The same change is seen to a lesser degree in control embryos and this is possibly related to endoderm regeneration since previous studies[19,20] and our own unpublished observations show that endoderm regeneration begins in the primitive streak. It is possible that before the rest of the mesoderm can begin to regenerate endoderm its cells may have to lose some of their more specialised mesoderm features and revert to a condition more like that of the primitive streak. Neuraminidase appears to exaggerate this normal response. Ruthenium red staining is not a sufficiently sensitive method to detect any loss of surface coat in the untreated specimens. In other cell types the surface coat is constantly renewed to replace losses during cell activities[21,22]. However, during development it is possible that the rate of synthesis may be varied by external stimuli or internal genetic factors, leading to altered surface properties of the cell concerned. These may then be demonstrated histochemically[11] or as changes in cell behaviour or shape[23].

Changes in cell shape, are produced by the action on the cell membrane of an internal cytoskeleton of protein microfilaments[24–27]. These filaments have insertions on the plasma membrane and may even attach to the intracytoplasmic protein end of surface coat glycoproteins[4,27]. By cross linking membrane glycoproteins Rees et al.[27] stabilised the shape of cultured cells. Thus they demonstrated a functional link between the relationships of adjacent glycoproteins in the surface coat and the activities of the cytoskeleton by altering the composition of the surface coat we may similarly have altered the arrangement or degree of contraction of cytoskeletal filaments in the mesoderm cells to one characteristic of the primitive streak, thereby changing the cells' shape and reversing the normal developmental process.

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Structural differences of cone 'oil-droplets' in the light and dark adapted retina of *Poecilia reticulata* P.

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Summary. The vast majority of 'oil-droplets' in the dark and light-adapted retinal twin-cones of *Poecilia reticulata* is of the 'matrix-type'. In bright light (day light + overhead strip light) there occurs in some regions a very pronounced numerical change from 'matrix' to 'cristate-type', whereas other regions remain unaffected. The functional significance of these differences is discussed.

Oil-droplets are abundant in the retinal cones of amphibia, reptiles and birds. They are thought to constitute intraocular filters which may serve to increase contrast, reduce glare and lessen chromatic aberration[1–3]. Oil-droplets were, for a long time, considered to be absent in teleosts, until Berger[4] described globular structures, which he called 'oil-droplets', in the twin-cones of *Poecilia reticulata* (*Lebistes reticulatus*). Subsequently, 'oil-droplets' were observed in the cones of other teleosts, all members of the Cyprinodontoidei and the closely related Exocoetoedei[5–7].

In teleosts, with 'oil-droplets', the ellipsoidal mitochondria mature in a vitreoscleral direction, and the 'oil-droplets' are considered modified scleral-end mitochondria[5–8]. *P. reticulata* has 4 types of cones arranged in tiers, which, in accordance with their distance from the membrana limitans externa, are called outer, middle and inner cones. The outer tier is made up of twin-cones and the 2 other tiers contain single cones[9]. The 'oil-droplet' is observed only in the twin-cones, and only in their shorter accessory member. There are 2 types of 'oil-droplets': a) 'matrix', with the cristae limited to the periphery and the lumen filled with a densely staining granular material; b) 'cristate', which contains vesicular membranes, clumps of fibrous material and a matrix similar to that of a mitochondrion[4,8]. The principal member of the twin-cones and the single middle cones also show a maturation of mitochondria, following a vitreo-scleral gradient to become progressively denser[4,8]. The single, inner cones display a mitochondrial size gradient from the periphery to the centre[8]. However, none of these cones develop an 'oil-droplet'. Various classical histochemical tests failed to reveal lipids in the lumen of the 'oil-droplets' of *P. reticulata*[10]. It appears that none of the 'oil-droplets' of other teleosts have been tested histochemically.

Between cristate and matrix type 'oil-droplets', intermediate stages have been observed. This would suggest that 'cristate' and 'matrix', rather than being 2 different types of droplets, may in fact merely represent different metabolic states of the cones. To test this hypothesis, eyes of fish kept in the dark and in the light were compared.

Material and methods. Adult fish (eye diameter > 1.6 mm) were used. Group 1 was dark adapted for 3 h. Group 2 consisted of daylight adapted fish taken from stock aquaria.

