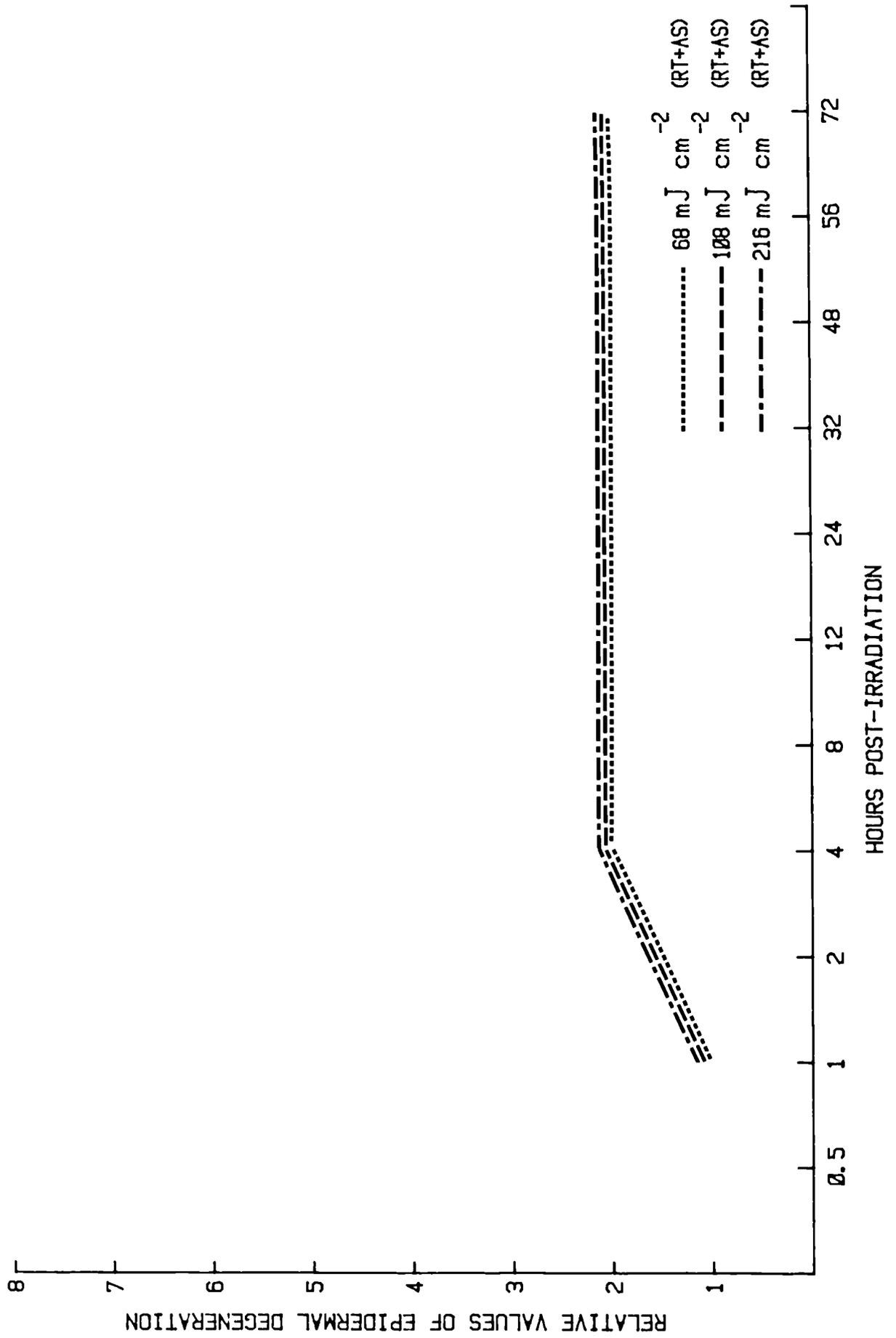
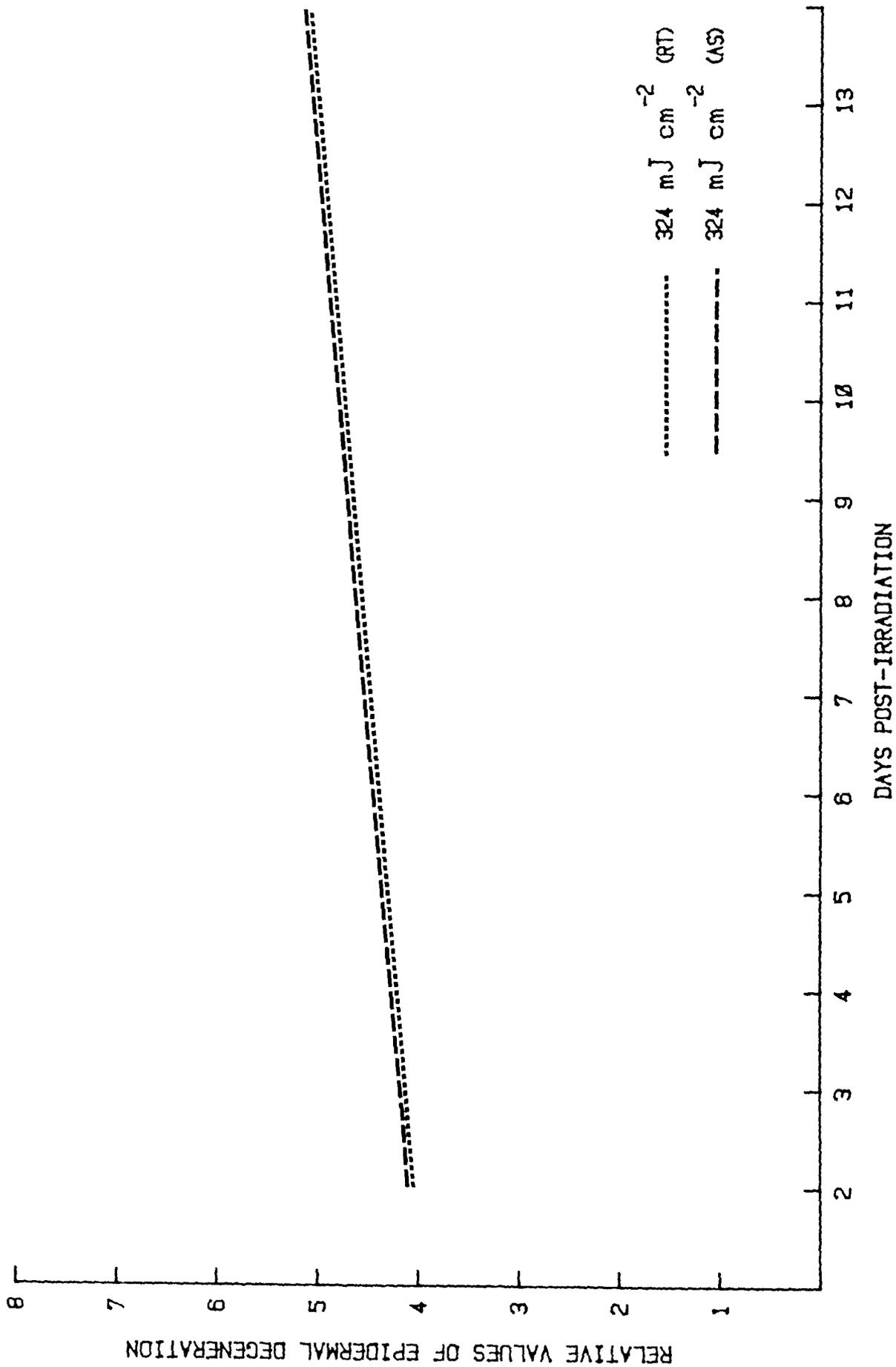


Figure 72

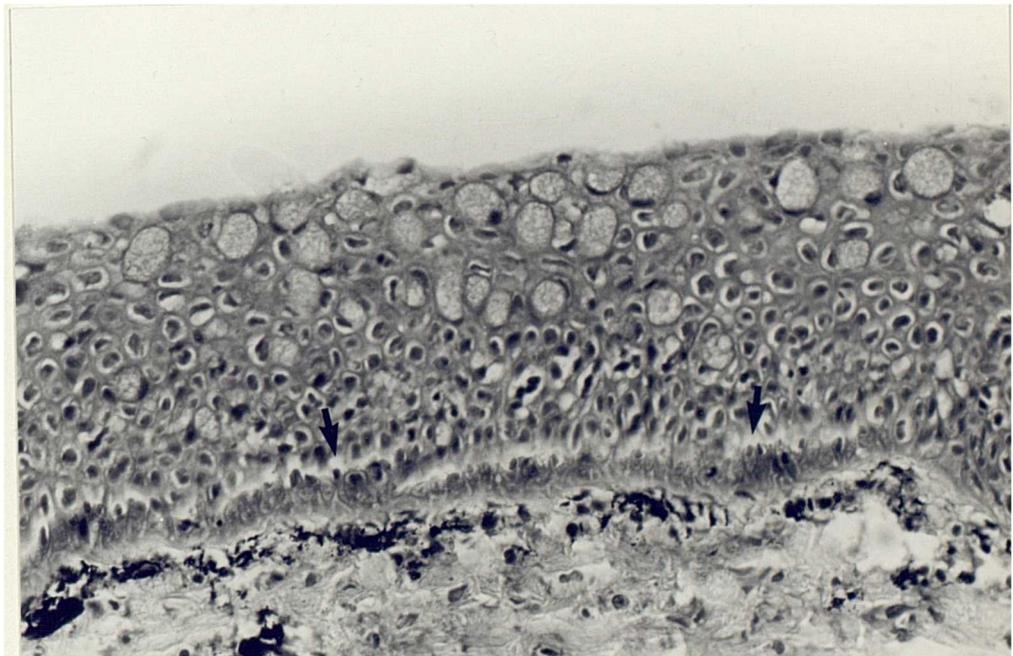
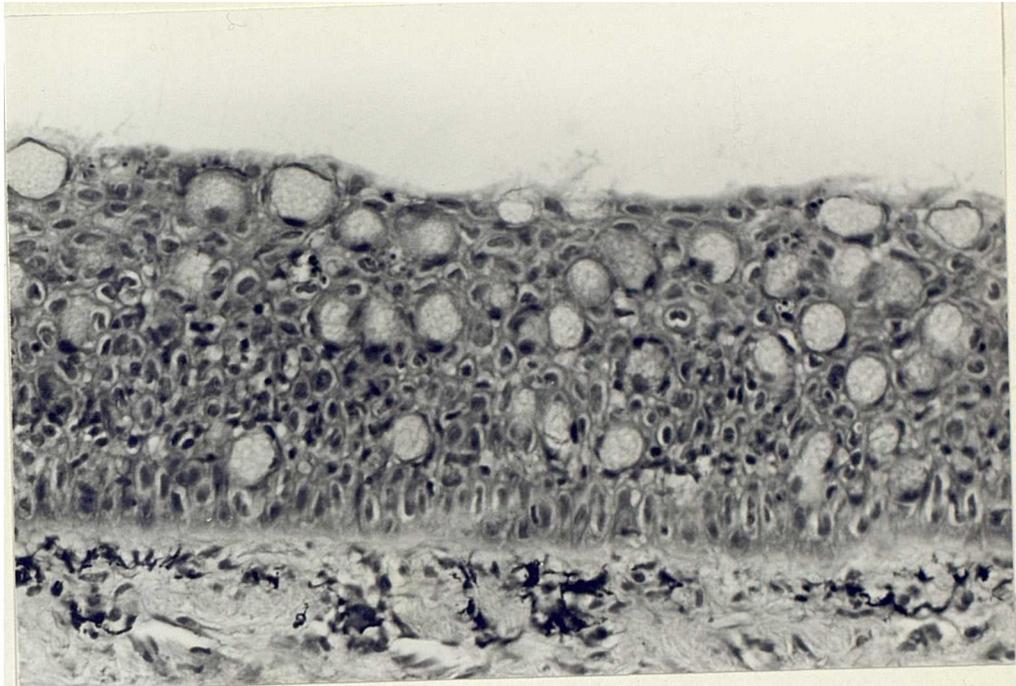
Fig 72 The effect of a cumulative dose of 324  
mJ cm<sup>-2</sup> for both rainbow trout and Atlantic  
salmon is shown. The sequential response  
is identical for both species.



Figures 73 and 74

Fig.73 324 mJ cm<sup>-2</sup>, day 2. The epidermis exhibits a moderate hyperplasia whilst the malpighian cells vary considerably in size and appearance.

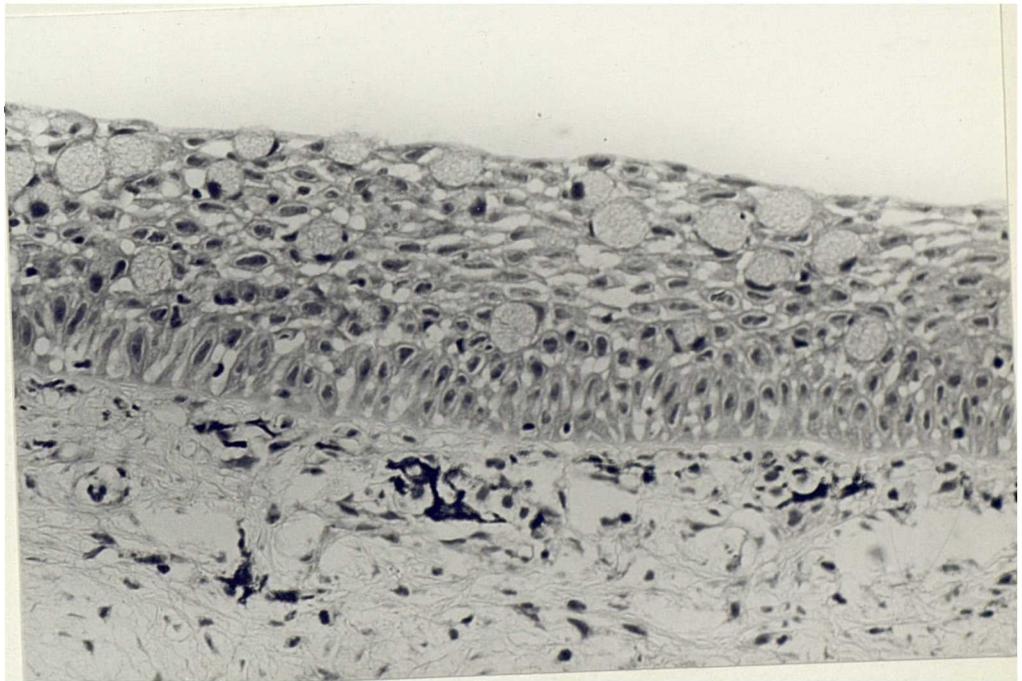
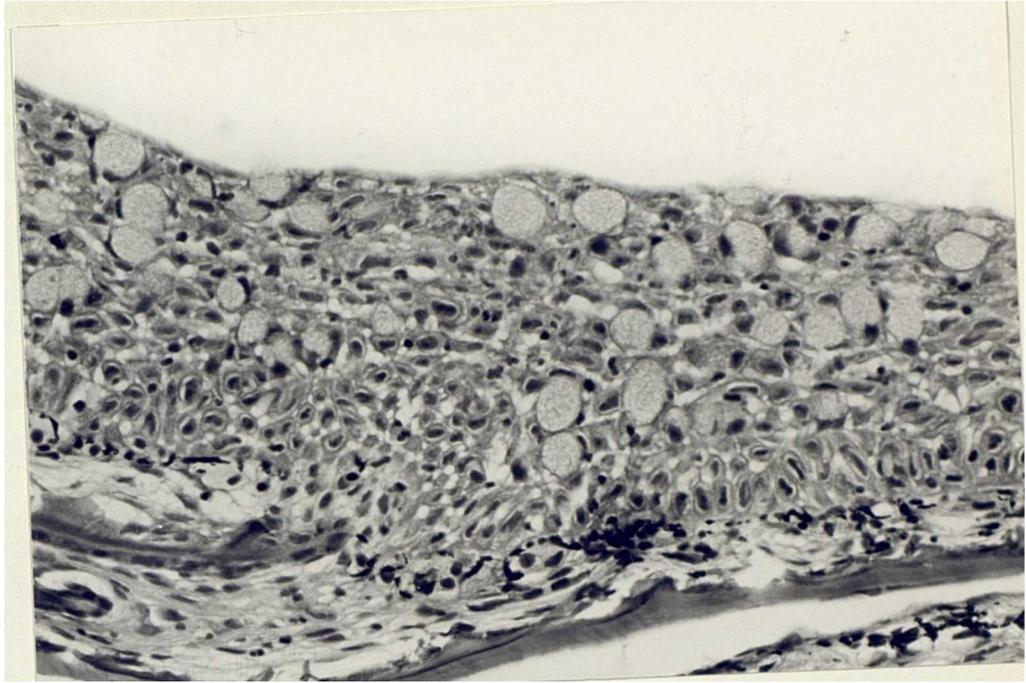
Fig 74 324 mJ cm<sup>-2</sup>, day 5. A focal separation between the cells of the basal and supra-basal layer can be detected (arrowed).



Figures 75 and 76

Fig.75 324 mJ cm<sup>-2</sup>, day 9. A generalised acantholysis is present throughout the body epidermis.

Fig.76 324 mJ cm<sup>-2</sup>, day 9. The head epidermis exhibits a similar acantholytic appearance.



Figures 77 and 78

Fig.77 324 mJ cm<sup>-2</sup>, day 13. The head epidermis has relatively few mucous cells and is markedly acantholytic. Individual malpighian cells are well-defined by their perinuclear haloes.

Fig.78 324 mJ cm<sup>-2</sup>, day 14. The body epidermis exhibits a similar response to the head epidermis with numerous pyknotic cells (arrowed) at all levels.

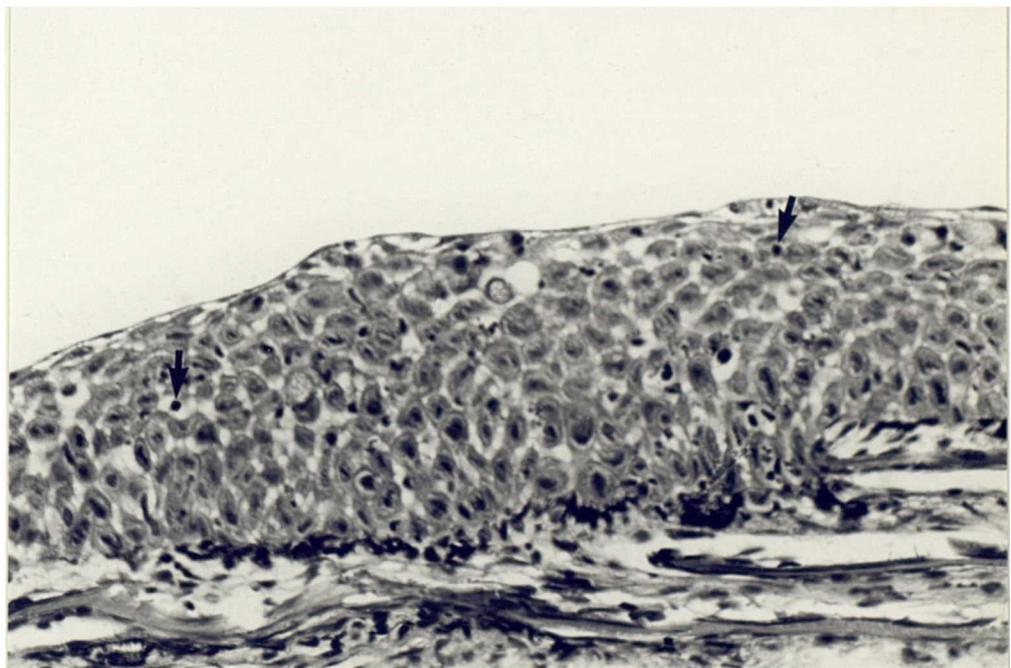
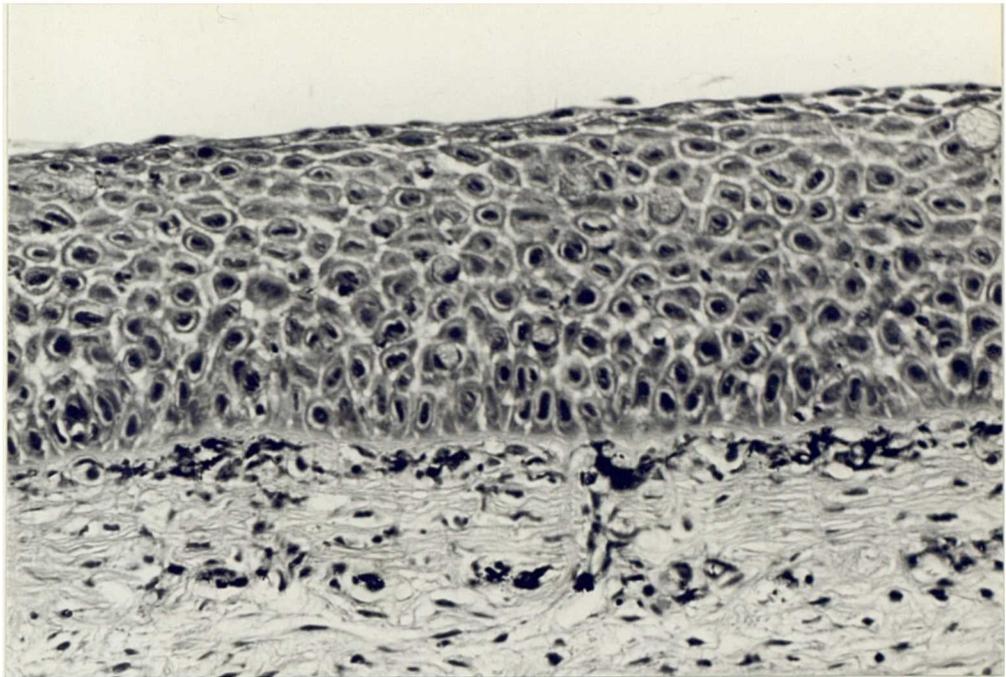
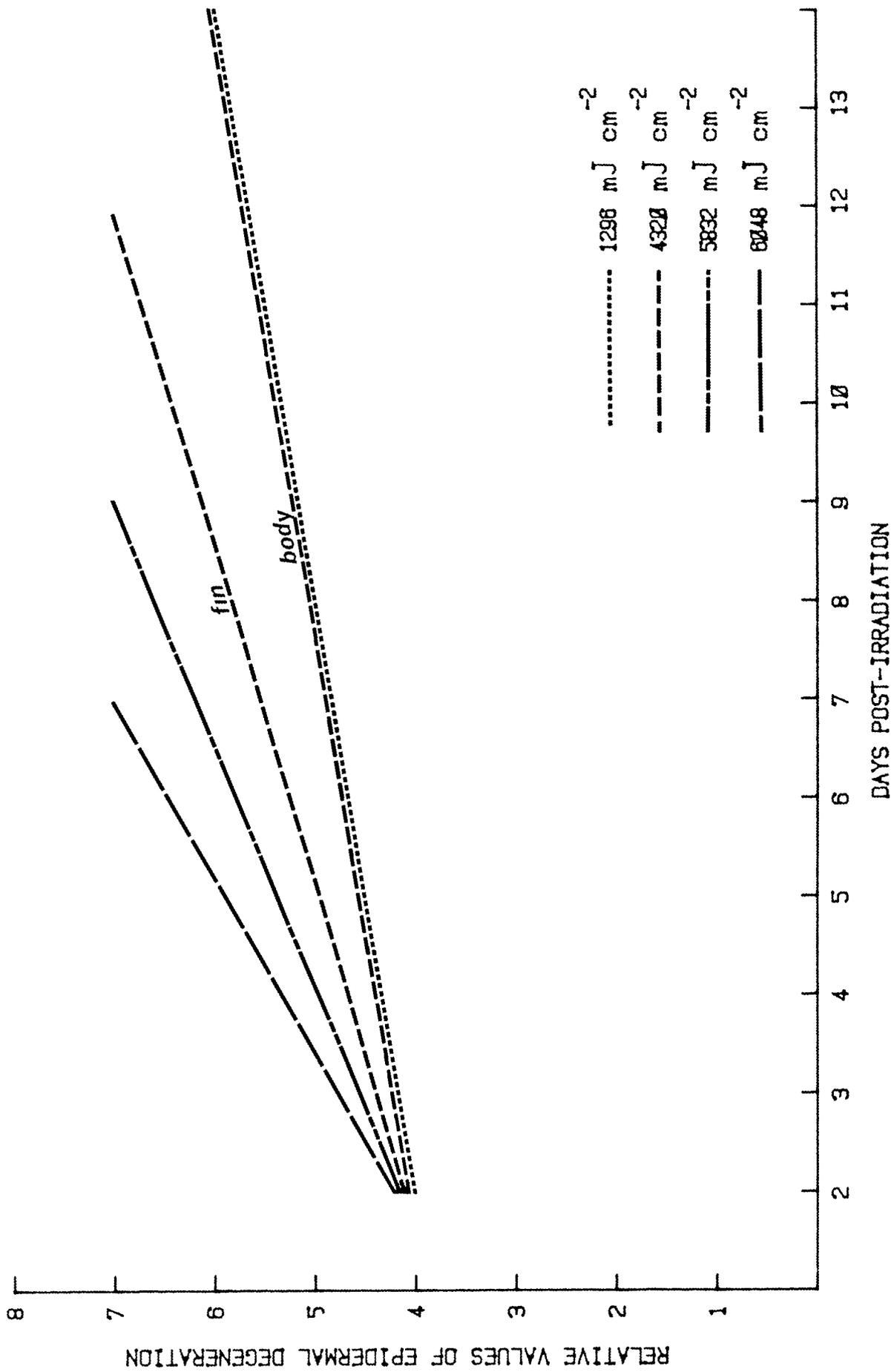


