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The effect of actinomycin D on the nucleolus and on pigment synthesis in pigment cells of *Xenopus laevis*: an ultrastructural study

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INTRODUCTION

The antibiotic, actinomycin D (AMD) (a DNA-dependent RNA synthesis inhibitor) inhibits pigment formation in neural crest-derived pigment cells of *Xenopus laevis* (Kramer, 1972); accompanying this inhibition is a reduction in RNA synthesis in these cells (Kramer, 1978). These effects of the antibiotic are reversible (Kramer, 1979).

An ultrastructural study of normal melanogenesis in the species concerned was undertaken to show in what way the morphology of melanogenesis was affected by this antibiotic. Also, the effect of the antibiotic on the nucleolar components was examined to assess whether the morphological relationships of these components were altered when cellular differentiation was inhibited.

The nomenclature used in this study for the ontogenetic stages in the formation of melanin, is that proposed by Fitzpatrick *et al.* (1966). According to their definition a *melanosome* is "a discrete melanin-containing organelle in which melanization is complete; (which is) shown to be more or less uniformly 'electron dense' by electron microscopy (and in which) tyrosinase activity is not usually demonstrable." *Premelanosomes* are defined as "all particulate stages in the maturation of melanosomes, (in which the) electron density is variable (and which) possess an active tyrosinase system after the onset of melanin synthesis." The enzyme tyrosinase has not been tested for in the present study: identification of the various stages has been based on the structural features only. Premelanosomal stages are divided into *early*, *intermediate* and *late* in order to reflect increasing amounts of melanin and decreasing visibility of structural features within these organelles.

MATERIALS AND METHODS

Neural crest of *Xenopus laevis* was cultured by means of the hanging drop technique according to the method described by Andrew & Gabie (1969). Embryos of stages 19–21 (staging according to Nieuwkoop & Faber, 1967) were used. Trunk neural crest, from which the outer layer of surface ectoderm had been removed as previously described (Kramer, 1972) was excised. Pieces of excised tissue were cultured in Niu & Twitty's (1953) balanced salt solution (BSS) at 18 °C.

Treatment of the cultures with actinomycin D (Merck, Sharp & Dohme) began at between 22 and 24 hours post-explantation, once at least 10–12 recognisable pigment cells had migrated from the explant, i.e. the drug was not administered until

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differentiation of the pigment cells had definitely begun. The concentration of AMD used was 10 μg/ml BSS, except for one small batch of cultures which was treated with a dose of 2 μg of AMD/ml. The full duration of treatment was four and a half hours after which the medium containing the AMD was replaced by fresh BSS.

The procedure carried out concurrently on control cultures was identical to that used on the experimental ones, except that AMD was omitted when the medium was changed.

Photographs of the living control cultures and experimental cultures were taken immediately before treatment; at $\frac{1}{2}$, 1, 2, 3 and 4 hours, between 10 and 17 hours and between $62\frac{1}{2}$ and $72\frac{1}{2}$ hours (selected cultures) after the start of treatment.

Preparation of cultures for electron microscopy

Those cultures which were used for transmission electron microscopy were grown on coverslips initially coated with a layer of carbon and subsequently with gelatin. These cultures, still on the coverslips, were fixed in Karnovsky's fixative for one hour and post-fixed in 1 % osmium tetroxide for 30 minutes. They were routinely dehydrated in a graded series of alcohol, cleared in propylene oxide and then embedded in Araldite in Beem capsules. The Araldite was polymerised at 60 °C for 48 hours with the coverslip in position. The carbon film interposed between the Araldite-embedded cells and the coverslip facilitated their separation, which was effected by immersion of the coverslip and capsule in liquid nitrogen. Thin sections were stained with uranyl acetate and lead citrate and viewed in a Siemens transmission electron microscope.

Two batches of neural crest explants were fixed for scanning electron microscopy. One batch was fixed two hours after the start of treatment with AMD; the other was fixed 19 hours after the end of treatment. Control cultures for both batches were fixed at the same times. The cultures were fixed by immersion in Karnovsky's fixative for 10 minutes, washed in glass distilled water (Boyd, Weiss & Veseley, 1972) and air dried. The cells were then coated with a layer of gold in a vacuum chamber. Viewing and photography were carried out on a Cambridge Stereoscan S_4 scanning electron microscope.

RESULTS

In scanning electron micrographs, differentiating pigment cells of control cultures are seen to be flat and thin. Even in the region of the centrally placed nucleus the cells are only slightly thickened. The body of the cell tapers so that the peripheral cytoplasmic processes are extremely thin and do not contain melanin granules. These organelles, as well as the yolk platelets, cause bulges on the surfaces of the body and the thicker processes of the cells (Fig. 1). Transmission electron microscopy demonstrates the presence of Golgi apparatus and cisternae of smooth and rough endoplasmic reticulum in the cytoplasm. The planes of the cisternae are at right angles to the substrate surface (Fig. 2).