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Group 3 was light adapted for 3 h in a 12-l-tank, without gravel and plants, and with an additional light from a 30-W clear striplight overhead. The eyes were fixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in Araldite. Ultrathin and semithick (1 µm) dorsoventral sections were made. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed with a Philips 201C Electron Microscope. Of the serially sectioned semithick sections, every second batch of 10 was collected and stained with toluidene blue (the intervening batches of ten being discarded). Using a M 20 Wild Research Microscope, with drawing tube attachment and a pantograph, the 'oil-droplets' were mapped and 2 polystyrene models, showing their exact distribution, constructed. 4 different types of droplets, ranging from type 1 (fully cristate) to type 4 (fully matrix) were distinguished. Since the rim of the eye only contains immature cones, it was not included in the mapping.

Results and discussion. Dark adapted eyes (group 1): The vast majority of 'oil-droplets' were of the matrix type (i.e. types 3 and 4) (figures 1a, 2). On average, 100 cristate droplets (mainly type 2) were found in each eye. The distribution of these was not consistent in the eyes tested but, as a rule, coincided with the area covered by the chorioid gland.

The twin-cones with cristate droplets make up only 0.01% approximately of all the twin-cones. This percentage is calculated, using the data provided by Müller[9], and by taking into account that only half of the sections of each eye were analyzed. (The total number of cones in the adult eye of *Poecilia* is 266,400, and the percentage of twin-cones, counted in dorso-ventral sections, is 73% of all cones.) The percentage of twin-cones containing cristate droplets would be higher, if only the area over the chorioid gland, where they occur, were considered. Moreover, this area has a lower density in cones and a lower ratio of twin-cones to single cones than has the ventral region.

Light adapted eyes (group 2): The results were similar to group 1, both in regard to distribution and number of cristate droplets. No differences between eyes, taken at different times of the day, were observed (figure 1a).

Light adapted eyes (group 3): The majority of droplets were of the cristate type (types 1 and 2). A definite regionalization was observed. Cristate droplets (type 1) were abundant in the dorsal region of the eye. The fundic region, around the exit of the optic nerve, contained all types. The ventral area was composed almost exclusively of matrix droplets (mainly type 4) (figures 1, 3, 4).

These observations of the light adapted retina explain the light microscopical findings of Müller[9]. He stated that oil-droplets are absent in *Poecilia* but described the accessory member of the twin-cones as having either 'unstained, clear spheres' (helle Kugeln) or 'barrel-shaped masses which stain light red with azan'. He also noted the absence of the 'clear spheres' in the ventral region. Accord-

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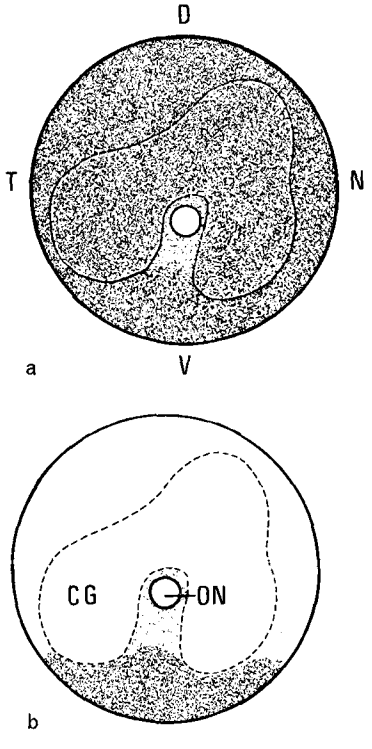

Fig. 1. Diagram of eye bulbus (rear view) demonstrating regional differences. *a* Dark and daylight adapted. *b* Adapted to bright light (group 3). CG, Horseshoe shaped chorioid gland, D, dorsal; N, nasal; ON, optic nerve; T, temporal; V, ventral.
▩ Predominance of matrix 'oil-droplets'; ▒ mixture of 'oil-droplets'; □ predominance of cristate 'oil-droplets'.