Figure 79

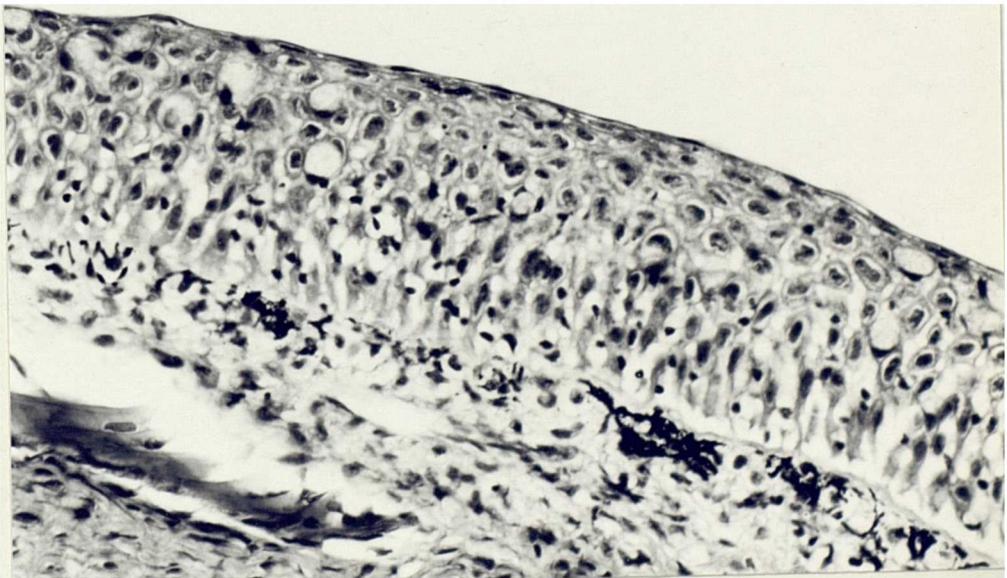
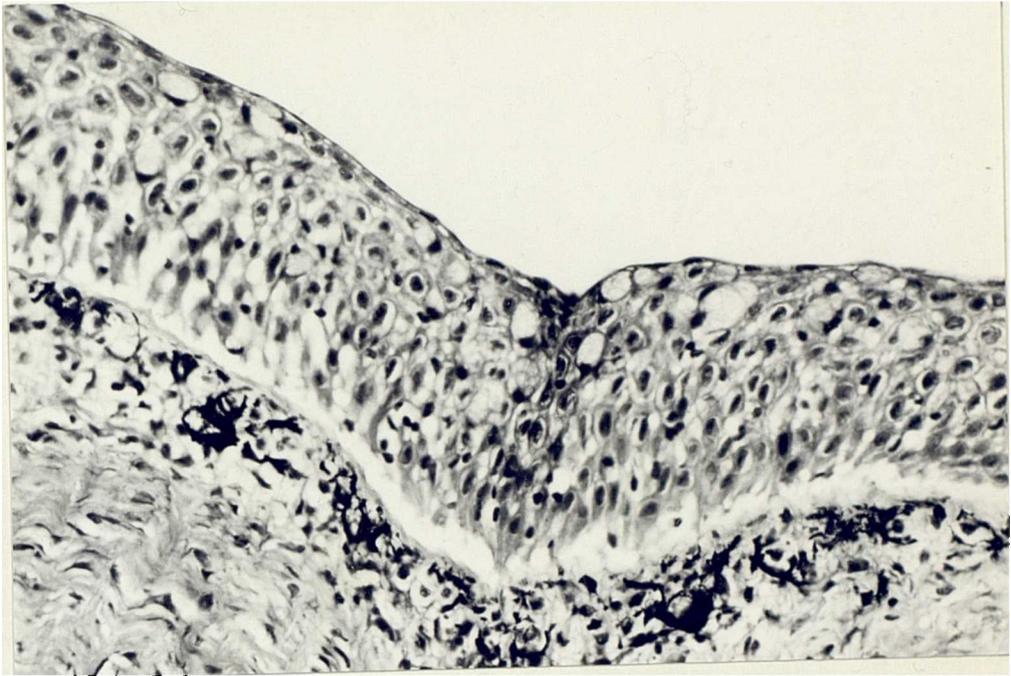
Fig.79 The effect of cumulative doses of 1296, 4320, 5832 and 6048  $\text{mJ cm}^{-2}$  filtered UV-B are shown. In both species the relative values of epidermal degeneration are the same.



Figures 80 and 81

Fig.80 1296 mJ cm<sup>-2</sup>, day 14. The head epidermis shows advanced oedema and separation from the basement membrane.

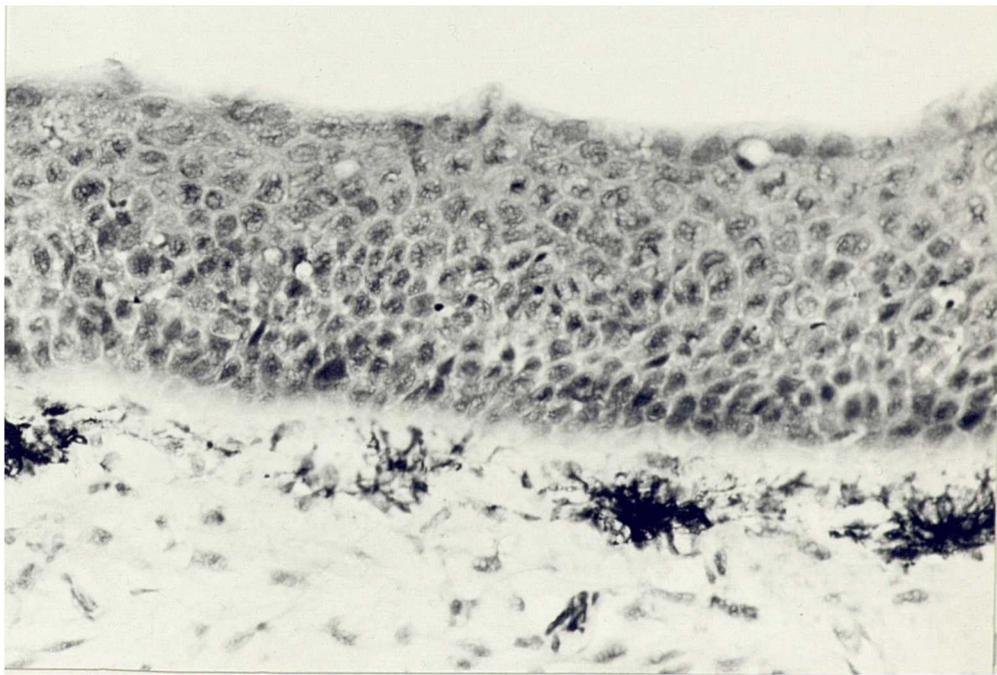
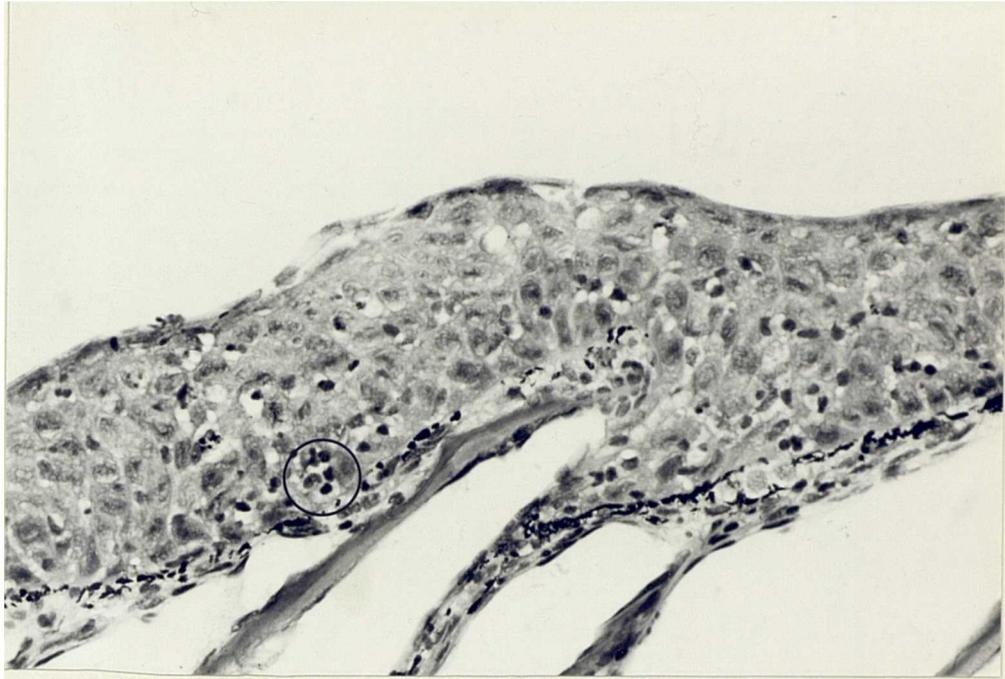
Fig.81 1296 mJ cm<sup>-2</sup>, day 14. Although similar in appearance to the head epidermis the body skin does not show the focal separation between the basal layer and the underlying basement membrane. Some leucocyte activity is apparent within the lower epidermis.



Figures 82 and 83

Fig.82 4320 mJ cm<sup>-2</sup>, day 5. The epidermal layer appears ill-defined and exhibits a foamy appearance. Within the basal zone a small foci of leucocytes can be seen (circled).

Fig.83 4320 mJ cm<sup>-2</sup>, day 6. The head epidermis shows a similar response to that seen at day 5.

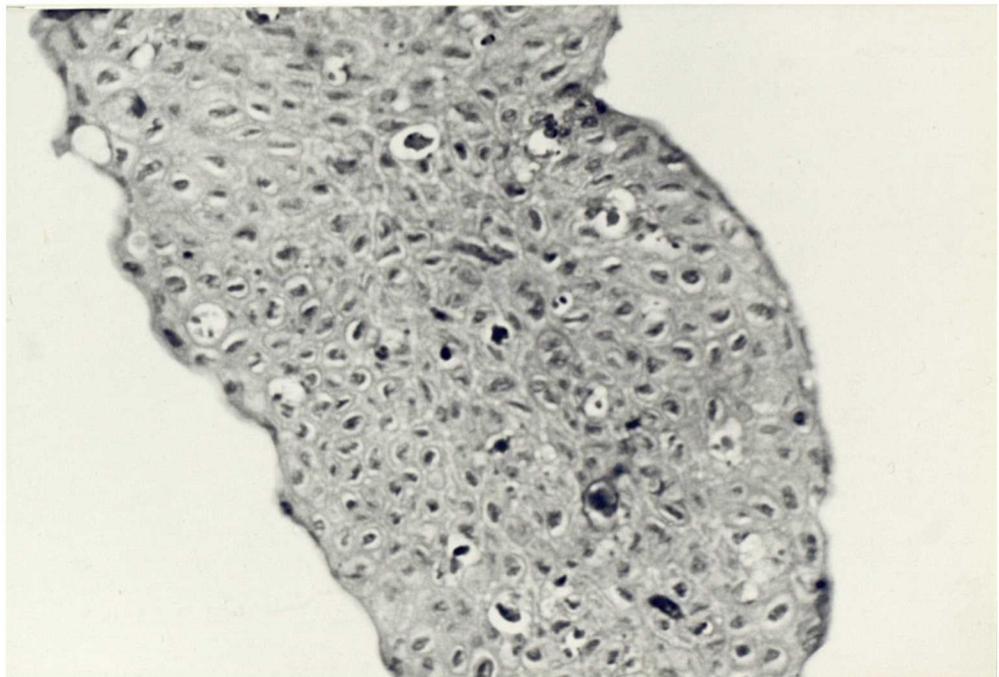
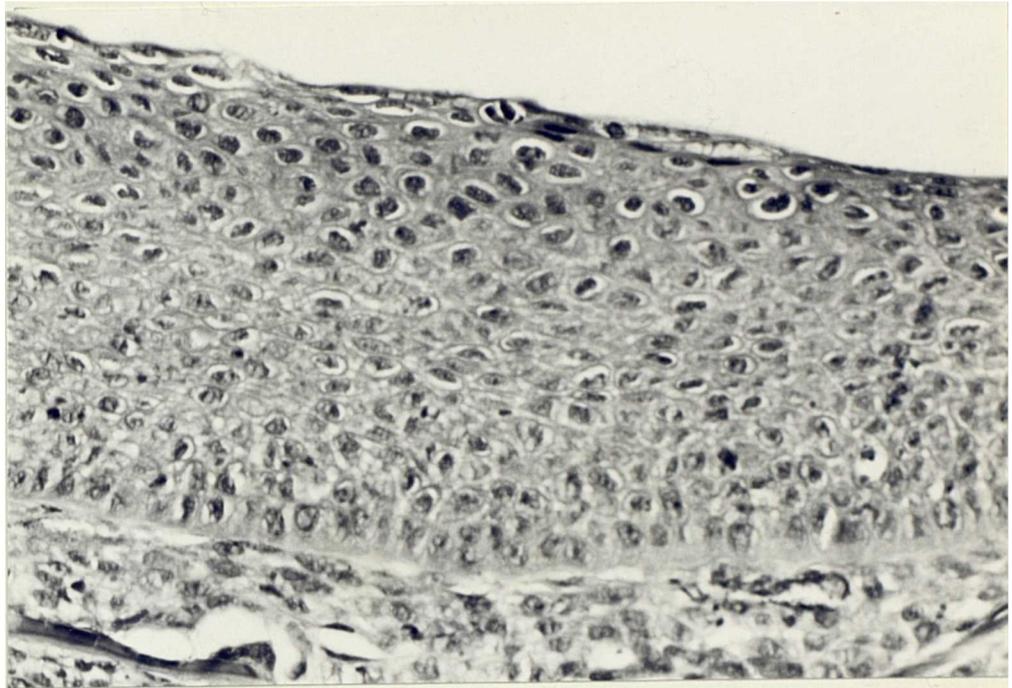


Figures 84 and 85

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Fig.84 4320 mJ cm<sup>-2</sup>, day 9. The malpighian cells now assume the characteristic perinuclear haloes associated with the UV response.

Fig.85 4320 mJ cm<sup>-2</sup>, day 9. Dorsal fin epidermis. Several *Type A* and *B* sunburn cells can be seen in association with a thickening of the epidermis.

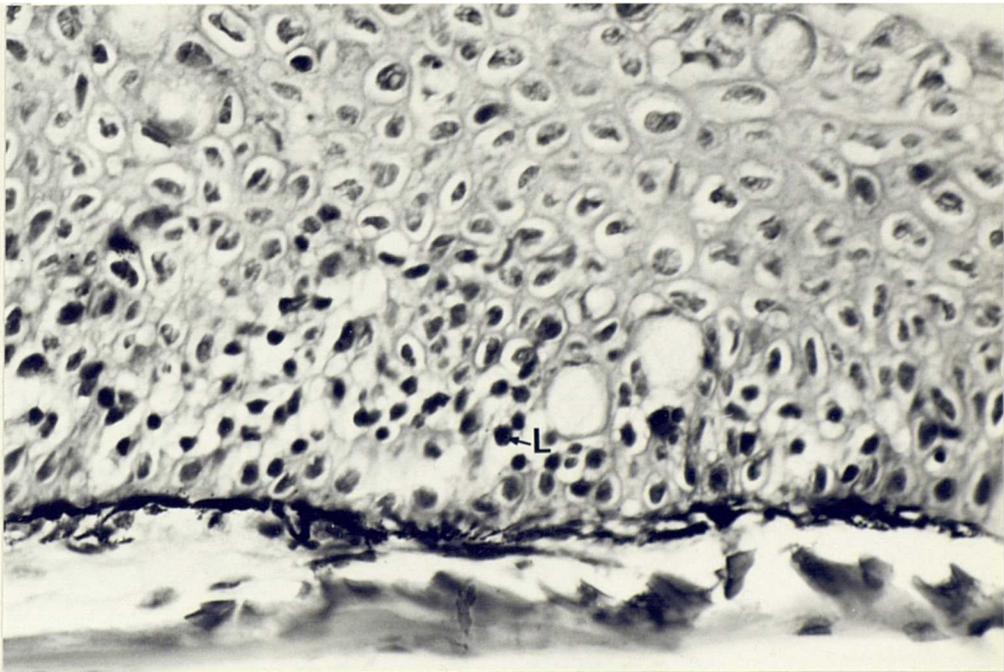


Figures 86 and 87

Fig.86 4320 mJ cm<sup>-2</sup>, day 9. Pectoral fin epidermis.  
Numerous nuclear droplets can be seen throughout the layer.

Fig.87 4320 mJ cm<sup>-2</sup>, day 11. The body epidermis exhibits a moderate leucocyte (L) activity within the basal/supra-basal zone.