The early premelanosomes are ovoid or spherical in shape and consist of an electron-lucent matrix containing elongated structures (Fig. 3) or, less often, small dense vesicles. It is not possible to determine whether the elongated structures seen within a premelanosome are filaments or laminae. As so much of the length of the structure is seen within a section, it seems unlikely that filaments have been sectioned, but rather laminae.

The intermediate stage of the premelanosome is characterised by the electron-dense nature of the laminae which have a periodic substructure as seen in Figure 4. The increased deposition of electron-dense material almost entirely obscures the internal structure of the late premelanosome. The melanosome is somewhat larger in size than the premelanosome, is rounded in shape and has no visible internal structure (Fig. 2); the outer membrane is not often seen.

Prior to treatment, differentiating pigment cells contain one or two large nucleoli composed of two portions – an inner, smaller, dense, fibrillar area and an outer, ovoid, less dense, granular area (Fig. 5). In the cytoplasm numerous yolk platelets, mitochondria, free ribosomes, polyribosomes and a few early premelanosomes are present. Well-developed Golgi complexes, rough endoplasmic reticulum and many smooth-membraned vesicles are also seen.

AMD markedly affects the structure of the nucleolus in differentiating pigment cells. The larger dose of AMD (10·0 μg/ml) has a much more dramatic effect on nucleolar size, shape and structure than the smaller dose (2·0 μg/ml). At the higher concentration the nucleolus disappears or where still present is 'subdivided' or reduced in size, while at the lower concentration 'subdivision' and/or less marked reduction in size occurs.

In general a comparison of experimental and control pigment cells reveals the features shown in Table 1. The nucleoli of experimental cells are markedly different: their morphological components vary from peripheral granular masses or blebs associated with large condensed inner masses, to small central condensed areas with undivided surrounding granular areas (Figs. 6–9). A comparison of the nucleoli in Figures 6–9 with nucleoli of control cells at a comparable time (Fig. 10) clearly demonstrates the greater condensation of the inner zone and irregular clumping of the outer granular area of the nucleoli in treated cells, as well as their smaller size. Yet other nucleoli were not composed of two distinct zones, but of a granular substance which varied in density (compare Figs. 11, 12) and appeared to be a confluence of the two zones. In a large number of nuclei, no nucleoli are evident (Fig. 13) but small masses of condensed chromatin are present. The nucleoli of untreated cells however, retain two distinct zones throughout (Fig. 14).

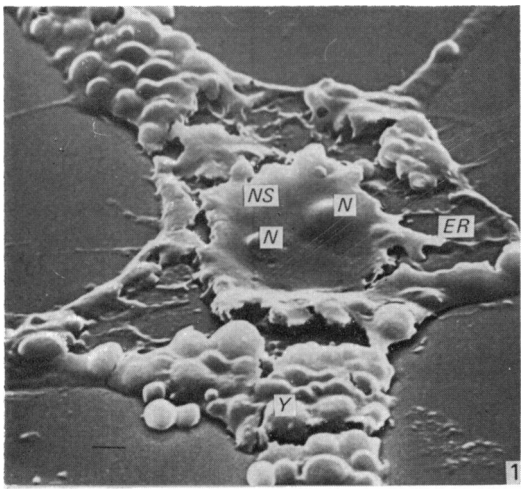
If a nucleolus is present at all in a treated cell at 3 hours it is much smaller than that of a comparable control cell, as well as being slightly but consistently smaller than the nucleoli of cells treated for two hours (compare Fig. 15 with Fig. 16 and Figs. 6–9).

The Golgi apparatus of treated cells is more distended than in untreated cells, and there is a marked reduction in the amount of rough endoplasmic reticulum. Numerous smooth-membraned vesicles occur in treated cells, while fuzzy-membraned vesicles are mainly apparent in the more differentiated untreated cells (Figs. 17, 18).

Treated cells contain far fewer melanosomes and only the later stages of pre-melanosomes (Fig. 19) while untreated cells contain both in rapidly increasing numbers (Fig. 20).

Yolk platelets are more numerous and larger in the treated cells while fewer free ribosomes are present in treated cells.