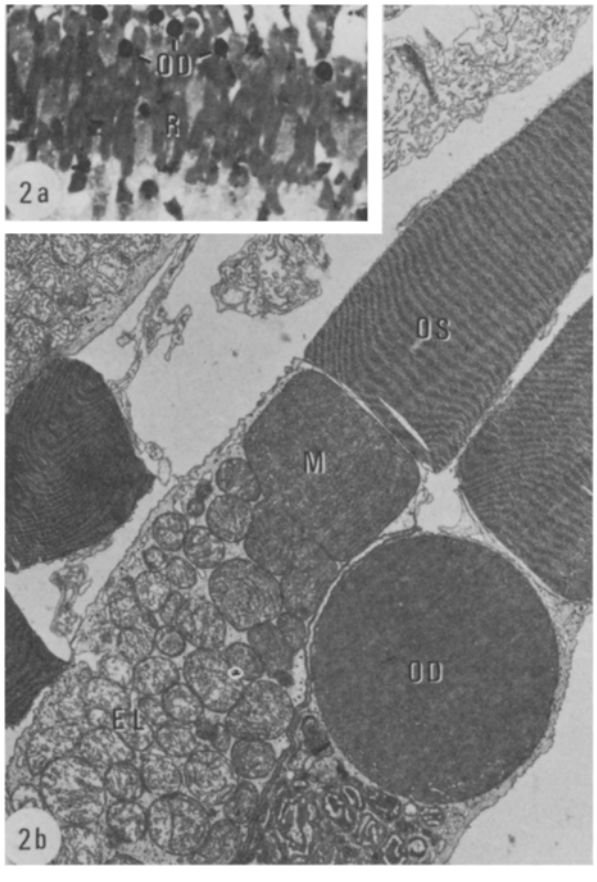

Fig. 2. Dorsal area of dark adapted eye. (Note: rods [R] in vitreal position and pigment granules retracted outside the frame of the photograph.) *a* Semithick section with 'oil-droplets' (OD) of matrix type. ×600. *b* Electron micrograph of twin-cone showing vitreoscleral size gradient of mitochondria. The accessory member contains a matrix droplet (OD) and the principal member a large mitochondrion (M) with concentrically arranged cristae. ×9000.

ing to Wagner[11], the twin-cones of *Poecilia* are unequal in the central and equal in the ventral area. Our observations indicate that it is the absence or presence of cristate 'oil-droplets' which, at light microscopic level, imparts equality or inequality to the twin-cones.

Bearing in mind that histological pictures give a static account of kinetic processes, it can be stated that bright light (group 3) results in a predominance of cristate droplets in the dorsal region, whereas in its absence the matrix droplets are the abundant type. The ventral region seems to remain unaffected. This agrees with the observations by Müller[12] who showed that during dark adaptation the 'colourless spheres' of the dorsal area are gradually replaced by granular material with affinity for azan stain. Thus, while the oil-droplets in amphibia, reptiles and birds have an optical function[1], in *Poecilia* they seem to be mainly an expression of the metabolic state of the photoreceptor.

The argentea of *Poecilia* shows regional differences with regard to structure and colour[13]. The ventral region, characterized by 5 layers of iridophores, is congruent with the ventral photoreceptor region, showing matrix droplets in any light conditions tested (figure 1b). The differences resulting from exposure to bright light seem to reflect functional differences between areas. The dorsal region receives only light reflected from the bottom. It has large, loosely packed cones and the relatively highest number of rods, which are all characteristics of dim light vision. The ventral region is liable to possess high visual acuity, since its short cones are densely spaced[9, 14, 15]. Since fish feed by cone vision and *Poecilia* is a surface feeder, its ventral region is mainly engaged in food detection and food catching. The main visual axis (general direction of gaze) passes through the fundic region, which is characterized by cones intermediate in size and density[9]. In eyes exposed to light, the visual pigments of the outer segments are continuously bleached and regenerated. Photon capture by the outer segments must trigger metabolic events in the ellipsoids, which are densely packed with mitochondria. The 'oil-droplet' is a scleral-end, modified mitochondrion, and it would seem that in *Poecilia* there is a continuous changeover between cristate and matrix state. The electron-dense deposits in the matrix droplets probably participate, in some way, in the regeneration of visual pigment. In the fully differentiated, but as yet hardly functional twin-cones of the *Poecilia* embryo (which develops within the maternal ovary), only cristate type 'oil-droplets' are observed[16]. Under the conditions of bright light (group 3), the cones of the dorsal region, which have probably a low visual threshold, are no longer able continually to replenish the deposits of the matrix droplets and the equilibrium gradually changes towards the cristate state. It has been