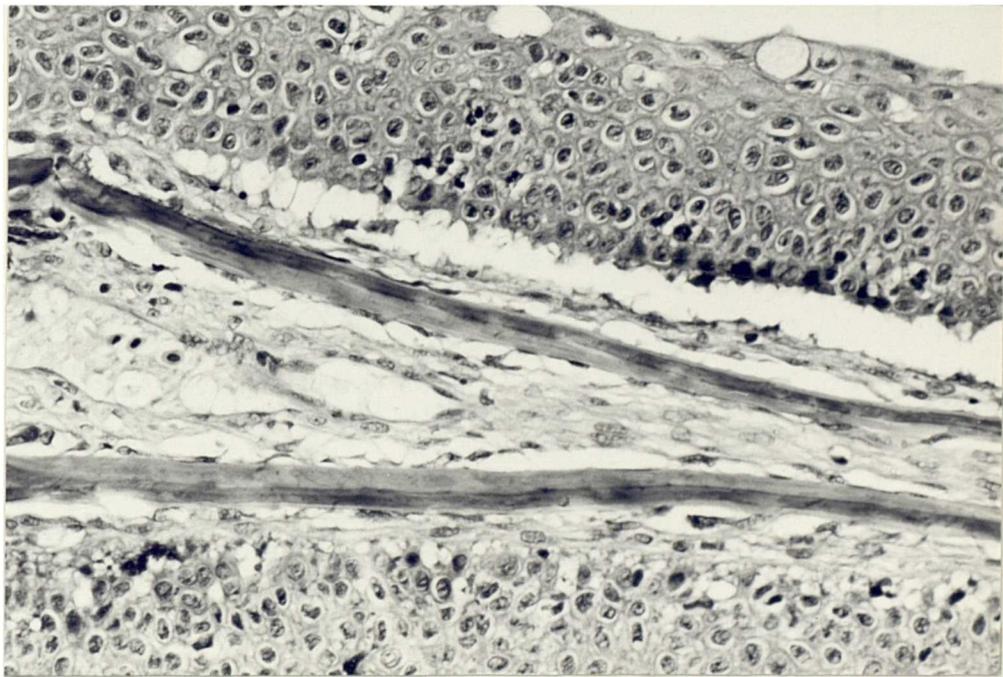
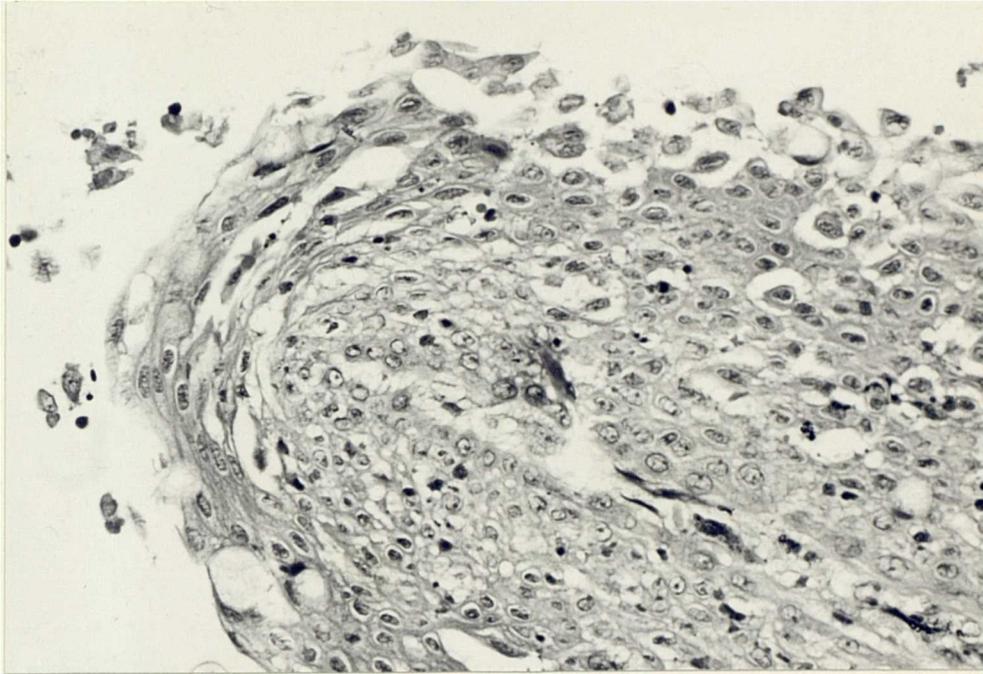
x 675



Figures 88 and 89

Fig.88 4320 mJ cm<sup>-2</sup>, day 11. Pectoral fin. The tip exhibits advanced necrosis with major sloughing of the outer zone.

Fig.89 4320 mJ cm<sup>-2</sup>, day 11. Mid-zone of pectoral fin. Note the separation of the basal layer from the basement membrane.

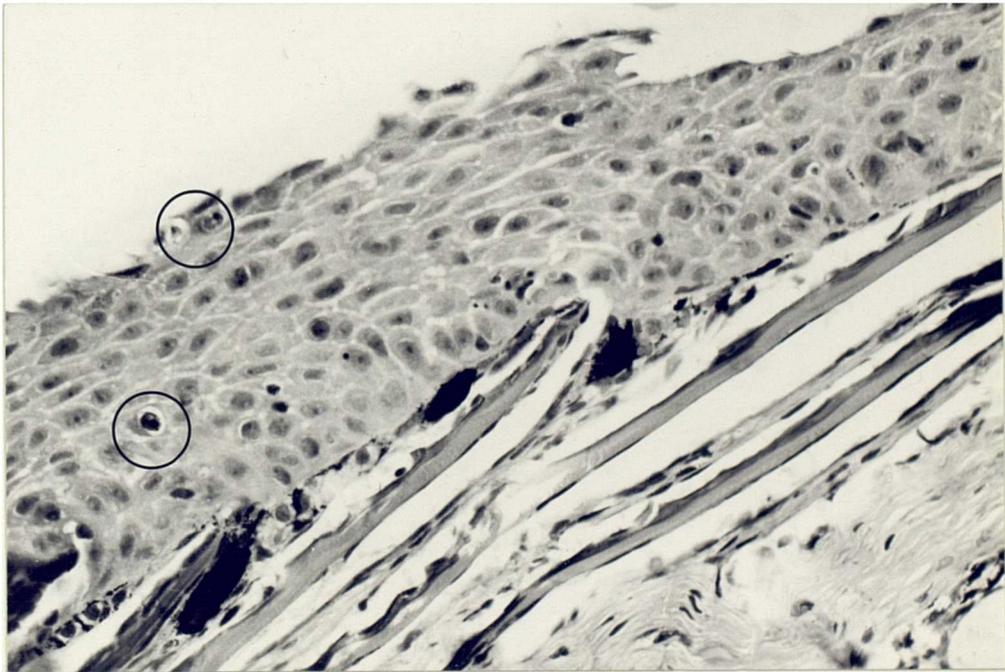
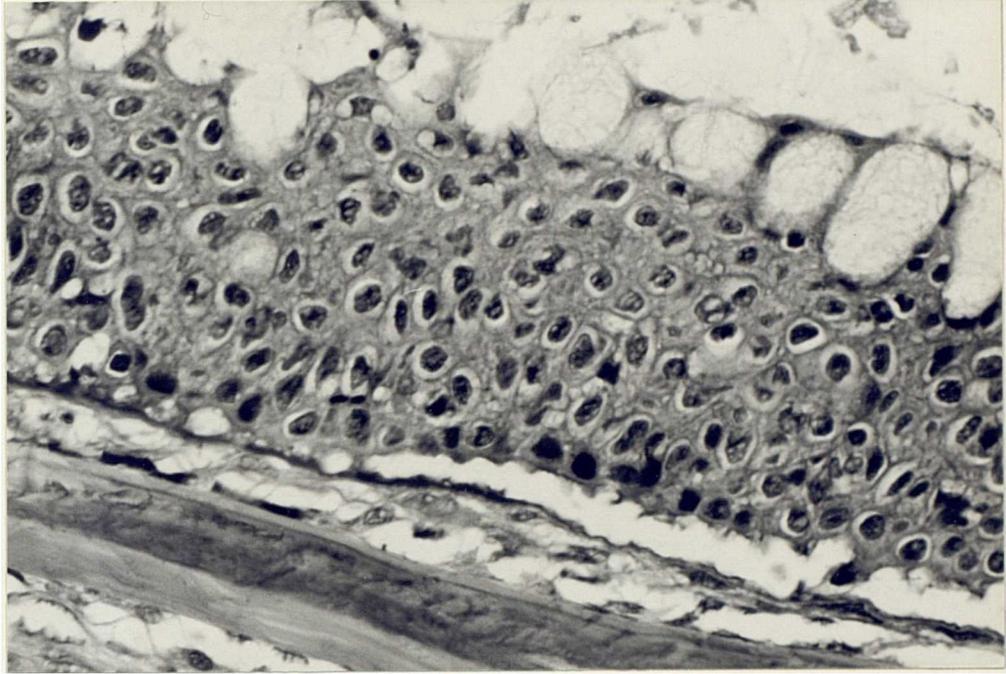


Figures 90 and 91

Fig.90 4320 mJ cm<sup>-2</sup>, day 14. The body epidermis now exhibits the separation within the basal layer observed in the fin epidermis at day 11.

x 675

Fig. 91 4320 mJ cm<sup>-2</sup>, day 5. Body skin of Atlantic salmon. The malpighian cells are pale staining often with prominent nucleoli. *Types A and B* sunburn cells can be seen (circled).



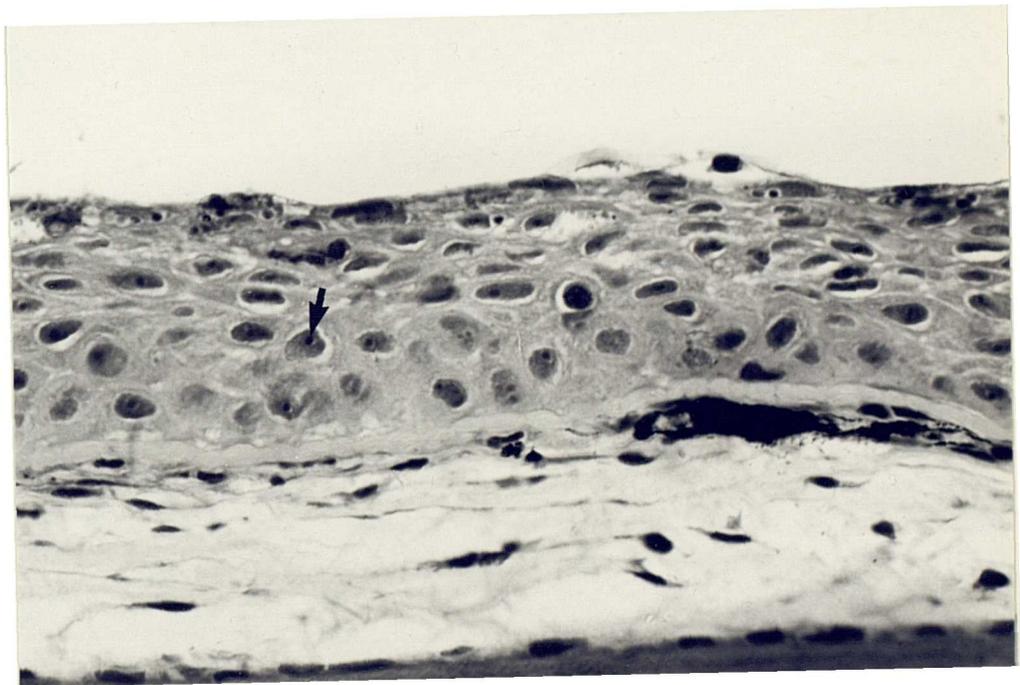
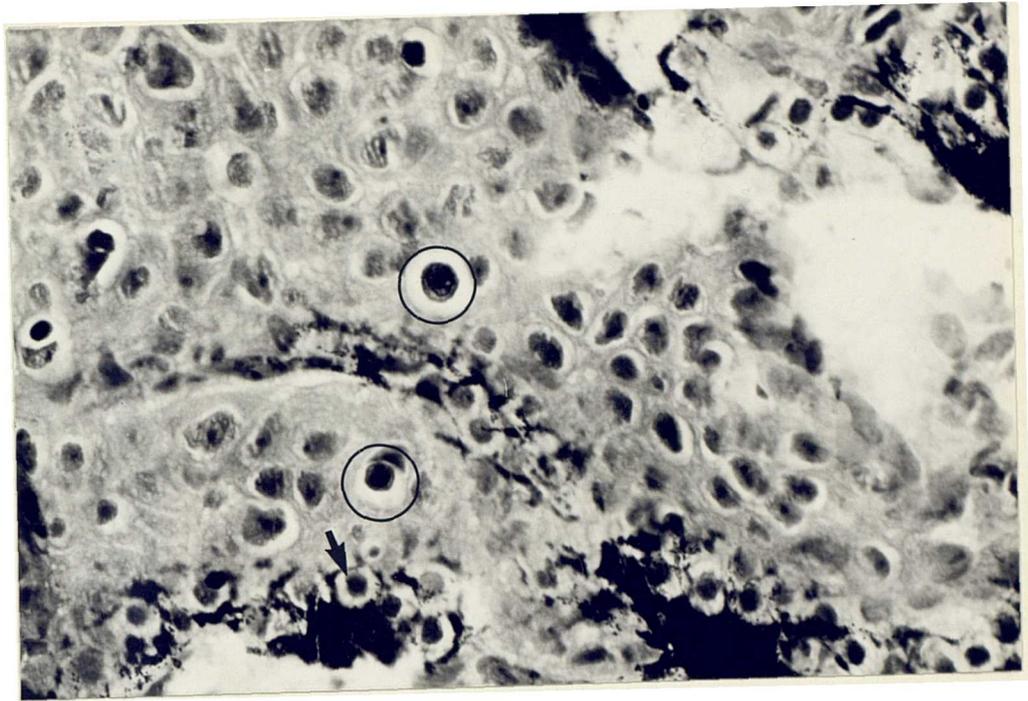
Figures 92 and 93

Fig.92 4320 mJ cm<sup>-2</sup>, day 14. Tangential section of the skin at the base of the dorsal fin. Note the presence of *Type A* sunburn cells (circled) and the swollen nuclei of the dermal melanocytes (arrowed).

x 675

Fig.93 4320 mJ cm<sup>-2</sup>, day 4. Nucleoli (arrowed) are prominent within the nuclei of many malpighian cells. Also present within the centre of the section, a *Type A* sunburn cell.

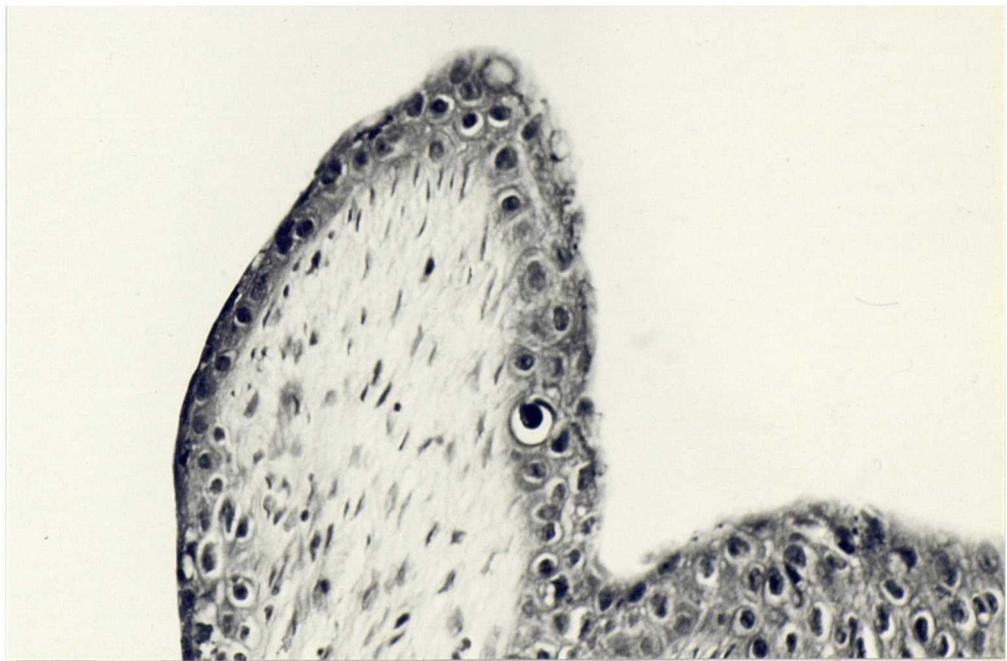
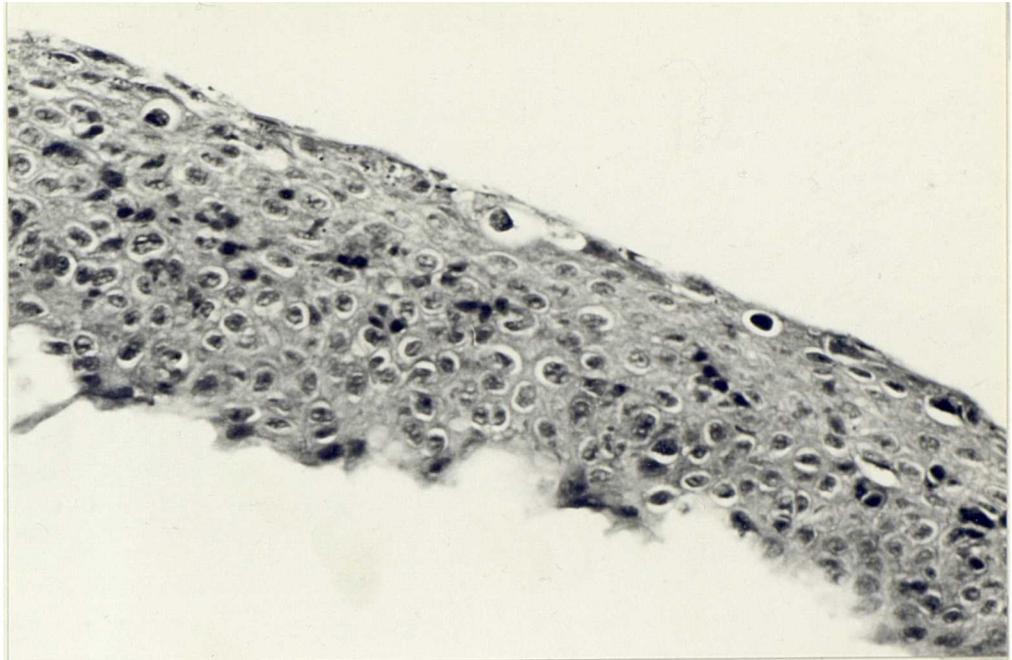
x 675



Figures 94 and 95

Fig.94 4320 mJ cm<sup>-2</sup>, day 5. Head skin. The epidermis now exhibits the foamy vacuolated response observed in rainbow trout skin at the same point on the timescale.

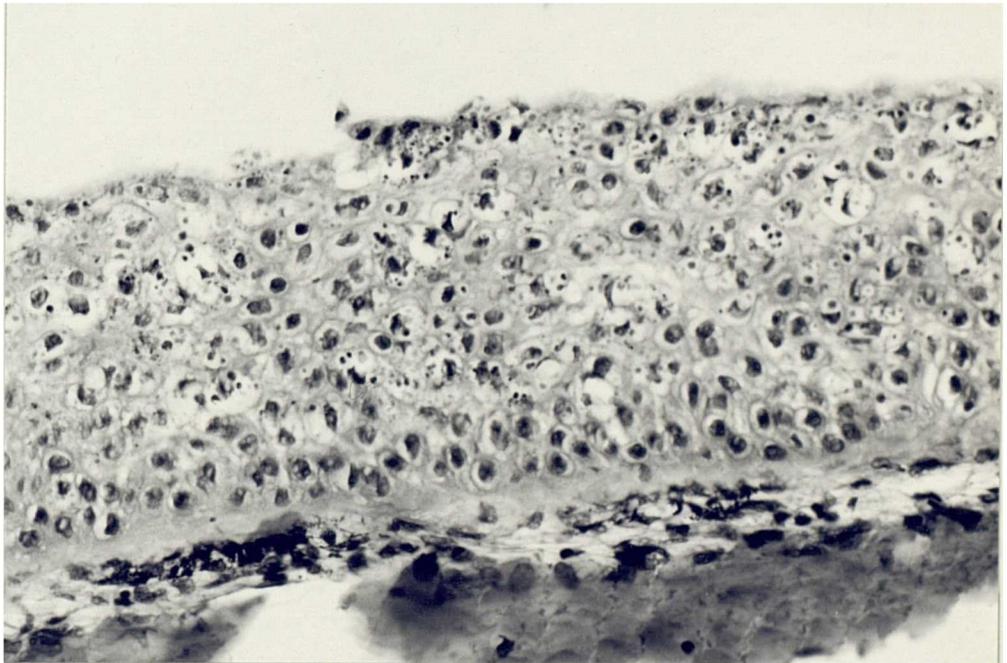
Fig.95 4320 mJ cm<sup>-2</sup>, day 6. Typical *Type A* sunburn cell within the dorsal skin at the base of the fin.



Figures 96 and 97

Fig.96 4320 mJ cm<sup>-2</sup>, day 6. Midway towards the tip of the dorsal fin. Note the hyperplastic response in association with the pale staining malpighian cells. *Type A* sunburn cells (circled) can be seen within the outermost layer.

Fig.97 4320 mJ cm<sup>-2</sup>, day 8. The epidermis now contains both types of sunburn cells and also exhibits an advanced state of necrosis.



Figures 98 and 99

Fig.98 4320 mJ cm<sup>-2</sup>, day 10. Section of pectoral fin. Note the marked differences in response between the upper and lower surfaces.

Fig 99 5832 mJ cm<sup>-2</sup>, day 12. Head skin of rainbow trout showing advanced oedema and necrosis.

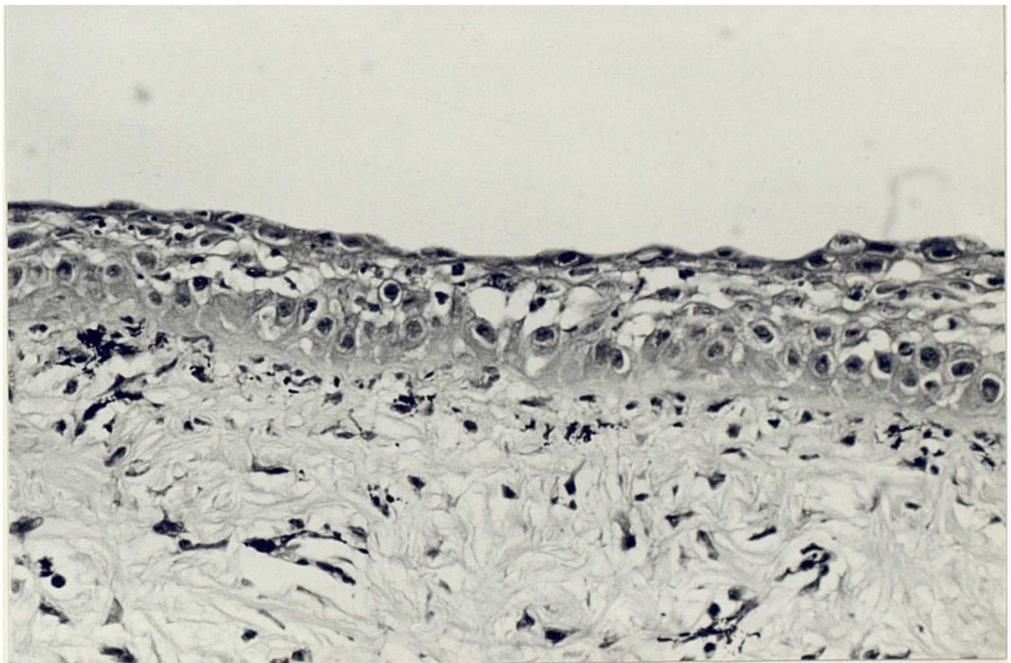
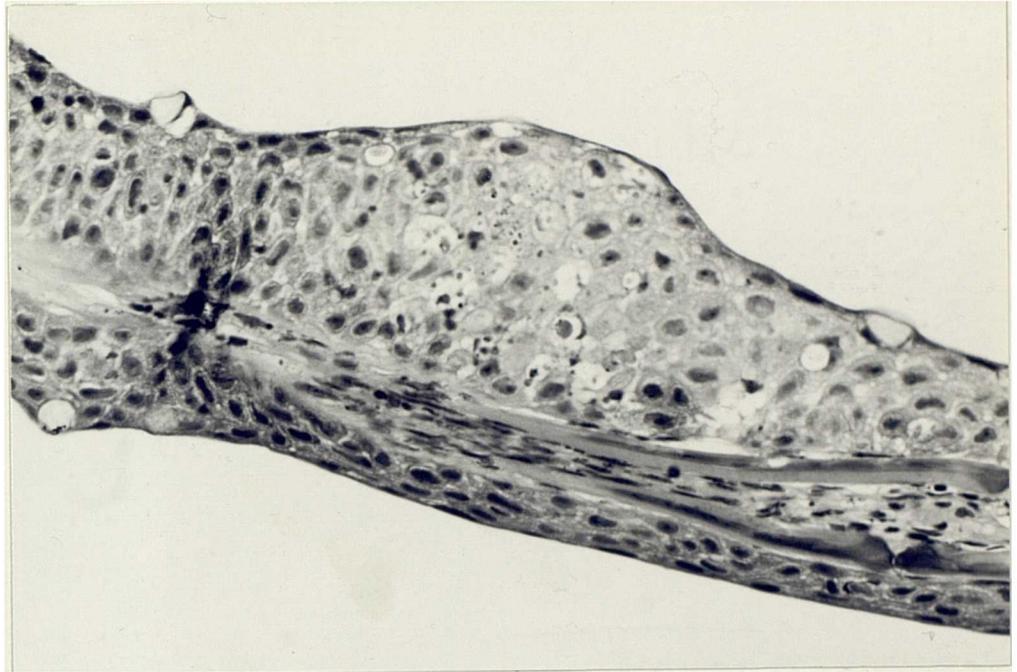
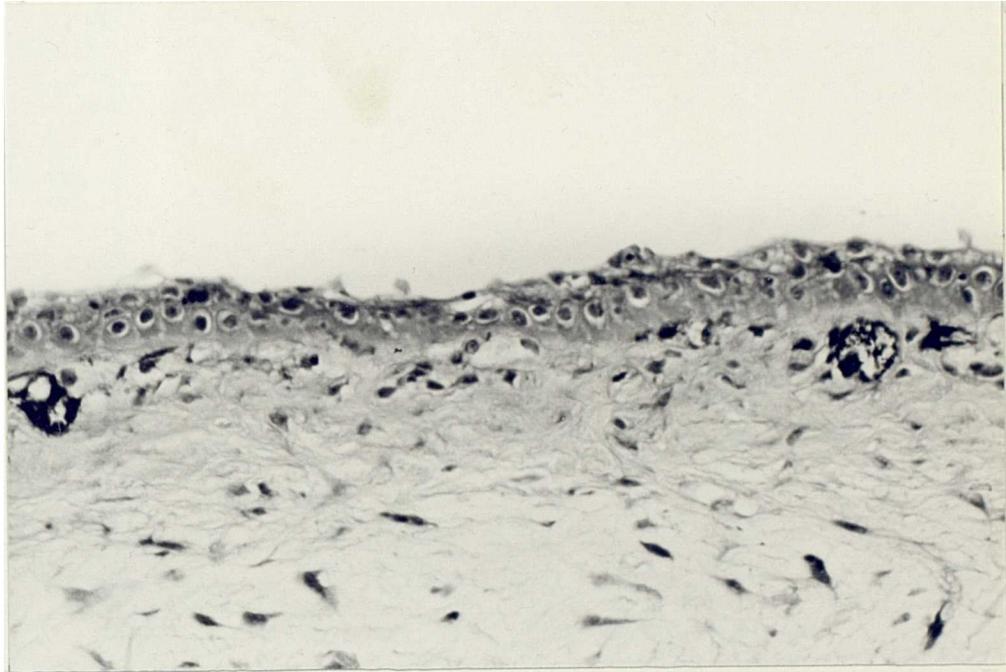


Figure 100

Fig.100 5832 mJ cm<sup>-2</sup>, day 14. Head skin of Atlantic salmon. Sloughing is well underway leaving only a thin layer of cells many of which are necrotic.



SECTION V

THE IMPACT OF SIMULATED 'SOLAR' UV  
RADIATION UPON THE SKIN OF RAINBOW TROUT

*Introduction*

From the data presented in Section IV the higher tolerance threshold of Atlantic salmon and rainbow trout to ultraviolet radiation, by comparison with that of two species of flatfish, has been demonstrated.

Throughout these experiments gross observations of the sequential radiation response in plaice revealed a progressive fading of the natural colouration over the dorsum as the lesion developed. By such time as epidermal erosion was complete, radiation damage was well defined by the roughened texture of the underlying collagenous layer and was usually delineated by a narrow margin of slightly darker tissue, giving the impression of a 'water mark' upon the skin surface. Although these findings were limited principally to plaice, they were also seen to a lesser extent in turbot skin which, following irradiation, more generally exhibited a diffuse greyish appearance over the entire surface with an associated loss of natural colouration.

Neither salmonid species, by contrast, showed much evidence of any macroscopic change in either skin texture or colouration over the dorsum. Towards the termination of the 14-day experiments, however, a small number of fish, upon close inspection, exhibited a barely detectable focal grey/

grey patch of necrosis often limited in size to a pin point. Sometimes the response occurred around the base of the dorsal fin but was more often seen anterior to the dorsal fin roughly midway towards the crown of the head.

In aquaculture such minor changes would almost certainly go unnoticed until the response had progressed to a point where the lesion, upon cursory inspection, became apparent to the naked eye. By this time a secondary infection might well have occurred with a subsequent rapid breakdown in epidermal integrity.

In an attempt to reproduce the effect of prolonged exposure to sunlight a further experiment was conducted over a 12-week period using the apparatus described in Section II (Regime B). For this experiment the irradiation array contained six tubes emitting in the visible spectrum, four in the UV-A and two in the UV-B. By linking the tubes in series to automatic time switches it was then possible to produce a light mix which provided an approximate simulation of natural sunlight. Using the data of Diffey (1982) the UV-A tubes were programmed to provide a daily fluence of  $40 \text{ Wm}^{-2}$  over a five hour period and the UV-B component a daily fluence of  $2 \text{ Wm}^{-2}$  over a four hour period, both sequences being centred upon midday. These values represented the anticipated irradiance impinging upon an unshaded horizontal surface at noon during the summer in the U.K. The remaining six tubes provided/

provided sufficient light in the visible spectrum for maximum photorepair to occur (Hunter et al , 1981) and were 'stepped in' over a 12 hour period overlapping the UV irradiance equilaterally, followed by 12 hours' darkness thus also providing an approximate diurnal rhythm. A schematic diagram of the irradiation regime is shown in Fig.101. Twenty rainbow trout averaging 20 cm in length were irradiated daily throughout the duration of the experiment and any changes in their appearance or behaviour noted.

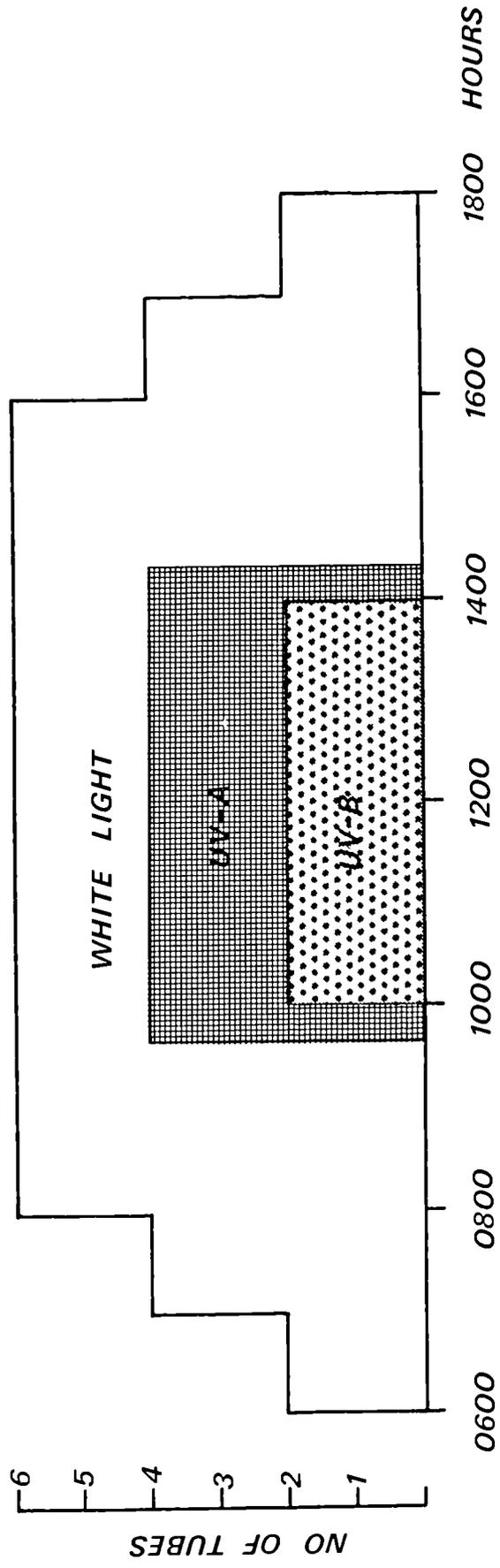
### 9.1 Results

The most striking feature was the variability in response of individual fish to radiation. By the end of the third week four fish exhibited an intense darkening of the dorsal skin along the entire length of the fish, when compared to the remainder which appeared to retain their normal colouration. All continued to feed voraciously. By week six the first visible evidence of acute radiation damage was apparent in two of those fish which first exhibited a radiation reaction. In general the dorsum now took on a greyish hue overlying the darkened areas giving an almost velvet appearance to the skin. There was, however, no direct evidence of epidermal sloughing.

Of/

Figure 101

Fig.101 Schematic representation of the irradiation regime used in the simulated 'solar' UV experiment.



Of the remaining fish a further eight by this time had begun to show signs of darkening. Throughout this period those fish most affected tended to remain low in the water column often swimming in short bursts towards the surface then subsiding towards the base of the tank and made little effort to feed. At this point one of the worst affected was removed, photographed (Fig.102) and sampled for histology.

By week ten the most susceptible fish, upon close examination, revealed some sloughing of the thickened epidermis primarily around the base of the dorsal fin (Fig.103) and also over the crown of the head.

At the termination of the experiment 14 out of the remaining 19 fish showed severe signs of skin trauma ranging from a frank sloughing (Fig.104) to a chronic hyperplasia in association with a patchy sloughing of specific areas (Fig.105). Although the major response was noted over the immediate dorsum a bullous reaction within the flank skin was also noted (Fig.106). Upon close inspection the dorsum of the five remaining fish, apparently unaffected by radiation, showed a similar bullous effect but there was no major thickening nor evidence of necrosis. This apparent resilience remains unresolved but suggests perhaps an enhanced photoprotective factor of/

of perhaps a genetic rather than species specific origin.

In section radiation damage was most dramatic. Over the crown of the head the lesion exhibited a massive acantholytic hyperplasia producing a four-fold increase in epidermal thickness (Fig.107). Perinuclear haloes were very pronounced throughout the majority of the layer, each cell being clearly delineated. At the base of the epidermis, however, a layer of cells 3-4 deep gave a pale eosinophilic staining reaction, the nuclear component scarcely distinguishable from the surrounding cytoplasm (Fig.108). By comparison the immediately adjacent layer exhibited densely staining nuclei. Mucous cells first appeared within the middle layer, sporadic in distribution, their contents showing an affinity for basic dyes. Towards the surface, the outer zone exhibited a rapid transition from the densely staining cells beneath to a layer of cells with large pale staining nuclei (Fig.109).

Within the dermis the dendritic processes of the melanocytes were grossly distended, their melanosomes often clearly evident as individual granules. Below, the fibres within the collagenous matrix of the *stratum compactum* stained intensely with some shrinkage of the bundles apparent, their associated fibroblasts/

fibroblasts often adopting bizarre forms varying considerably in morphology (Fig.110).

Histological examination of the body skin over the dorsum showed the lesion to be essentially similar to that of the head, although it could be more easily categorised into five discrete layers (Fig.111). There were also subtle differences apparent primarily above the supra-basal zone where the malpighian cells tended to lose their closely defined organisation, appearing necrotic and often breaking down into nuclear droplets. On occasion a *Type B* sunburn cell could be seen within this zone.

Above this layer the general appearance was similar to that of the head epidermis although the malpighian cell nuclei often exhibited bizarre forms. It was within this layer that the mucous cells first appeared, their distribution similar to that seen on the head. The upper layer contained numerous mucous cells whilst the surrounding malpighian cells were large by comparison with those below, their nuclei showing a progressive loss of staining towards the surface. The outermost layer, some 2-3 cells in thickness, had the foamy vacuolated appearance seen in the skin during a number of the short term UV experiments. Occasionally the outer surface (Fig.112) was invested by an intensely eosinophilic staining layer of amorphous/

amorphous material in close proximity to the surface cells. This was generally assumed to be an excess of cuticular secretion from the irradiated surface cells.

Upper flank skin also showed radiation damage. In these areas the epidermis appeared in section as a thickened layer of well-defined large pale staining cells. Throughout, numerous types of sunburn cells could be seen (Fig.113) confined mainly to the upper half of the epidermal layer.

Irradiated dorsal fin skin varied considerably in its appearance. Often there was a discrete separation of the epidermis from the basement membrane (Fig.114). The epidermis stained weakly and occasional sunburn cells could be seen. In other areas the layer assumed the thickness associated with the head epidermis giving a similar multi-layered appearance. Dermal necrosis was also apparent with a massive dilatation of the blood vessels a frequent occurrence.

Those fish appearing less affected by irradiation nonetheless showed changes within their epidermis also. In general, these were limited to a slight oedema within the supra-basal zone immediately above the columnar basal cells (Fig.115). Within the same section, however, hyperplasia was also evident (Fig. 116) with *Type B* sunburn cells present.

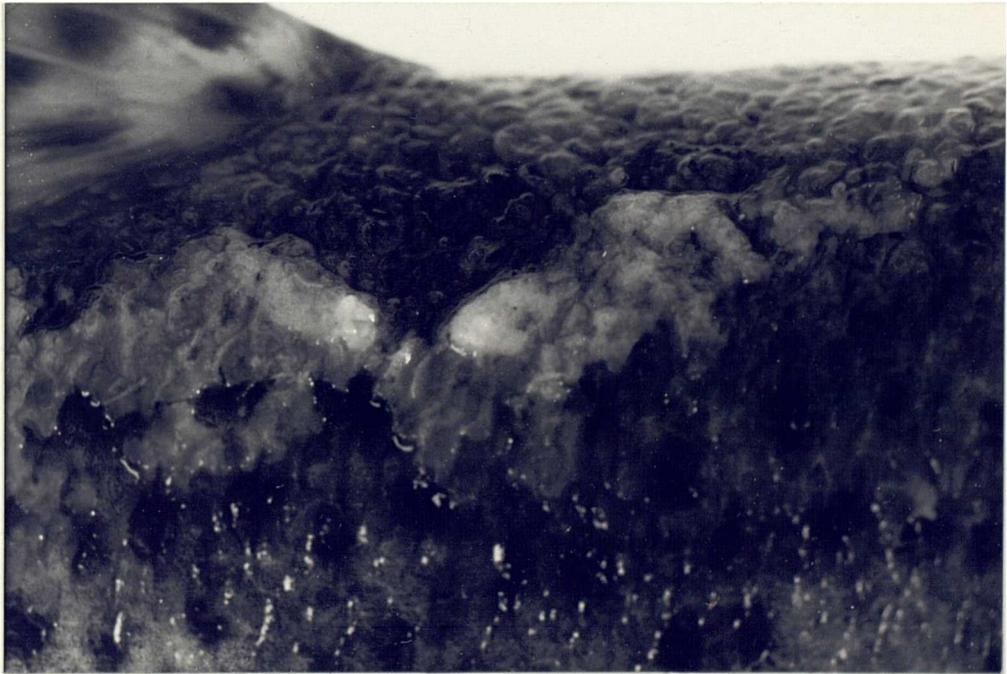
Upon/

Upon handling the skin was found to be extremely friable and often in section appeared only as an irregular mass of degenerating cells (Fig.117). As the lesion progressed it was found that in many instances the scales remained *in situ* (Fig.118) although complete erosion of the epidermis had occurred. By this stage the underlying dermis usually lacked differentiation and the fibroblasts were destroyed. Microscopically the most conspicuous feature of the open lesion was the laminated appearance of the *stratum compactum* (Fig.119) which no longer retained its normal form whilst within its upper confines there was considerable local oedema. Examination of the later stages of the open lesion showed a high bacterial loading. The infection was not limited to the surface but often appeared as localised microabscesses within the *stratum compactum* (Fig.120).

Figures 102 and 103

Fig.102 Dorsal skin of rainbow trout following six weeks' irradiation. Note the generalised blistering of the skin.

Fig.103 Epidermal sloughing around the base of the dorsal fin.



Figures 104 and 105

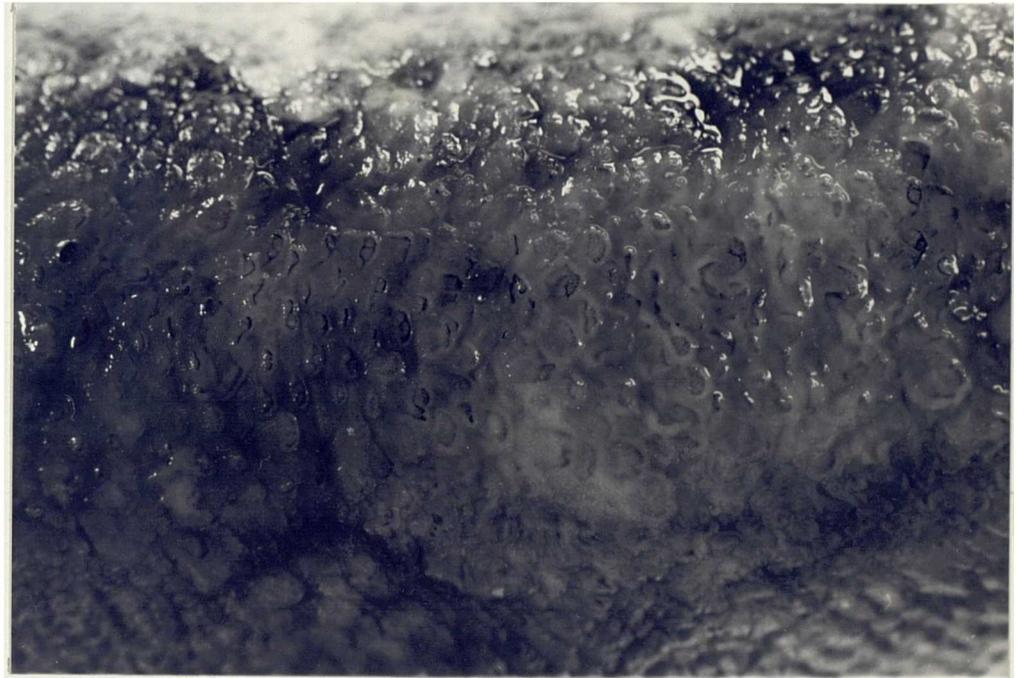
Fig.104 Complete sloughing of the epidermis has occurred over the dorsum.

Fig 105 Acute hyperplasia and sloughing following 12 weeks' irradiation. The epidermis gives the appearance of psoriasis seen in human skin.



Figure 106

- Fig.106 Upper flank skin. Note the swelling of the epidermis culminating in numerous serous bullae.