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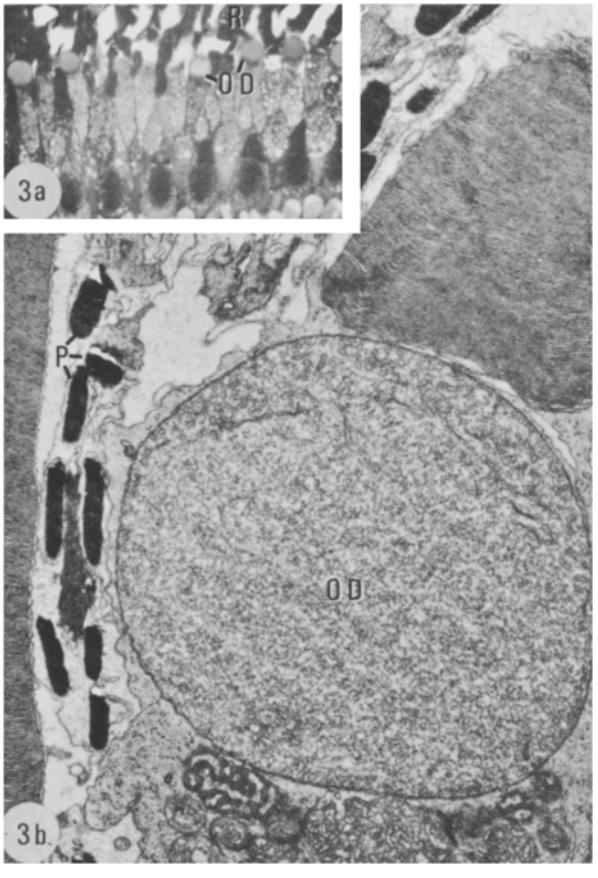

Fig. 3. Dorsal area of light adapted eye (group 3). (Note: rods [R] in scleral and pigment granules [P] in vitreal position.) *a* Semithick section revealing 'oil-droplets' (OD) of cristate type. ×600. *b* Electron micrograph of accessory member of twin-cone with cristate 'oil-droplets' (OD). ×13,500.

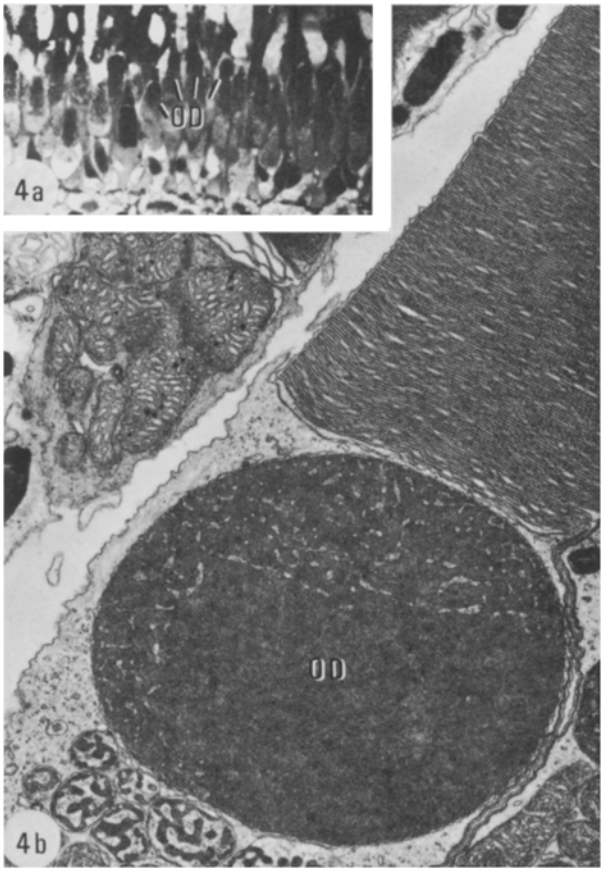

Fig. 4. Ventral area of light adapted eye (as for figure 3). *a* Semithick section with matrix 'oil-droplets' (OD). ×600. *b* Electron micrograph with accessory member of twin-cone showing matrix 'oil-droplets' (OD). ×13,500.