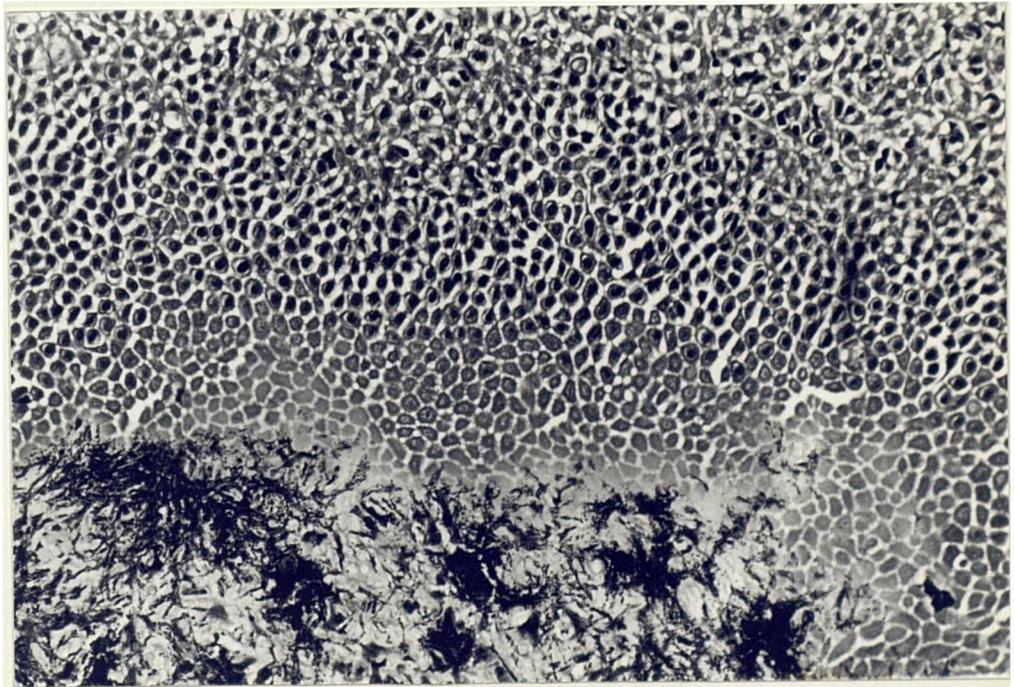
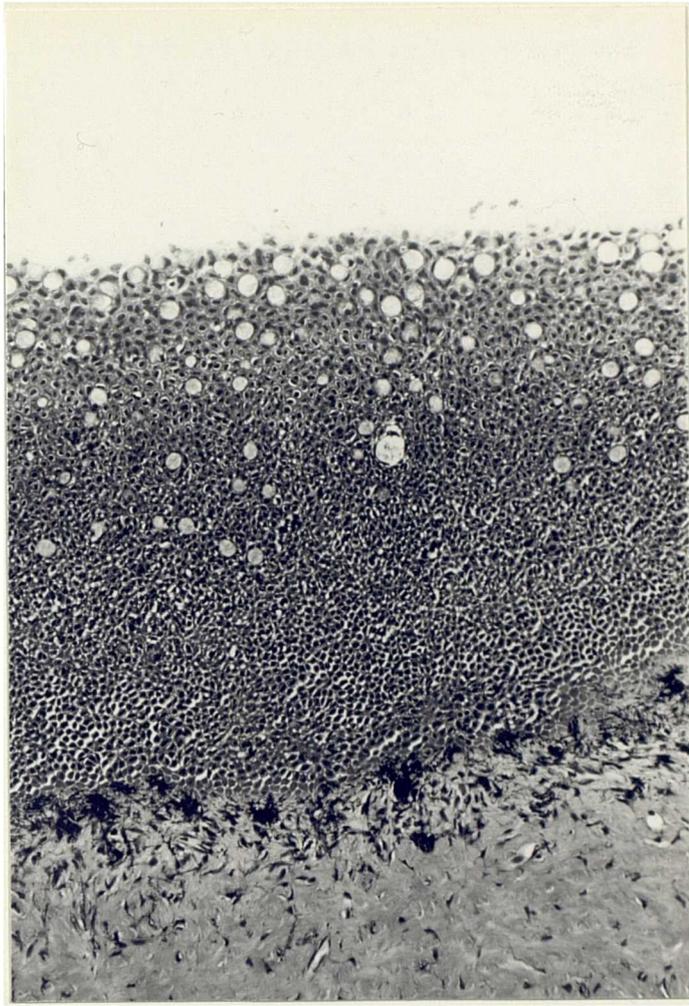
Figures 107 and 108

Fig.107 Low power photomicrograph showing the massive increase in epidermal thickness following prolonged irradiation.

x 160

Fig.108 Basal and supra-basal epidermis. A severe acantholysis is evident in association with a notable lack of staining propensity within the basal layer.

x 320

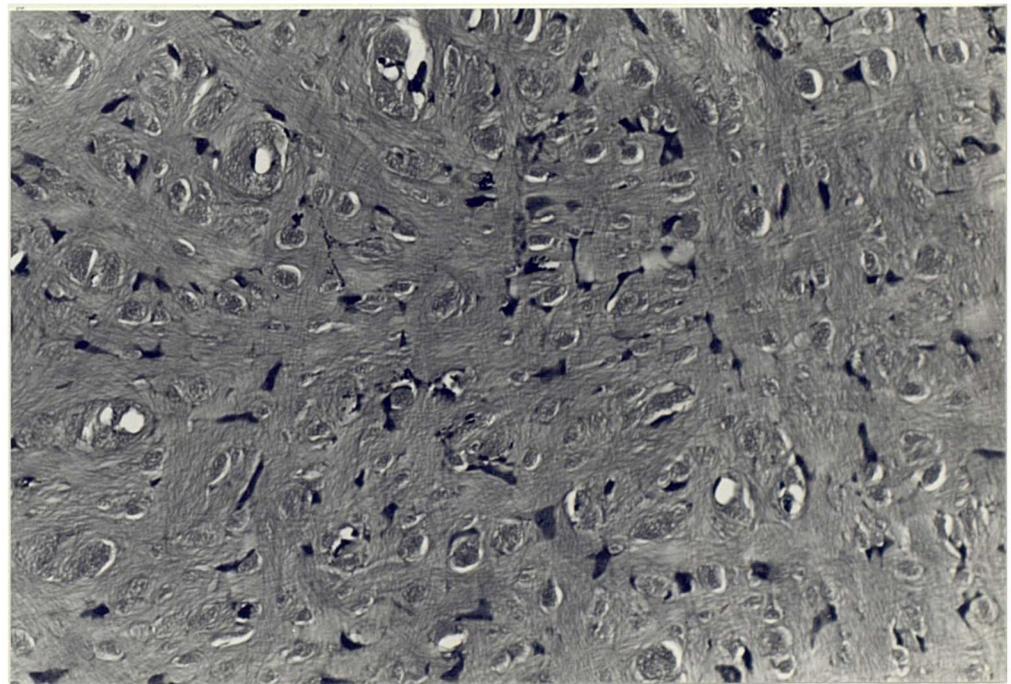
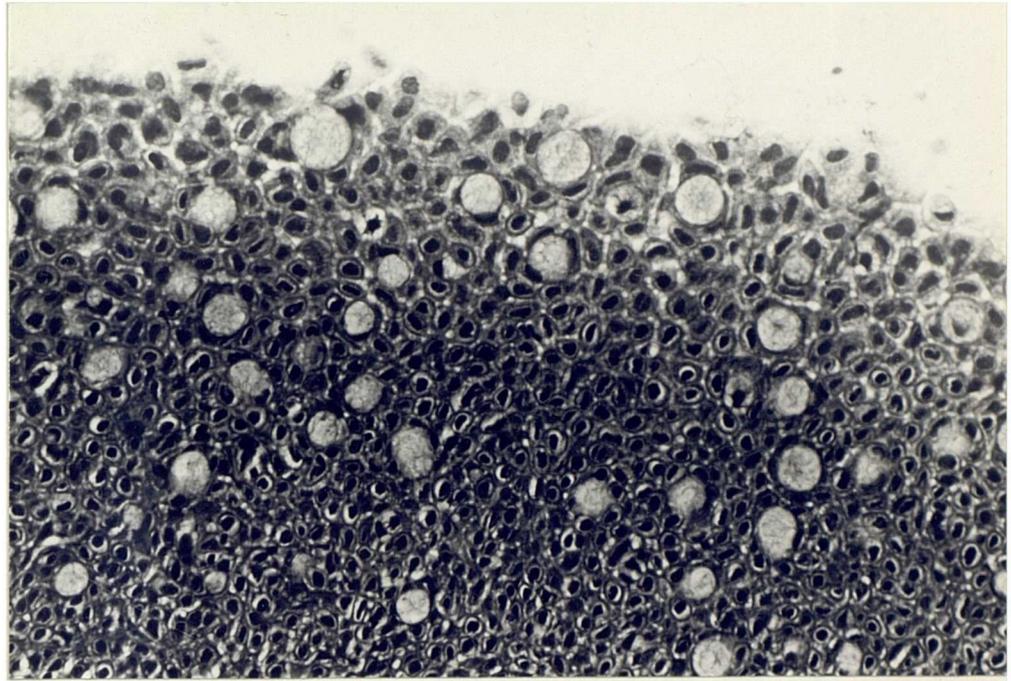


Figures 109 and 110

Fig.109 The outer layer of irradiated epidermis.  
The uppermost malpighian cells are enlarged  
and pale staining by comparison with those  
below.

x 320.

Fig.110 Section of the *stratum compactum*. Note the  
shrinkage of the collagen bundles and the  
necrotic appearance of the fibroblasts.



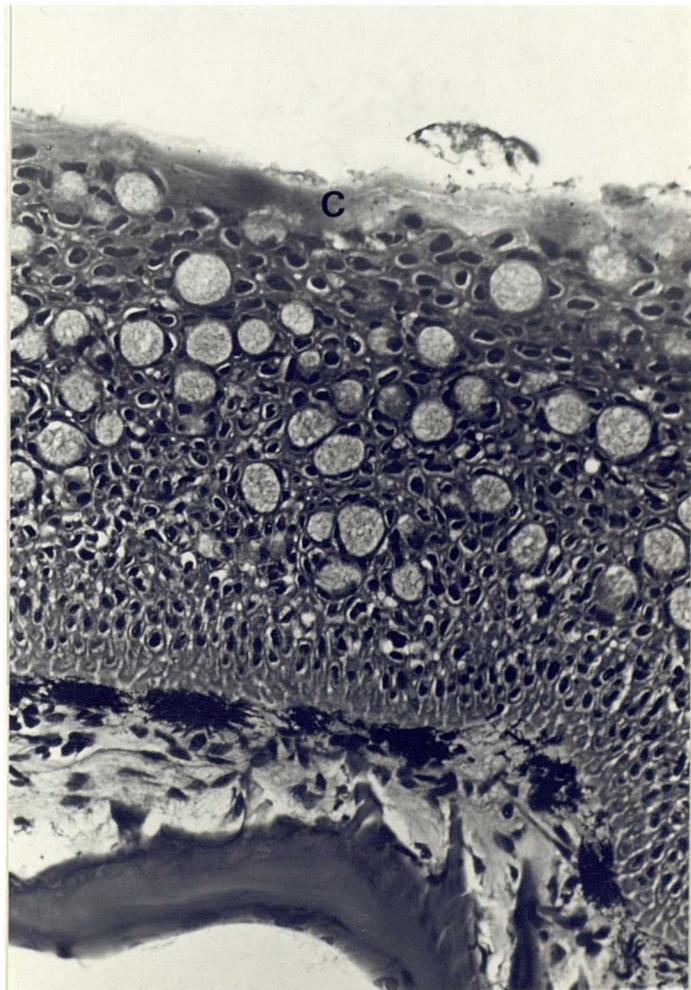
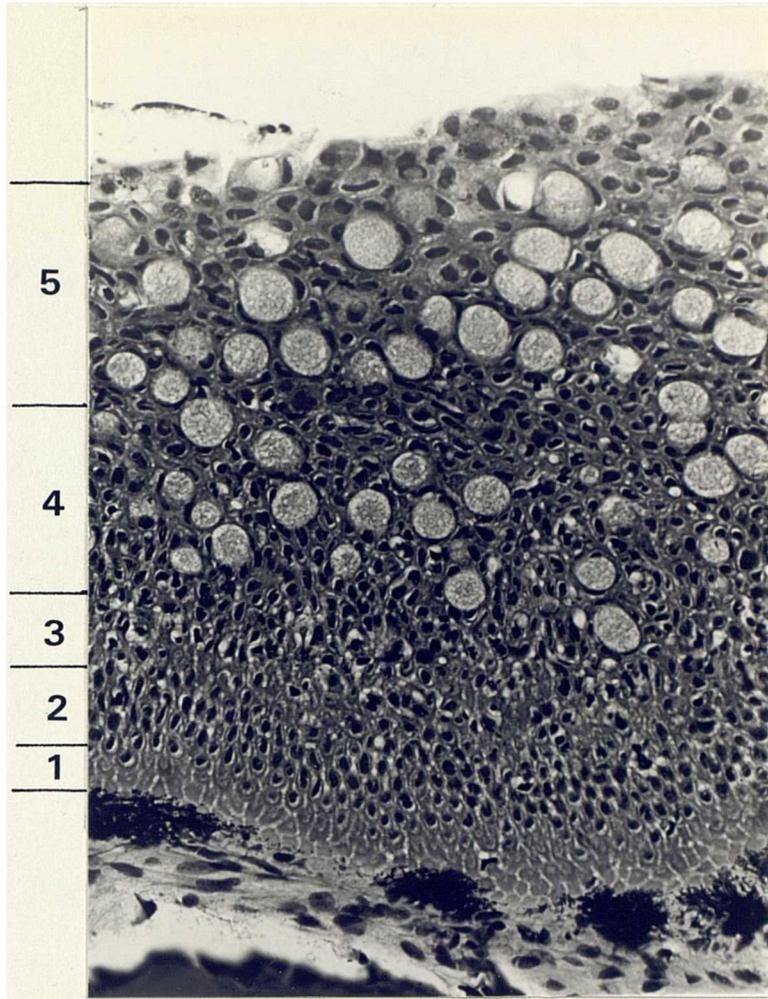
Figures 111 and 112

Fig.111 Section through body skin. The epidermis subdivides into five layers.

x 320

Fig.112 Cuticular secretion (C) upon the outer layer of the epidermis.

x 320



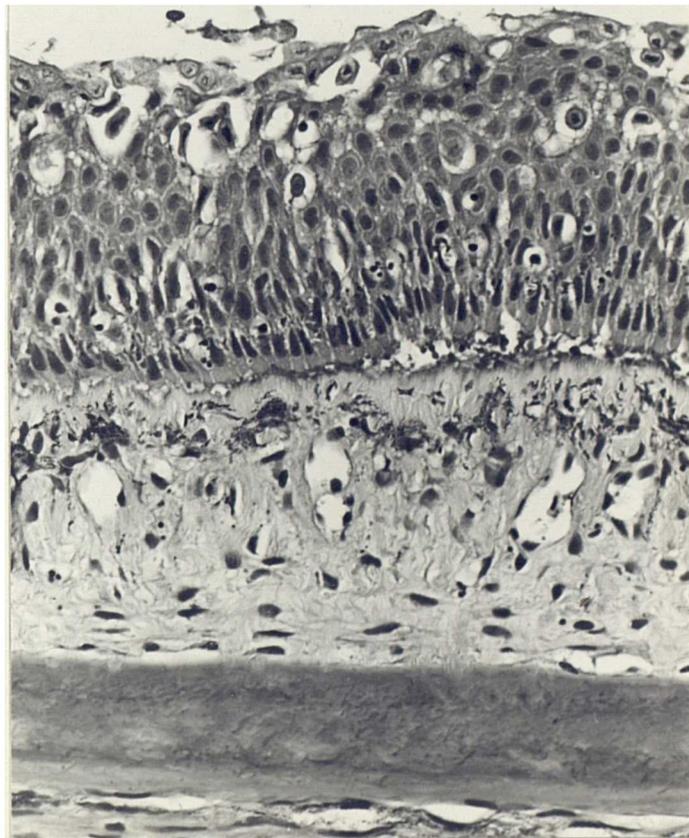
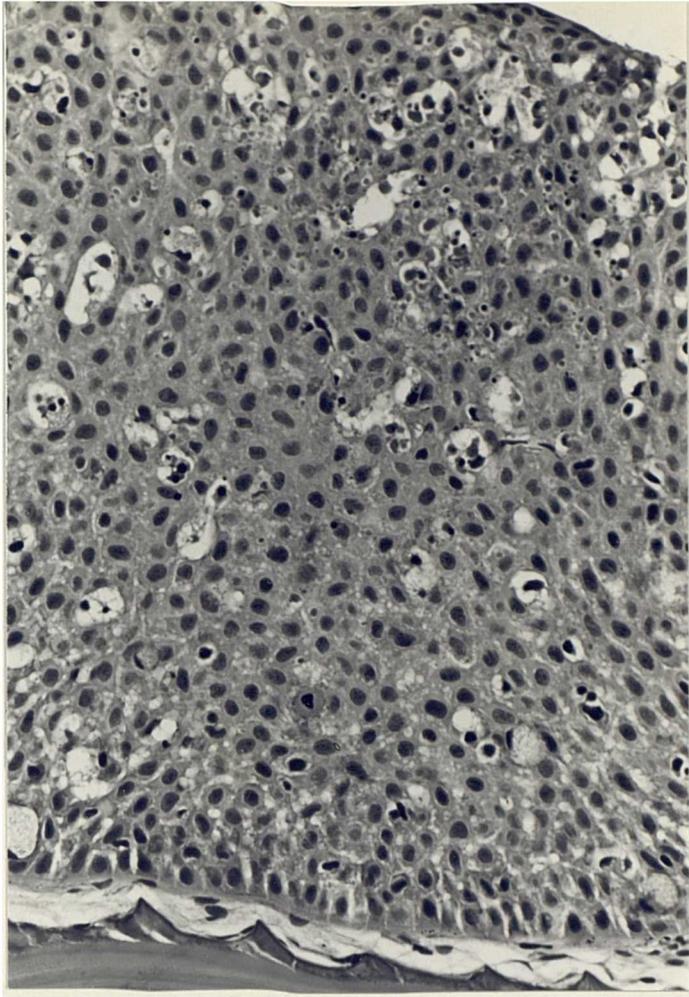
Figures 113 and 114

Fig.113 Upper flank skin. Numerous sunburn cells can be seen at all levels.

x 320

Fig 114 Section of dorsal skin. Note the focal separation of the epidermis from the basement membrane.

x 320



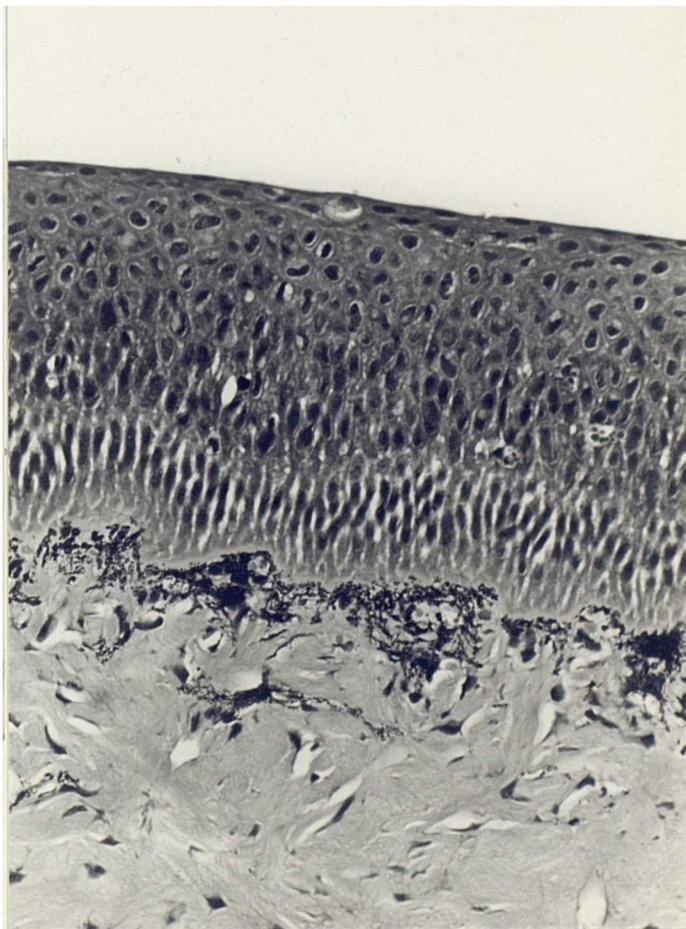
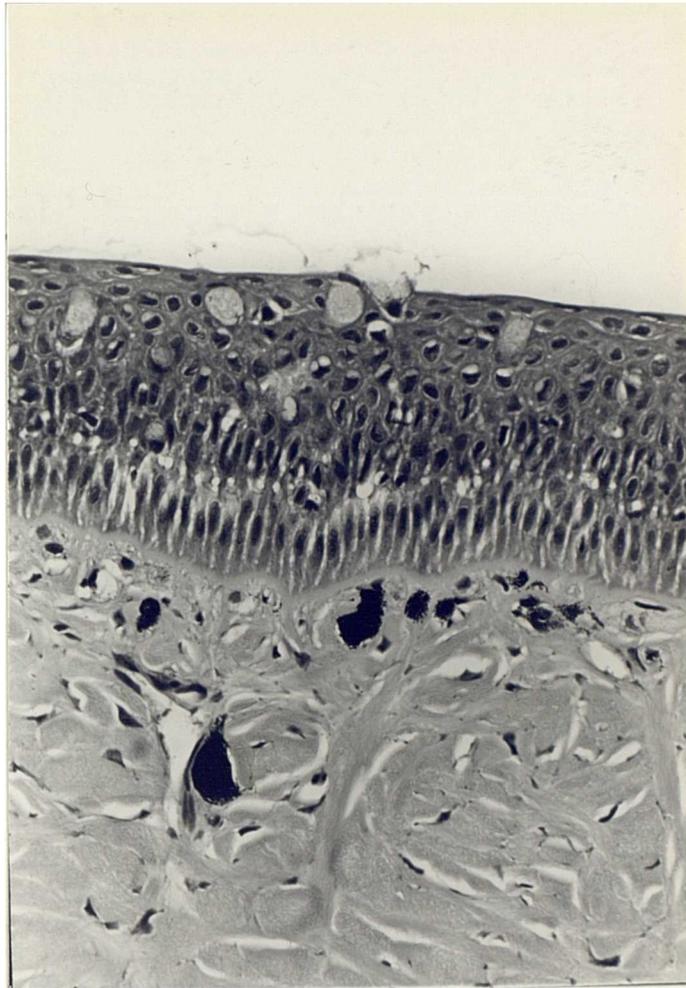
Figures 115 and 116

Fig.115 Section through the head skin of a fish less severely affected by radiation. In this instance the changes are limited to a slight oedema within the supra-basal zone.

x 320

Fig 116 *Type B* sunburn cells present within the epidermis of the head skin featured in Fig.115

x 320



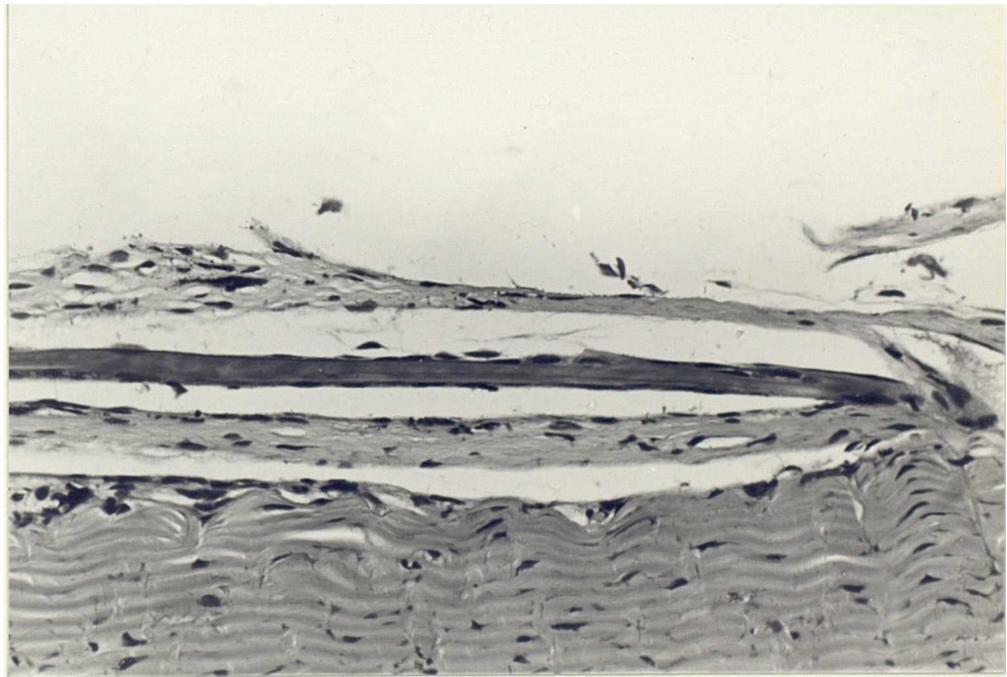
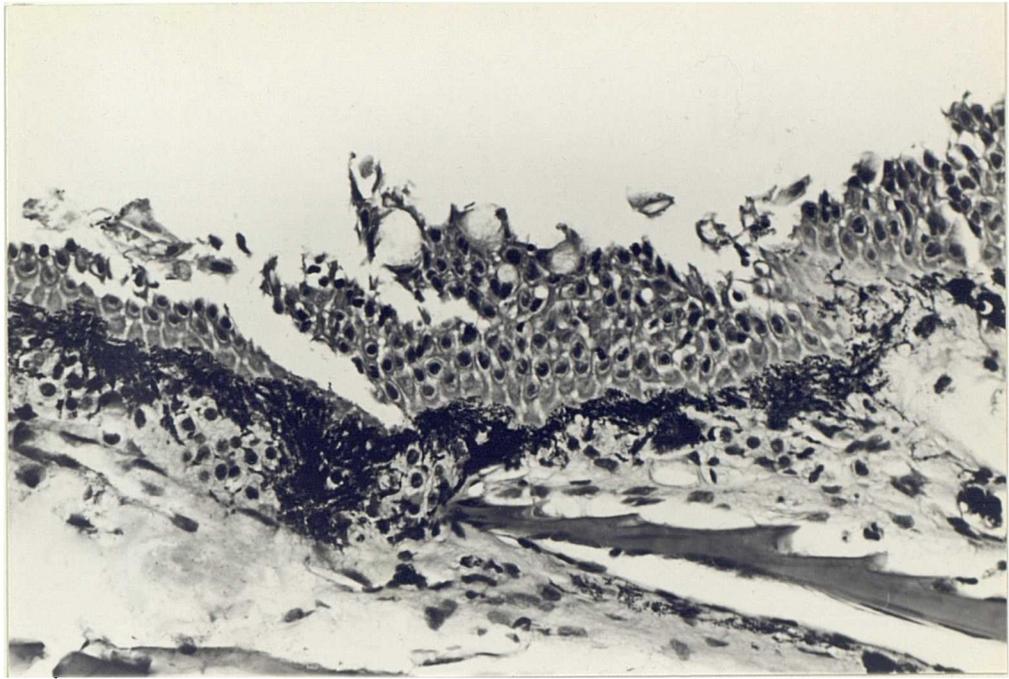
Figures 117 and 118

Fig.117 Section of body skin following minimal handling. The vulnerability of irradiated skin to physical trauma is thus apparent.

x 320

Fig.118 Section through an open wound. The scales remain *in situ* whilst the underlying dermis lacks differentiation.

x 320

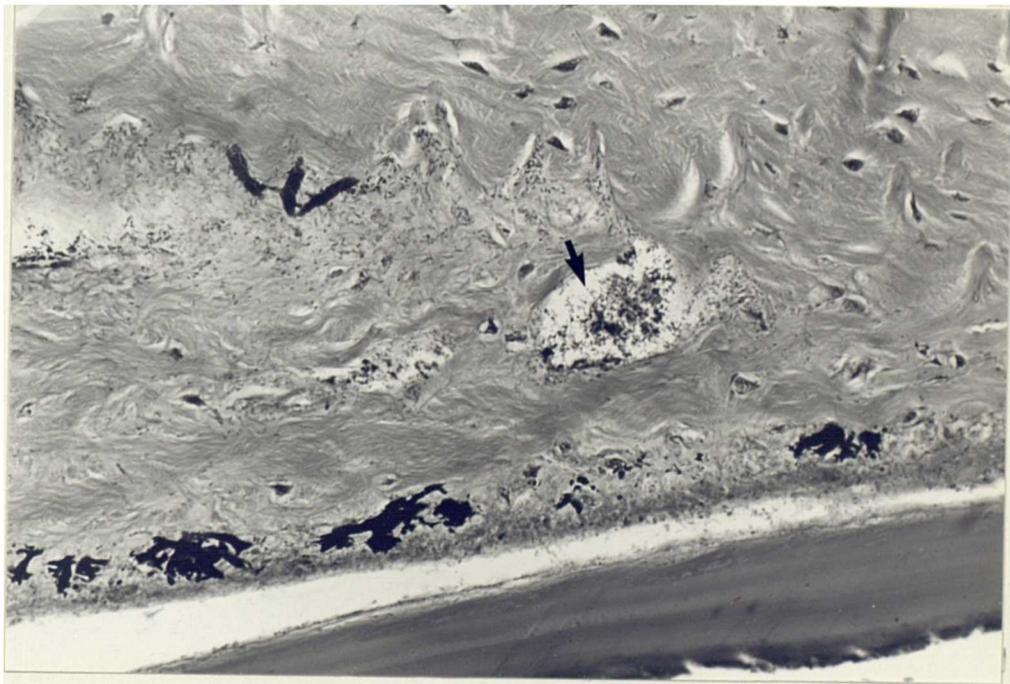
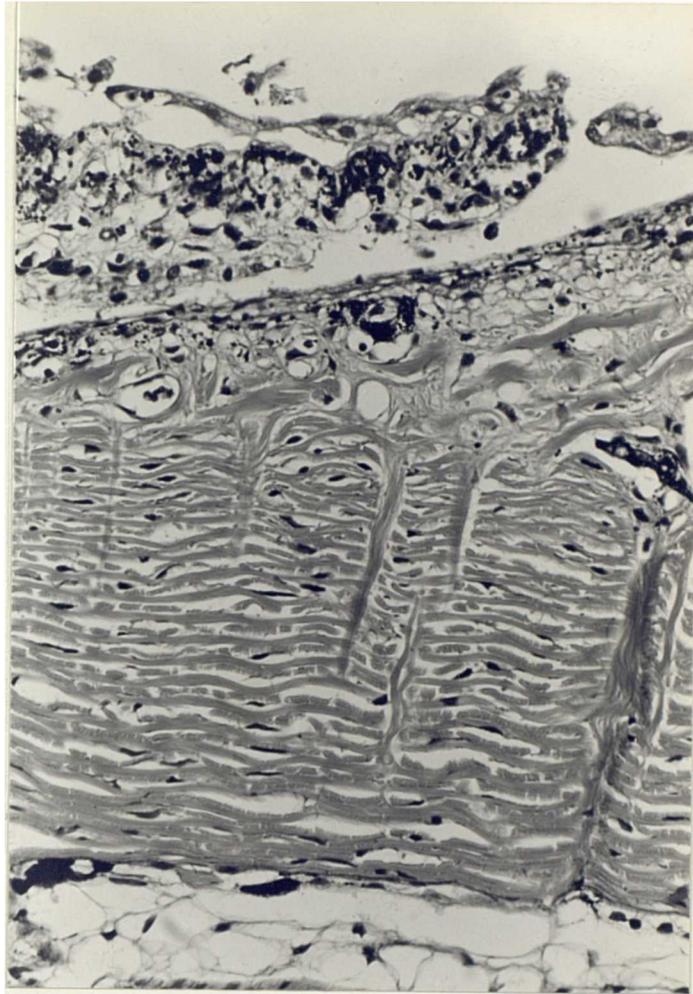


Figures 119 and 120

Fig.119 Advanced necrosis of the irradiated skin. The *stratum compactum* exhibits a laminated appearance, whilst above the *stratum spongiosum* is markedly oedematous.

x 320

Fig.120 A microabscess (arrowed) within the *stratum compactum*. Numerous bacteria can also be seen throughout the surrounding tissue.



SECTION VI

CASE HISTORIES

### *Introduction*

During the course of this study a number of clinical outbreaks of 'light exposure' related dermatopathies were drawn to the author's attention. In most instances these appeared to be of a straightforward solar UV induced nature although one case involved the administration of a drug in the diet invoking a phototoxic response. In another, the importance of recognising sunlight as a contributory factor in the breakdown of the skin, culminating in a major bacterial outbreak, was demonstrated.

All such cases are highly relevant to the present study and are, therefore, briefly outlined in this section.

### Case A

During the early summer of 1980 a rainbow trout hatchery, newly established on a high quality water supply in the south of Scotland, held fry in fibreglass or metal tanks, at standard stocking densities for a period of two months after swim-up and before stocking into earth ponds. Due to building of the hatchery being incomplete only half of the total number of tanks were indoors, the remainder being held outside with a south-west facing aspect. All tanks held trout from a mixture of origins, and a variety of fry foods were being fed.

Within/

Within two weeks of transfer, during a prolonged period of continuous sunny weather, some of the fish in the outdoor tanks started to show the development of white pectoral and dorsal fins. The numbers gradually increased in proportion and despite treatment by the farmer with formalin, hyamine and copper sulphate the affected fish started to die in small numbers.

After advice, he covered over two of the four outside tanks to prevent exposure to solar radiation and within two days all mortalities ceased and the condition of the affected fish began to improve. The number of affected fish in the two still unprotected tanks continued to rise, as did mortalities. Convinced of the efficacy of protection from sunlight, the farmer covered the remaining tanks and the condition completely resolved. Histological examination of the damaged fins revealed a characteristic UV induced response, the malpighian cells appearing swollen and pale staining with prominent perinuclear haloes. *Type A* sunburn cells, although few in number, were present primarily towards the base of the fin.

#### Case B

During the same period, an almost identical condition developed on a farm situated in the north-west of Scotland approximately 800 km distant from that referred to in Case A. In this instance, the affected fry were Atlantic salmon/

salmon, which had been transferred from the hatchery to outdoor tanks and held at a water depth of approximately 5 cms, several days prior to the initial lesions being observed. Shading screens were in regular use and adjusted daily to allow one-third of each tank to receive full daylight from 0900 to 1700 hours. This was done to encourage a more even distribution of fry within the tanks.

Eight days following first removal of the screens small numbers of fry exhibited an unusual behavioural pattern and were seen to drift, apparently unable to hold station within the shoal. The general tendency of the affected fish was to move towards the wall of the tank into the stronger current. Despite their impaired activity they continued to feed well. Upon close inspection macroscopic lesions were visible upon the dorsal and pectoral fins (Fig.121). In some instances the upper part of the tail fin was also affected. The general appearance was of a white or creamy-coloured thickening of the fin. No lesions were observed elsewhere and the pectoral fins of fish examined from completely shaded tanks appeared normal. The condition of the affected fish continued to deteriorate whilst others developed similar signs and eventually mortalities in the tanks rose from an anticipated 150 per day at a stocking density of 40,000/

40,000 per tank to approximately 300 per day. Shading of the tanks was recommended and within 15 days the mortalities had subsided to normal proportions. Microscopical examination of the fins showed no evidence of any pathogen except in the most severely affected fish where a layer of myxobacteria was often present on the fin surface. As in Case A, the malpighian cells were enlarged and pale staining with, in many instances, prominent nucleoli. *Type A* sunburn cells were also present (Fig.122).

#### Case C

In the late spring of 1983 a large on-shore salmon rearing unit situated on the west coast of Scotland reported dorsal skin lesions on mature (500-1000 gm) fish. Skin damage was first observed over the snout, but was also evident, to a lesser extent, over the dorsum particularly around the dorsal fin region.

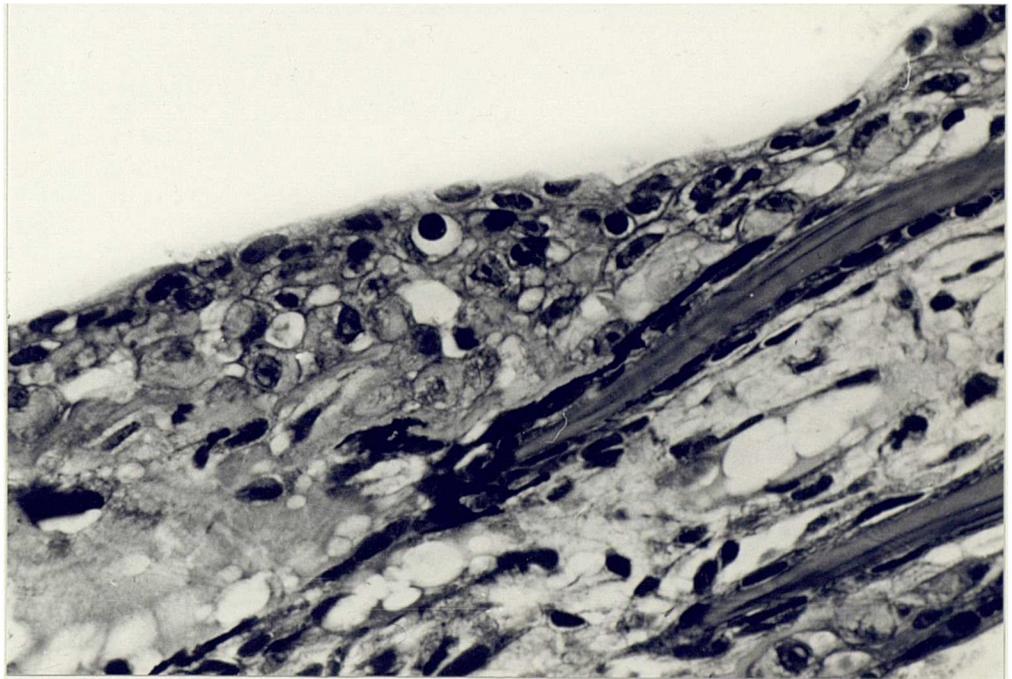
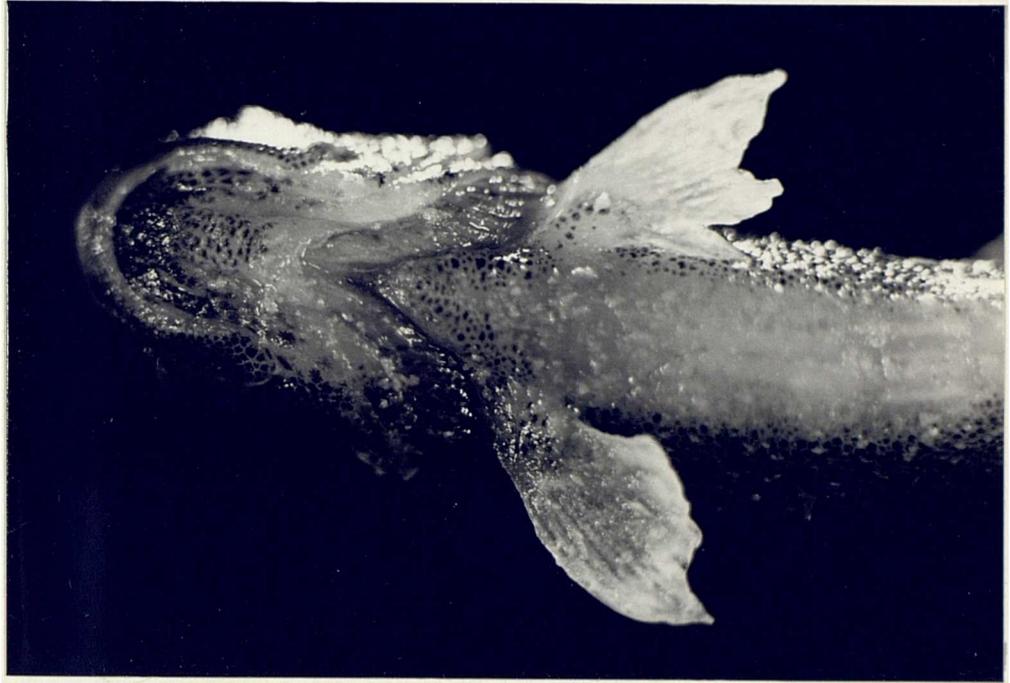
Prior to the lesions developing the fish had been overwintered in tanks which were quite heavily fouled and the water relatively turbid. Following transfer to clean tanks providing a light background and a continuous supply of exceptionally clear seawater of approximately 2 m in depth, a number of fish exhibited skin damage within 7-8 days. Throughout this period high sunlight conditions prevailed.

Subsequent/

Figures 121 and 122

Fig.121 Pectoral fins of salmon fry affected by solar UV radiation. The white colouration indicates thickening and general necrosis of the epidermis layer with subsequent destruction of fin structure.

Fig.122 Section of fin showing *Type A* sunburn cells. The surrounding malpighian cells are pale staining and exhibit prominent nucleoli.



Subsequent investigation of the lesions showed the presence of moderate numbers of sunburn cells within the periphery of the open lesion. Before shading could be provided, however, the outbreak rapidly progressed and a bacteriological investigation showed that an infection of *Aeromonas salmonicida* was present within the stock. Following chemotherapy the condition was resolved.

Although the presence of *A. salmonicida* was undoubtedly the major feature of this case the combined presence of high sunlight levels concurrent with transfer stress, clear water conditions, and the evidence of radiation damage within the lesion, all suggest that exposure to prolonged solar UV might well have been partly responsible for the initiation of skin trauma, the bacterial infection being a subliminal factor until such time as the fish succumbed.

#### Case D

Following a report in the Norwegian literature by Hastein et al (1980) of sunburn affecting salmon fry the author was given the opportunity to examine sections of the skin of affected fish and found that they were morphologically identical to those lesions observed in the preceding case histories and also certain of the UV-B experiments contained in this study.

The/

The Norwegian outbreak occurred on a large salmon hatchery in early July of 1980. During the period 5th-12th June batches of 65,000 and 120,000 fry were transferred outdoors into two uncovered circular concrete tanks, 10 metres in diameter, with a water depth of 60 cm. Water conditions were noted as being exceptionally clear. A further transfer of 10,000 fry into each of six similar tanks took place over the period 19th June until 5th July. Following transfer, the original groups received full sunlight for eleven hours per day over a 30-day period, the remainder receiving a similar daily irradiance over 2-18 days before lesions were first observed in the original groups.

Clinically the lesion was seen to develop as white patches between the head and dorsal fin, affecting only those fish first transferred. In certain cases the dorsal fin was also affected appearing whitish and curled. Behavioural changes were noted in that the affected fry tended to clump together. Initially, mortalities were moderate but gradually increased until the total losses in the most densely stocked tank reached 30%, with 10% losses in the tank holding 65,000 fry. It was noticeable that the fish involved in the later transfers at low stocking density showed only minor losses.

When/

When the cause of the losses was verified histologically the water level was increased, the water surface agitated, and partial shading provided. Four weeks later the condition had resolved to a point where only a few fish were affected.

#### Case E

In February 1981, during a visit to Kenya, the author was able to investigate the potential impact of solar UV radiation upon rainbow trout fry on a fish farm in the foothills of Mount Kenya. The farm was situated near Naro Moru, close to the equator at an altitude of 2,100 m.

From the literature (Diffey, 1982) it is known that for every 300 m increase in altitude above sea level, there is an overall increase of 4% in global UV-B. Therefore, under such circumstances on this farm the incident solar UV-B radiation would be in the region of 28% greater than that anticipated at sea level. With such an increase in incident radiation it might be expected that farming at high altitude would result in a considerable increase in the occurrence of radiation induced dorsal skin lesions. On this farm, however, the condition was relatively unknown, due in all probability to the attenuating properties of the highly turbid water; Secchi disc measurements of less than 30 cms were not uncommon in the ongrowing ponds.

Hatchery/

Hatchery water by contrast was relatively clear having passed through a graded gravel/sand filter prior to entering the raceways. In addition, adequate shading from solar radiation was provided over the entire hatchery area.

For experimental purposes six groups of 10 fry were held in separate containers in full sunlight at a water depth of 10 cms. Owing to the limitation on suitable containers irradiation was carried out in deep bins which had the effect of providing partial shade throughout the day apart from a two-hour 'window' when the sun was directly overhead. Nonetheless cumulative daily irradiation on average would have exceeded that anticipated at sea level by a significant factor.

Over the 10 day exposure period it was noted that the fish had a distinct preference for the shaded areas within the containers, moving into the brightly lit zones only if food were offered. No macroscopic evidence of radiation damage was seen either upon the fins or the dorsum. At the end of the experiment all fish were killed and preserved in chilled formalin for 48 hours, prior to transportation.

Detailed microscopical analysis of the skin showed that no major breakdown of the epidermis had occurred in any of the irradiated fish although, such areas as the base of the dorsal fin seemed to be especially vulnerable (Fig./

(Fig.123) exhibiting an advanced oedema and the presence of *Type A* sunburn cells. In many fish the dorsal epidermis also exhibited a massive intercellular oedema (Fig.124) the nuclei adopting bizarre forms with an occasional *Type B* sunburn cell within the layer.

Examination of the pectoral fins provided further evidence of radiation damage (Fig.125) where numerous *Type B* sunburn cells were noted. In contrast to the UV-B experiments whereby the lesions were observed primarily over the upper surface of the fin, under natural irradiation the response appeared to be equally distributed over both sides of the fin.

#### Case F

Following a visit to Bolivia during October/November 1982 Beveridge (pers. comm.) brought to my attention the high incidence of a dorsally related skin disorder involving principally the dorsal and caudal fins in rainbow trout reared on a high altitude (3700 m) fish farm in the Altiplano zone of that country. Samples of the affected skin were taken by Beveridge and subjected to a detailed histological examination at this laboratory.