shown that ATP-ase activity in the retina is significantly accelerated by illumination[17]. Cations involved in ATPase activity, such as Ca^{++}, Mg^{++} and also inorganic phosphorus (P_i), are accumulated in relatively large amounts by isolated mitochondria. In vivo, mitochondria will rapidly accumulate i.p. injected ^{45}Ca and also ^{56}Mn and ^{89}Sr[18]. It was also suggested that Ca^{++} may play a role in the generation of the photoreceptor response to light[19]. It is hoped, therefore, that the planned elemental analyses of the *Poecilia* 'oil-droplet', under different light conditions, will provide a further insight into its functional significance.

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Acceleration of crypt cell proliferation by acoustic stimuli

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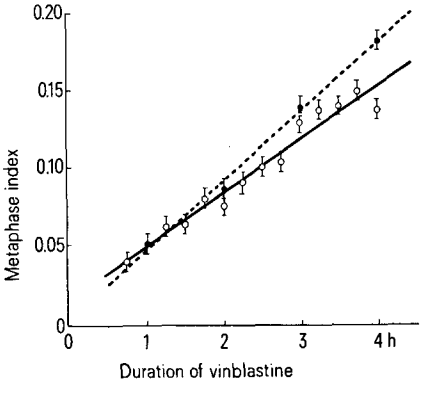
Summary. Cell proliferation rates in the bases of the crypts of Lieberkuhn in the jejunum of rat were measured using a stathmokinetic technique. In rats subjected to recurring loud noise cell proliferation was more rapid than in rats not subjected to the noise.

A wide variety of environmental stimuli, all of which could be considered to constitute forms of stress, have been shown to influence epithelial cell proliferation. For example, epidermal cell proliferation in mice is inhibited during both the acute stress of circulatory shock[1] and the chronic stress of overcrowding[2]. In the intestinal epithelium mild stress in the from of exercise promotes cell division[3] whereas more severe stress created by peritonitis[3] or electric shocks[4] inhibits cell division. In this communication the influence of auditory stress on jejeunal crypt cell proliferation is reported.

Materials and methods. Adult male Sprague-Dawley rats were used throughout the experiment. The mitotic rate in the bases of the crypts of Lieberkuhn in jejunum was measured using the stathmokinetic (that is, metaphase arrest) technique previously described[5]. All mitotic indices were corrected for sectioning artefacts[6]. In order to avoid errors attributable to the circadian rhythm in crypt cell proliferation[7], all estimates of mitotic rate commenced at 12.00 h. The mitotic rate was measured in 4 rats which were placed in a special cage and intermittently exposed to an acoustic stress of mixed frequency at 125 dB. This stress was applied for 1 min at the beginning of each 15-min-interval during the 4 h of the experiment. The mitotic rate was also measured in 14 rats not exposed to auditory stress.

Results and discussion. In rats not exposed to auditory stress (controls) the mitotic rate was 0.035 ± 0.002 (mean $\pm$ SE) mitoses per cell per h. In rats exposed to the auditory stress the mitotic rate was 0.045 ± 0.003 mitoses per cell per h. Analysis of variance shows that this value is significantly higher than that in control animals ($p < 0.05$). Graphs of mitotic index versus time after injection of vinblastine in control and stressed rats is illustrated in the figure.

Many hypothetical mechanisms could be proposed to explain the above result. One such proposal which is perhaps worthy of consideration is that the recurring loud noise simply leads to a general awakening of the rats from their usual daytime rest period. The animals used in the experiments are, of course, nocturnal and normally have their most rapid crypt cell proliferation between 0.00 and 4.00 h[7]. This nocturnal acceleration of crypt cell proliferation has been shown to be dependent upon the integrity of the sympathetic nervous system[8]. Thus, auditory stimuli may awaken the animal and promptly stimulate cell proliferation via a neural mechanism.



Accumulation of blocked metaphases as a function of duration of vinblastine treatment. ○——○, Controls; ●·····●, animals exposed to intermittent noise at 125 dB.

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