Invariably the lesions exhibited nearly all the features associated with UV radiation damage in salmonids as seen in other clinical cases, and also under artificial UV-B radiation. Within the immediate vicinity of the open/

Figures 123 and 124

Fig.123 Section through the base of the dorsal fin.  
Note the presence of *Type A* sunburn cells  
(circled) within the supra-basal zone.

x 320

Fig.124 Dorsal epidermis midway between the base of  
the dorsal fin and the crown of the head.  
Advanced intracellular oedema is evident with  
*Type B* sunburn cells (circled) also present.

x 320

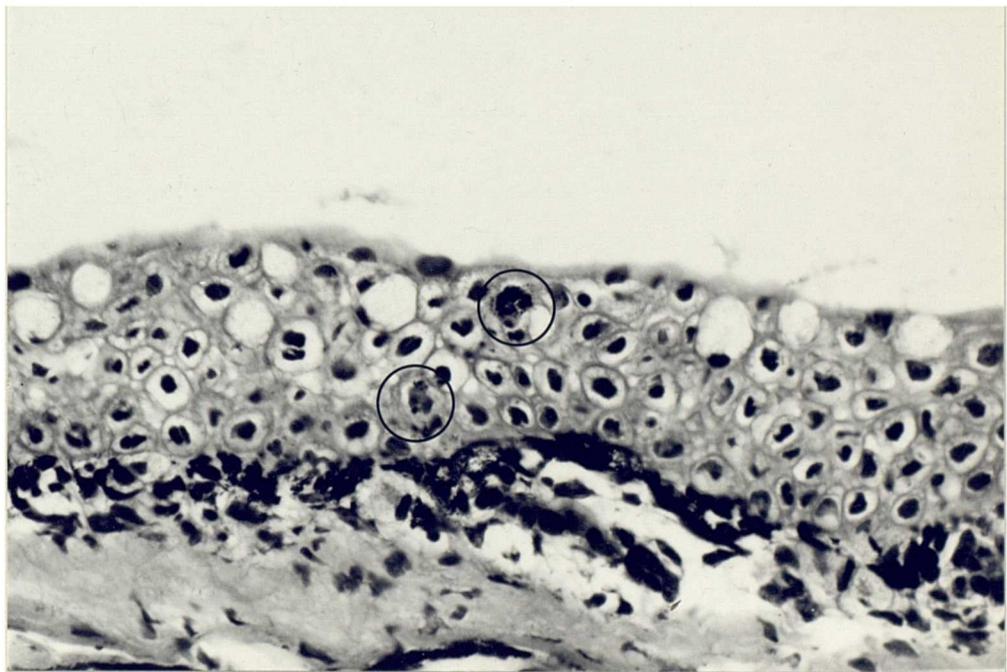
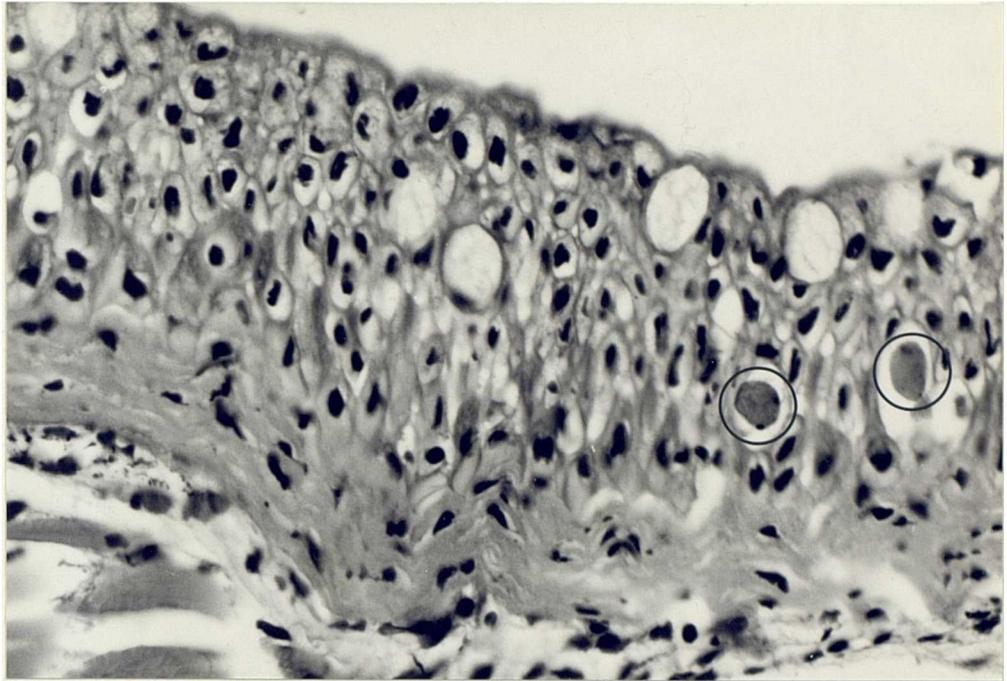


Figure 125

Fig.125 Section through the pectoral fin. Note the presence of *Type B* sunburn cells (circled) in both the upper and lower surfaces of the fin.

x 320



open wound the epidermis exhibited a bland, amorphous pale staining reaction within the cytoplasmic component of the malpighian cells (Fig.126). Where the nuclei remained relatively undamaged nucleoli were prominent whereas the more severely affected cells appeared as foci of nuclear droplets, somewhat characteristic of the *Type B* sunburn cell response. Within the basal zone there was often a considerable loss of attachment between adjacent cells identical to the focal separation associated with irradiated skin. Outwards from the lesion the epidermis showed a gradual but progressive hyperplasia and within its mid zone numerous bizarre nuclear forms could be seen. Prominent nucleoli remained a feature of many cells and over the surface at this point the cuticular layer could often be seen (Fig.127). At the outermost point in the response the epidermis was considerably thickened and throughout its entirety moderate numbers of *Types A* and *B* sunburn cells could be seen. Typical examples are shown in Figs.128 and 129.

Further reports from Bolivia (Coutts, pers. comm.) indicated that on such sites up to 60% of mature rainbow trout held in cages exhibited clinical signs of a necrosis of the dorsal fin and upper lobe of the caudal fin (Fig.130 and 131).

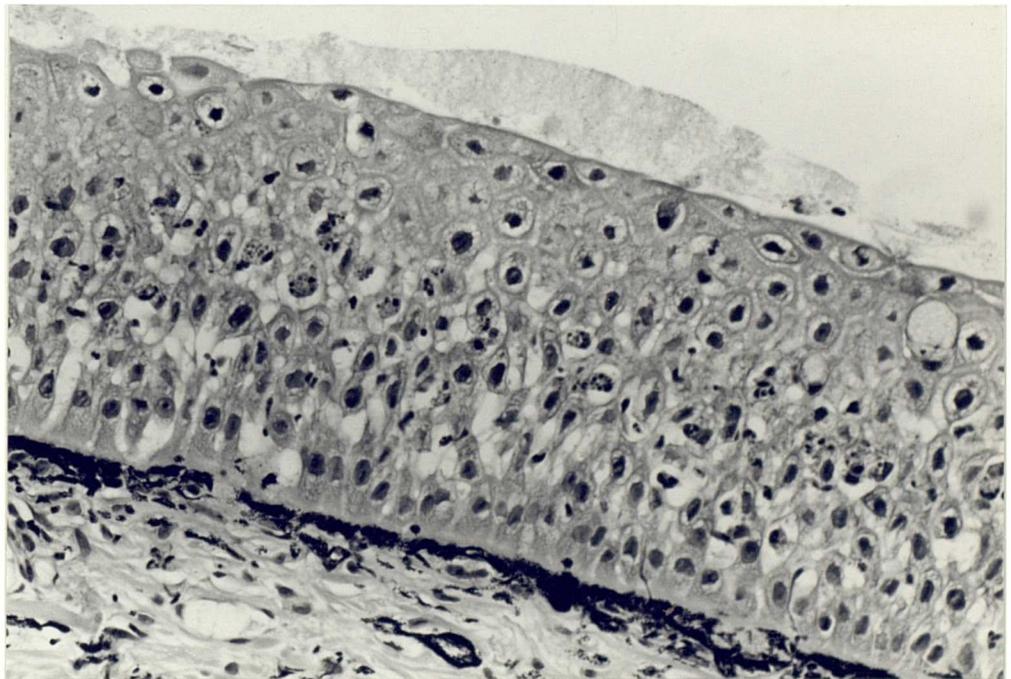
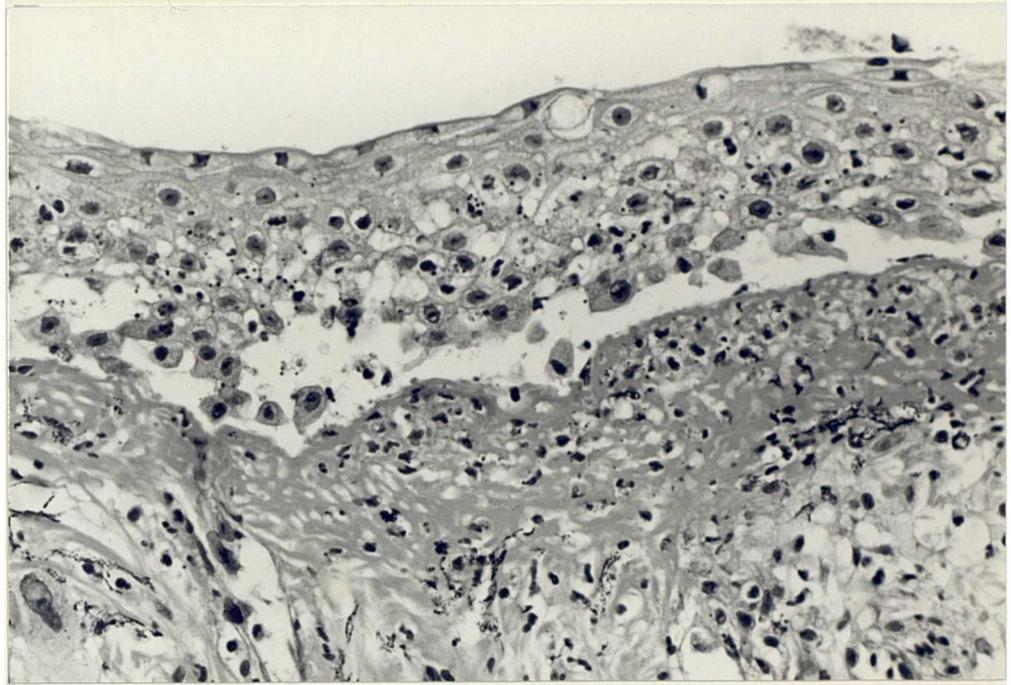
Figures 126 and 127

Fig.126 Section of epidermis immediately adjacent to the open wound. The malpighian cells, where visible, are pale staining and nuclear drop-lets are present within the central zone. A major feature is the loss of attachment between the basal zone and the underlying basement membrane.

x 320

Fig.127 Section showing the cuticular layer overlying the epidermal surface close to the open wound. Numerous *Type B* sunburn cells are also present.

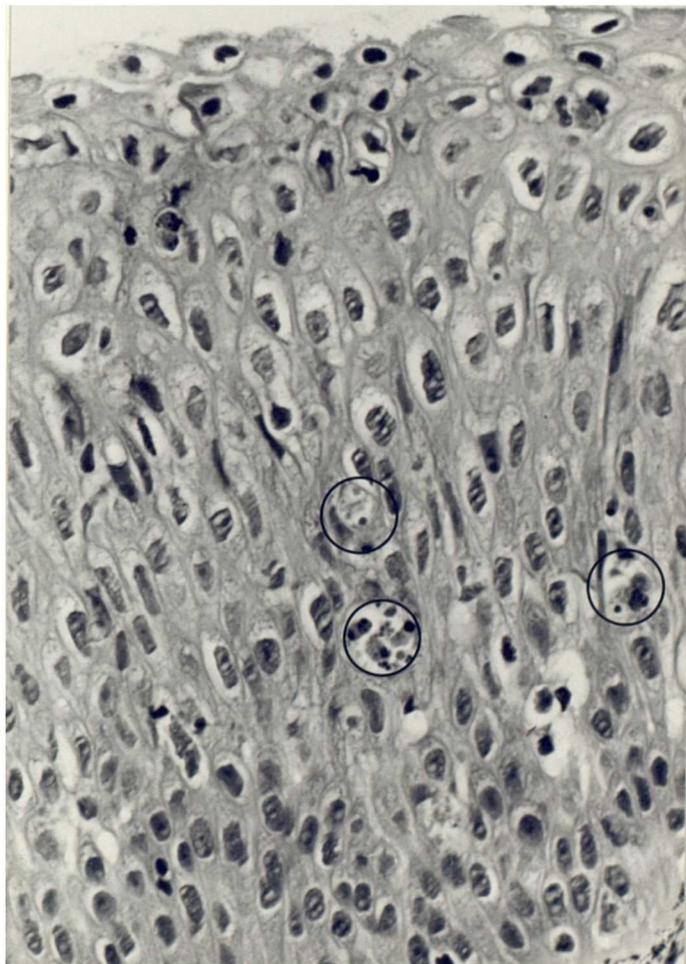
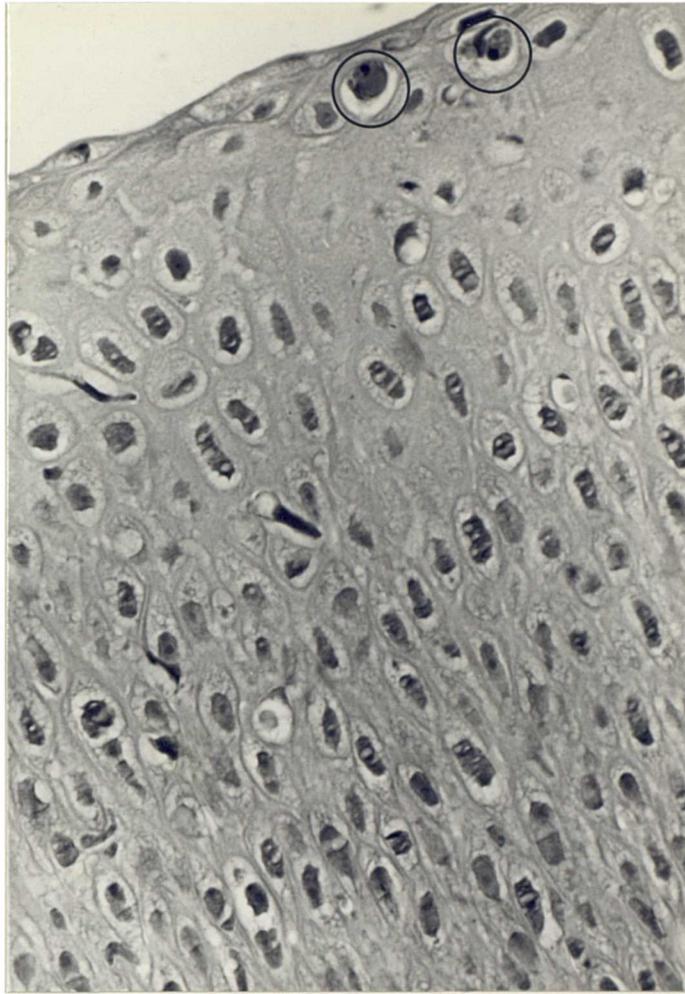
x 320



Figures 128 and 129

Fig.128 *Type A* sunburn cells (circled) within the upper layer of the epidermis.

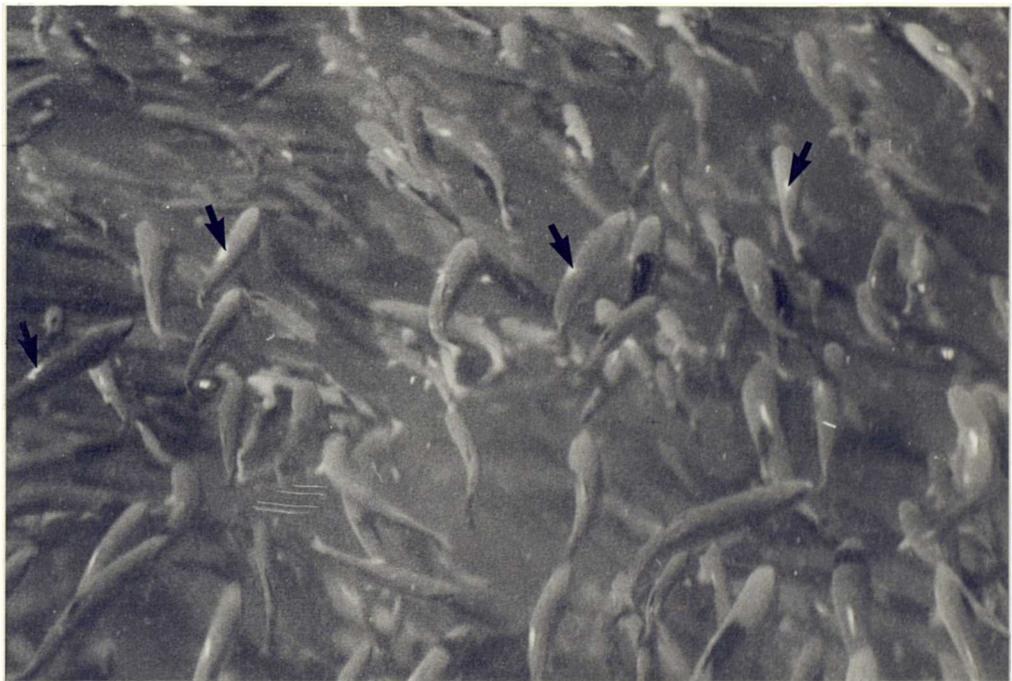
Fig.129 *Type B* sunburn cells (circled) more often associated with the middle zone of the epidermis.



Figures 130 and 131

Fig.130 Close up view of the affected dorsal fin.  
(Reproduced from a colour negative supplied  
by Mr. R Coutts)

Fig.131 General view of caged fish at Koryhuaya,  
Lake Titicaca. Dorsal lesions (arrowed) can  
be seen on numerous fish.  
(Reproduced from a colour negative supplied  
by Mr. R. Coutts)



Case G

Reports of solar UV induced dermatopathies were not found to be exclusive to salmonids. During the course of his studies upon flatfish larvae in the shallow water region of the North Frisian Wadden Sea (eastern North Sea) Berghahn (1983) investigated the movement of several species of postlarval fish, including plaice, over the tidal flats exposed at low tide where they remain in the puddles and drainage gulleys and thus are potentially vulnerable to high levels of solar radiation. In such circumstances the larvae generally bury themselves in the substrate thereby avoiding the direct rays of the sun. On warm, cloudless days temperature levels in these pools may increase to lethal thresholds and consequently the larvae attempt to escape from the higher tidal flats, near the shore, through the drainage gulleys. Berghahn reports a species specific change in their distribution pattern when this occurs with plaice seldom being found in near-shore areas and also absent at high and low tides. He postulates that at least some of the mortalities in postlarval flatfish might be due to solar radiation.

Following correspondence on the subject, samples of plaice from his collection were examined histologically. In order to avoid any bias in the interpretation of the results the samples were identified by prefix only.

Comparison/

Comparison of Berghahn's data and the results of the histological study showed a good correlation between those fish most likely to have received high radiation levels and skin damage. In section, fish which had been buried just prior to sampling exhibited a normal skin structure (Fig.132) whereas larvae exposed to prolonged periods of sunlight exhibited a somewhat differing response (Fig.133). In these fish, the general reaction was seen to be a thickening of the dorsal epidermis, the malpighian cells becoming oedematous with large pale staining nuclei which eventually sloughed. The characteristic sunburn cell, although present, was not a major feature, however. The fact that epidermal necrosis was limited to the dorsum with the ventral aspect appearing normal in all samples suggests that larvae in natural populations subjected to high solar radiation levels are prone to skin trauma which, if severe, could lead to an irreversible breakdown in the integrity of the epidermis resulting in the death of the fish.

#### Case H

Koi carp *Cyprinus carpio* are very valuable Japanese ornamental fish much sought after by enthusiasts for the distinctive colouration and patterning of their skin, both qualities being of fundamental importance in determining the value of individual fish.

During/

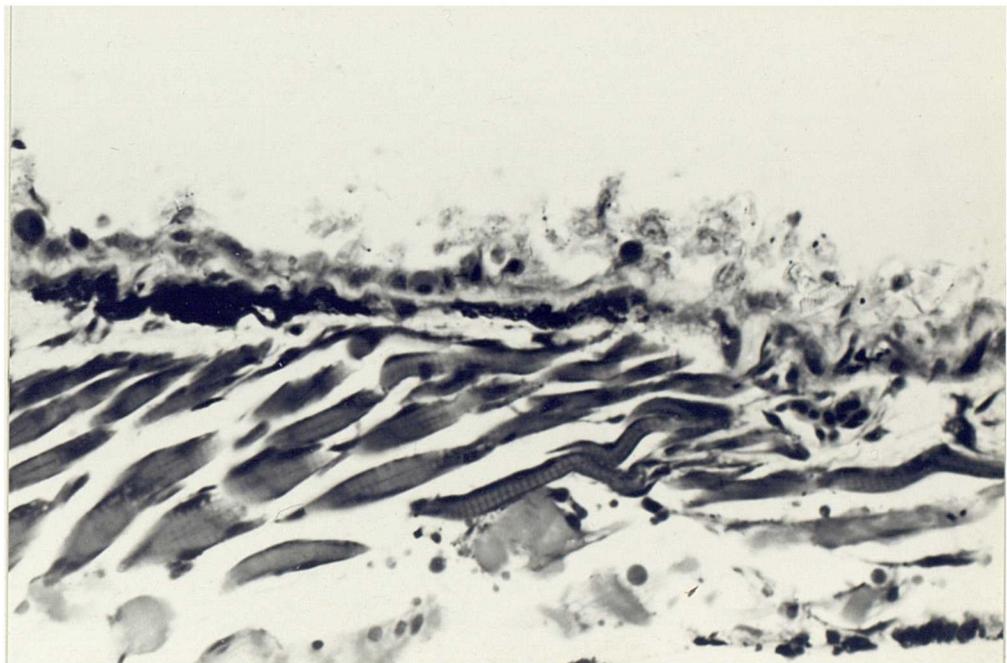
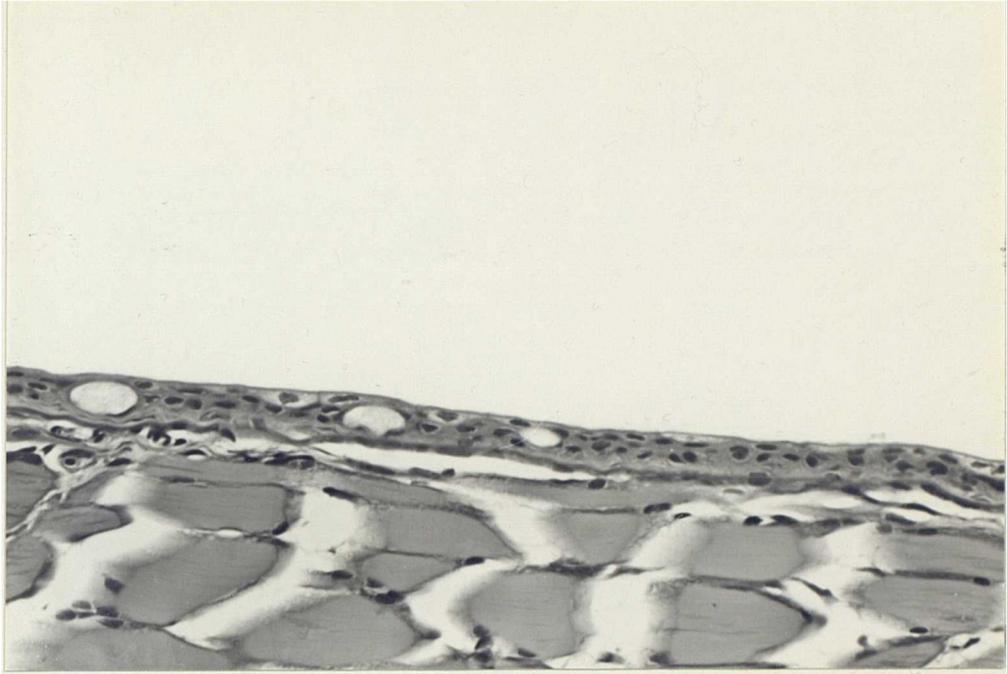
Figures 132 and 133

Fig.132 Section of the dorsal aspect of larval plaice skin showing its typical morphology.

x 320

Fig.133 Section of dorsal skin of larval plaice subjected to natural solar radiation.

x 320



During the course of this study a skin condition in this species was reported to the author by dealers and hobbyists in the U.K. which was associated with the fish being held outdoors in very clear water. Chemotherapy had little effect in most cases, as did treatment by formalin bath or malachite green. In view of the great variation in response and the very high value of individual fish, a detailed clinicopathological study was carried out in an attempt to define its aetiology more accurately.

The fish in the outbreak studied had been quarantined in an indoor pond for six weeks after importation from Japan, thereafter being placed outdoors. Within four days of transfer 20% of the fish developed skin lesions limited to the dorsal aspect. Clinically the lesions manifested themselves as congestion of the pectoral fins and thickening of focal areas of epithelium which appeared only within the white non-pigmented patches. As these zones became thickened they adopted a greyish mucoid appearance (Fig.134) and eventually sloughed leaving shallow, haemorrhagic ulcers. Generally the red and black pigmented dorsal areas remained unaffected. The fish continued to behave normally, fed well, but were obviously unsuitable for sale

During the outbreak, water conditions were exceptionally clear with a concurrent period of uninterrupted sunlight/

sunlight to which the fish were continuously exposed. Placing even severely affected fish into water which had greened up with algae or contained sufficient cover in the form of aquatic plants, produced rapid relief and in view of these findings a tentative diagnosis of ultraviolet radiation induced dermatitis was made.

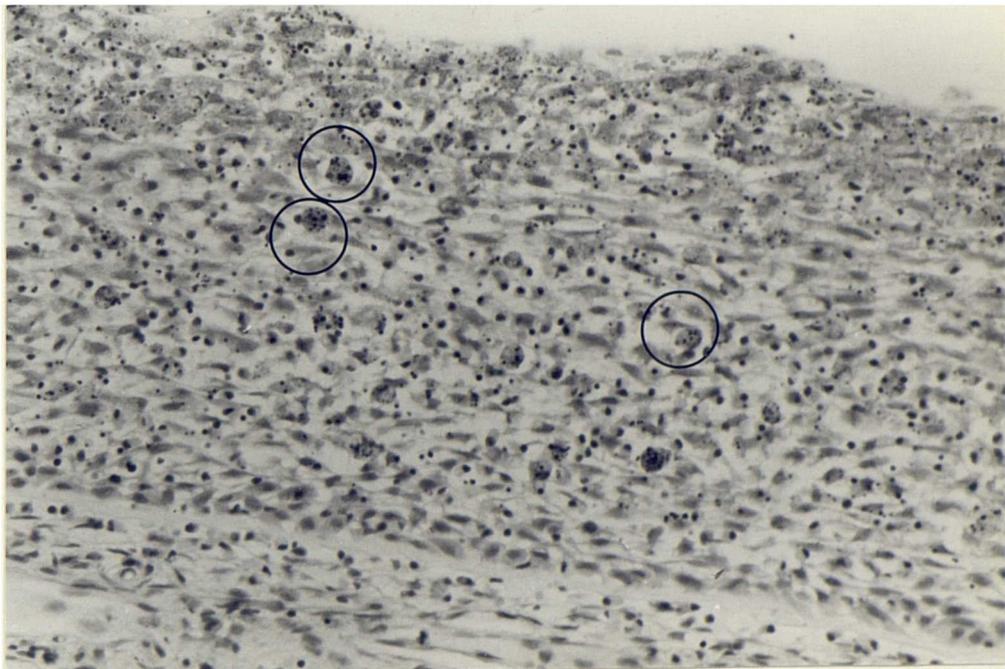
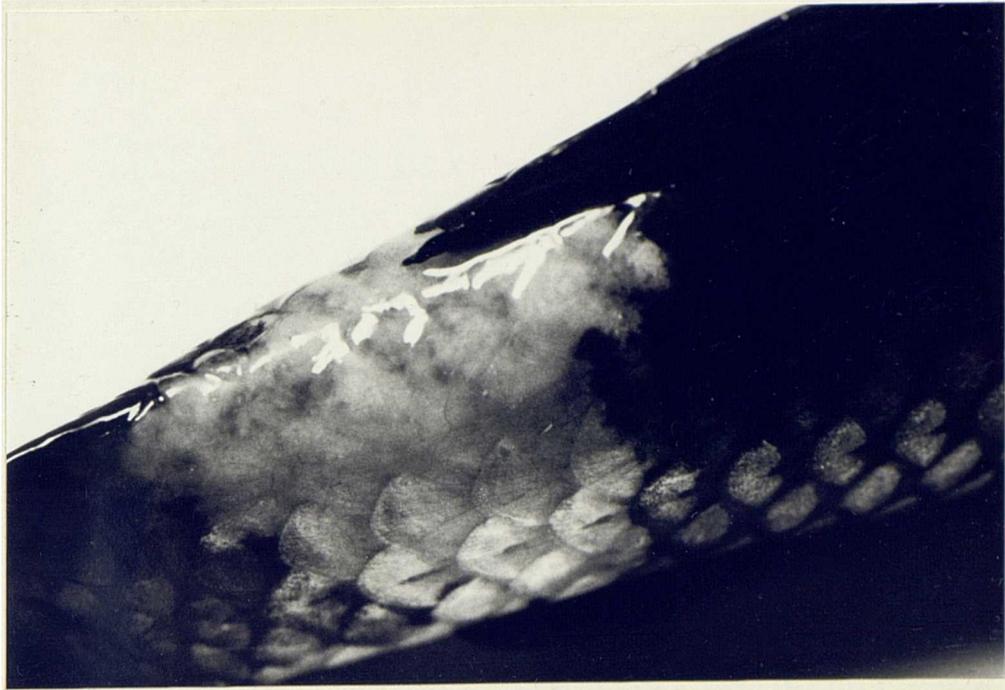
Histopathological examination of the peripheral zone surrounding the lesion confirmed this, with the epidermis exhibiting numerous *Type B* sunburn cells, particularly in the upper layer, characteristic of UV radiation damage (Fig.135). Close to the centre of the lesion the underlying dermal vessels were extremely hyperaemic with subepidermal exudate extending up into the basal layers of the epidermis. In general there was no evidence of the characteristic histopathological response associated with bacterial infection. Any bacterial activity observed in section was limited to the centre of the lesion where the epidermis had sloughed allowing access to opportunistic organisms. A diagnosis of primary sunburn was, therefore, considered justified.

Figures 134 and 135

Fig.134 Solar ultraviolet-induced dermatitis on the dorsum of a Koi. Note the thickening of the epidermis in relation to that on the flank.

Fig.135 Section of upper epidermis from the periphery of a sunburn lesion. The characteristic *Type B* sunburn cells are circled.

x 320



SECTION VII

DISCUSSION

Fish skin, by comparison with that of higher animals, is an extremely delicate membrane and in most species appears quite incapable of resisting even minor physical trauma. Its vulnerability relates to its functioning in a number of differing, but highly significant roles, all related to its presence as the limiting membrane at the interface between the fish and the aquatic external medium. These include the production of the cuticle, prevention of osmotic gains or losses of water or tissue fluids (depending upon the osmolarity of the ambient medium) and provision of a smooth, laminar flow inducing, surface to enhance locomotion.

Consideration of the traumatic potential of ultraviolet radiation for fish skin compared to that of the human suggests two major features of primary importance. In fish skin the malpighian cells, comparable to the keratinocytes of human skin, are capable of mitotic division at all levels within the epidermis (Bullock et al , 1978). As cells undergoing DNA synthesis or mitosis are the most liable to suffer radiation damage (Giese, 1976) then it would seem reasonable to speculate that the fish integument is likely to be more vulnerable than its human counterpart where mitosis occurs only within the basal layer. In addition fish epidermis lacks a photoprotective pigment-producing/

producing mechanism by which it can protect itself from the noxious effects of ultraviolet radiation, the pigment containing cells of fish skin are primarily sub-epidermal with the majority occupying the *stratum spongiosum*.

Because of the wide structural differences between fish skin and that of higher animals a comparison of the response following irradiation may appear somewhat specious. Nonetheless the cellular events leading to the development of the sunburn cell *per se* in human skin are of relevance to this study. Olson et al. (1974) observed the sequential development of the sunburn cell by light and electron microscopy and found that throughout the early stages of the radiation lesion a constant feature was the appearance of densely staining eosinophilic rings or haloes surrounding the periphery of each cell. The reaction, however, was limited to individual cells dispersed throughout the superficial epidermis and they interpreted the response as representing a progressive intracellular oedema resulting in a pronounced swelling of each cell, the cytoplasm being pushed outwards to the periphery thus forming a ring or halo. The nucleus retained its central position but altered in appearance as the lesion progressed often adopting bizarre forms before finally becoming pyknotic. Between the nucleus and the outer halo the cell showed little staining propensity and generally appeared in section as a relatively unstained area contrasting sharply with/

with the central nucleus and the peripheral ring.

Although an essentially similar response was observed in this study the intracellular oedema following irradiation in the early stages was not limited in fish skin to individual malpighian cells but was seen to occur throughout the entire epidermis in all species studied. Scanning electron microscopy of plaice skin showed that a progressive swelling of each malpighian cell occurred following irradiation with a loss of microplicate patterning over the cell surface. Observations by Olson *et al.* (1974) by transmission electron microscopy indicate that a loss of "firm attachments" (?desmosomes) between the sunburn cell and those adjacent was commonplace.

In human skin sunburn cells are considered to be dyskeratotic in nature, that is, they are thought to occur due to the premature or abnormal keratinisation of individual epidermal cells. Dyskeratosis is, however, common also to certain malignant and benign skin disorders such as Bowen's disease, a form of skin carcinoma, and also the benign familial pemphigus (Milne, 1972). Fish skin does not have a keratin component so, therefore, the presence of what would appear to be typical sunburn cells in fish skin must be attributable to some other factor.

If a comparison is drawn between the location of sunburn cells within human skin and fish skin, it becomes clear that their distribution varies greatly. In human skin/

skin they are limited primarily to the superficial layers of the epidermis whereas in fish skin they appear at all levels. Smith (1974) among others has drawn attention to the key role that DNA plays in cell mutation or death following irradiation. Cells undergoing synthesis are probably at their most vulnerable at this stage (Giese, 1976) which suggests that, in fish skin at least, those cells actively synthesising DNA may well represent the target cells. Autoradiographic studies (vide supra) have confirmed that the generative compartment in fish skin encompasses the entire epidermal layer and as the pattern of labelling closely follows that of the distribution of sunburn cells within the epidermis then such a hypothesis would seem valid.

If, however, this were to be so, then some other explanation must be offered for the apparent limitation of sunburn cells to the upper epidermis in human skin as DNA synthesis, and hence mitotic division, occurs only within the basal layer. One possible explanation is put forward by Olson *et al* (1974) who suggest that the affected target cells, although situated basally, are swiftly 'devitalised' following irradiation, lose their desmosomal attachments and thus advance towards the surface more rapidly than surrounding cells. Another possibility has been proposed by Johnson *et al*. (1972) who hypothesised that cells containing large quantities of pigment might/

might, paradoxically, be more susceptible to irradiation injury. Since most melanin is associated with lysosomal activity (Olson *et al.*, 1970), ultraviolet light, which is known to cause release of lysosomal enzymes, would thus damage cells containing melanin more than other cells (Johnson & Daniels, 1969). It is assumed by these workers that in negroid and other heavily pigmented skin enough melanin is present in dispersed form in the upper layers of the epidermis for protection to dominate.

Of the fish examined in this study only one species, the turbot, contains any significant amounts of intra-epidermal melanin, which, in the normally pigmented fish, is distributed randomly in the form of large dendritic melanocytes throughout the epidermis. In the irradiation experiments on turbot skin there was no evidence of an increase in sunburn cell numbers in cells in close proximity to the melanocytes although, on occasions, where two or more melanocytes were situated closely localised hyperplasia did occur; sunburn cells were not a major feature of the reaction. Hyperplasia of this type was interpreted as being an immediate localised response to radiation as the melanocytes swell and push their melanosomes through the dendritic processes. In contradistinction to the human tanning response (Quevedo *et al.*, 1974) there is no evidence to suggest that the melanosomes transfer through to the surrounding malpighian cells and provide protection. Thus/

Thus, the possibility of an interaction between melanin and the appearance of sunburn cells in fish epidermis seems unlikely.

The appearance of two quite distinct sunburn cell types within the epidermis of all species investigated has not previously been reported. The sunburn cell in human skin conforms to that described as *Type A* in this study, whilst the *Type B* cell is identical to that described by Hunter *et al* (1979) in brain lesions of larval anchovy and mackerel. Throughout this study both cell types were present in all species with *Type B* predominating in plaice and turbot. In salmonids there was a greater proportion of *Type A* cells. The reasons for this apparent selectivity remain speculative although the higher prevalence of *Type B* cells within the skin of the more vulnerable benthic species would suggest that they represent a more severe response to radiation. This is emphasised by the higher incidence of *Type A* cells within the more resilient salmonid skin. In general it seems valid to consider both cell types to be of the same origin but exhibiting quite differing degrees of sensitivity to radiation damage.

The importance of photoreactivation or photorepair in fish skin has been amply demonstrated in this study. The vulnerability of fish skin to UV-B in the absence of visible light was found to be much greater than its susceptibility/

susceptibility when UV-B irradiation was accompanied by high levels of white light throughout and subsequent to irradiation. These results compare favourably with the findings of Kaupp & Hunter (1981) who demonstrated that the photoreactive fluence rate (amount of white light) required to activate the photorepair mechanism in larval anchovy was less than 10% of that available from sunlight at latitude 33°N in spring; of particular relevance also was their observation that there was a greatly increased survival rate in larvae exposed to 6 h of white light, following irradiation, over those receiving UV radiation followed by darkness. A recent study by Hunter *et al* (1981) examined the impact of solar UV-B irradiation upon larval northern anchovy and their results indicated that biologically adverse conditions did indeed exist near the sea surface with mortalities of up to 13% of the annual production for this species at least being considered to be due directly to the detrimental effects of sunlight.

The much greater susceptibility of UV-B demonstrated in this study when filters were not used to eliminate the shorter wavelengths emitted by artificial UV sources indicates the importance of presenting a realistic spectrum in laboratory investigations. It also serves to emphasise the relative effectiveness of the shorter wavelengths not normally found in incident light in cell destruction.

Unwitting/

Unwitting use of fluorescent tubes emitting in the UV-B without adequate filtration has undoubtedly lead to a considerable over-estimation of the noxious effect of the UV-B bandwidth in the past, since in all of the previous studies on UV radiation and fish skin, with the exception of the elegant studies of Hunter and his colleagues little, if any, attention has been focused upon the need to consider carefully the spectral emission, radiant intensity and adequate filtration of the light source.

Observations on the response of the melanocytes within the skin were in accordance with those observed by Hunter and his colleagues in their study. In general the reaction at the lower doses was one of a dispersal of the melanosomes within the dendritic processes with a concomitant swelling of the melanocyte nuclei. At higher doses the melanocytes appeared to aggregate, forming dense patches. Such a response was noted also by Fujii *et al.* (1973) following irradiation with germicidal UV at 254 nm, and although this was interpreted as a depression of the response of the melanophores to pigment aggregating substances (norepinephrine and melatonin), it seems probable that the highly biocidal wavelengths emitted from the UV-C source were sufficient to cause cell death which, as this study has demonstrated, manifests itself as a dense aggregation of melanin, quite devoid of any morphological identity.

The/

The observations in the present study on fish held in unprotected situations both at high altitude in Bolivia and in equatorial tropical conditions in Kenya indicate that, in relation to aquaculture, altitude is of fundamental importance owing to the overall increase in solar UV by around 4% per 300 m elevation (Diffey, 1982). The studies carried out at Naro Moru, Kenya, would have been more satisfactory if circumstances had allowed a longer timescale for the experiments and difficulties were encountered in ensuring maximum daily exposure due to experimental field conditions but, nevertheless, the limited response obtained there to solar UV in conjunction with the extreme evidence of radiation damage at the macroscopic level induced in the fish in both shore based facilities and in cages on Lake Titicaca at 3,700 m serve to emphasise this point. Thus it can be confidently assumed that UV radiation trauma is a real and important factor to be considered in the design engineering for aquaculture projects at all elevations, but becomes progressively more significant with every 300 m of elevation.

The complex role of the skin in modulating the relationship of fish to their environment has already been emphasised, and it is a basic tenet of fish pathology that the maintenance of the integrity of the surface of the skin and gill is one of the main criteria for health. The aquatic/

aquatic environment, and indeed the cuticle itself, contains large numbers of potentially pathogenic organisms. Their numbers are greatly increased in enriched water systems and in farm conditions, although normally they are extremely limited in their pathogenic capability by their general lack of any invasive capacity in that usually they can merely attach to the relatively unattractive cuticular surface. Any factor which alters the nutritional state of the cuticular proteins, or which changes the surface characteristics of the cuticle, allows ready attachment, multiplication and growth, and possibly further penetration to a wide range of organisms. Often this may be a multi-stage process with relatively innocuous organisms such as myxobacteria beginning the colonisation, and, after a period, the limited pathological change they induce allowing them to be replaced subsequently by more significant pathogens such as the aeromonads. The type of trauma induced by even low levels of UV-B irradiation in this study would appear *prima facie* to be precisely the sort of moderation of epithelial surface to allow such infection and indeed this has proved to be the case. It is obvious from the scanning electron microscope study that bacterial infection of the altered surface can occur early in the lesion, the results of the clinical outbreaks described further this contention.

In/

In natural conditions there are reports of very characteristic lesions of the dorsal fin, known as 'saddleback', occurring in fish farms and impoundments where *Chondrococcus columnaris*, one of the more pathogenic myxobacteria is the predominant pathogen (Morrison et al. 1981). Similarly, in the reef silverside, *Atherina harringtonensis*, a condition of the dorsal fin culminating in *Vibrio parahaemolyticus* infection and death, is observed regularly in wild fish and aquarium specimens (Rand, pers. comm.). In both cases the initial lesion, by its location and association with high incident light opportunity, would seem to be strongly suggestive of a sunburn primary aetiology.

The evidence presented in this study and in the work of Hunter has demonstrated the significance of UV-B as a source of traumatic damage to fishes by virtue of its effect on nuclear DNA. Evidence exists for such effects occurring in wild populations and the experimental work of Hunter and his colleagues emphasised the vulnerability of planktonic larval species in particular.

The contribution that planktonic animals make in both marine and freshwater conditions to the food chains of the earth's ecosystem are immense and the effect of an even minor increase in incident UV-B on such systems could be profound. Nothing in the present study suggests that the widespread concern for the effects of various ozone/

ozone inhibitors ranging from aerosol sprays to supersonic aircraft on the ozone layer, which is the primary filter for such short wave radiation, is misplaced.

The involvement of ultraviolet radiation in integumental neoplasia in higher animals is already well documented (Blum, 1959). There is currently no extensive literature to suggest such an aetiology for fish neoplasia, but a number of well recognised skin tumours have been reported for the marine flatfishes (Peters *et al.*, 1978). These are usually found on the dorsal surface and take the form of papillomatous or carcinomatous circumscribed lesions, often occurring with a high frequency in certain populations or areas. No viral or other infectious aetiology has been adduced, and, whilst views on their aetiology must currently remain open it is pertinent to note that Hoffman (1976) recognised the significance of ultraviolet radiation as a possible agent in such conditions. In addition the pioneering work of Setlow & Hart (1974) in demonstrating that neoplastic transformations could be induced in fish by the use of short wavelength UV indicates that, however remote, the possibility of UV induced neoplasia occurring in wild populations subjected to ultraviolet radiation cannot be discounted.

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