Two to three hours after the end of AMD treatment, premelanosomes are once more evident in the cytoplasm although fewer melanosomes are present than in untreated cells at the same time (Table 1). Polyribosomes are again evident and the nucleoli are similar to those of controls in that they do not show two distinct zones.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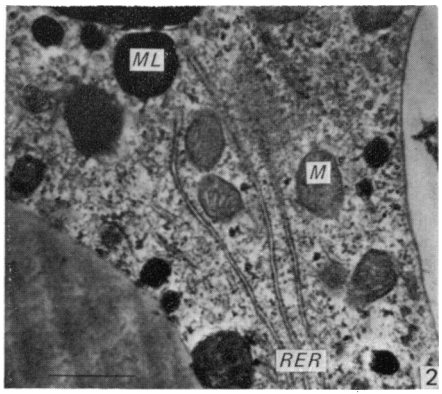
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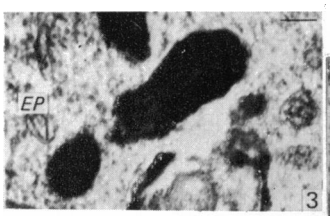
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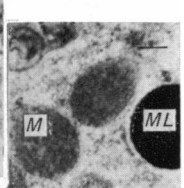
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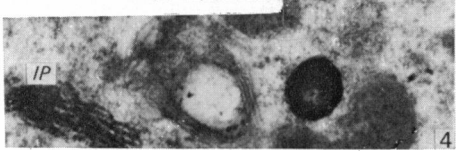
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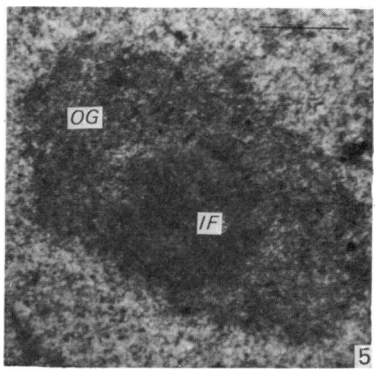
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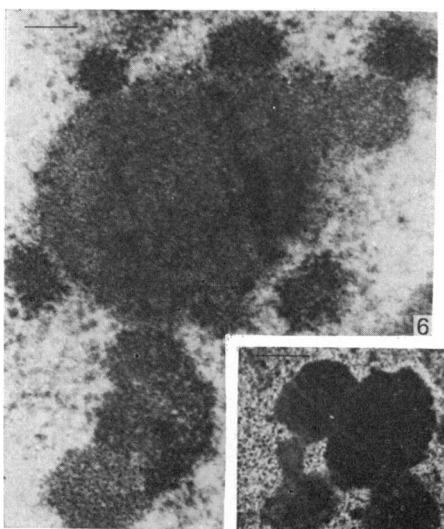
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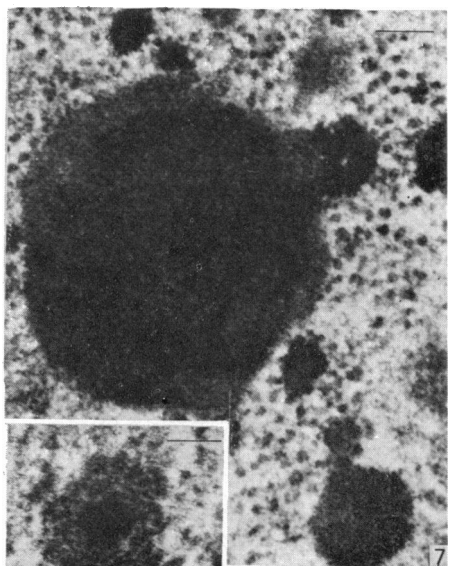
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DISCUSSION

The most marked effect of AMD on pigment cells was on the morphology of the nucleoli and on the formation of the melanin granules.

On occasion in the nucleoli of treated pigment cells there appeared to be 'confluence' of the two zones, which were previously separate, into one mass. In Chang liver cells treated with AMD, Reynolds, Montgomery & Karney (1963) also demonstrated redistribution and coalescence of nucleolar material, changes which are comparable – if not identical – to changes sometimes observed in the treated cultures. Reynolds *et al.* also described the appearance of electron-dense plaques which migrated toward the margin of the nucleoli, where they formed an irregular dark rim and finally a larger mass which they termed a 'dark nucleolar cap'. These dark nucleolar caps may be considered similar in distribution, if not in shape, to the blebs of material seen around the periphery of treated nucleoli in the present study.

The synthesis of rRNA is more sensitive to the inhibiting effects of nucleolar segregating antimetabolites than is that of other RNA species (Girard, Penman & Darnell, 1964; Wagner & Drews, 1970). This is interesting as diminution and disappearance of the granular nucleolar component would therefore imply impairment of rRNA synthesis. It has been shown that inhibition of rRNA synthesis by agents causing nucleolar segregation results in a depression of the large ribosomal RNA precursor molecules (Girard *et al.* 1964; Penman, 1966; Wagner & Drews, 1970; Recher, Briggs & Parry, 1971).

Approximately three hours after the end of treatment the nucleoli of control cells no longer consisted of two distinct zones but of a single homogeneous mass. The loss of a distinct granular region suggests less active nucleolar RNA synthesis.

Complete disappearance of nucleoli in some AMD-treated cells as seen in the present study has been observed by Jacob & Sirlin (1964) on salivary gland cells of *Smittia parthenogenetica* (Chironimidae) and by Schoefl (1964) on baboon kidney cells. Thus it seems that in the normal functional state the various nucleolar components must have a definite spatial relationship. Once the nucleolar functions (e.g. synthetic processes) have been interrupted, the ordered framework collapses as happens when the cells are treated with AMD. Izawa, Allfrey & Mirsky (1963) reported disappearance of loops of lampbrush chromosomes of amphibian oocytes

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Fig. 1. Pigment cell, prior to treatment. Note central, flat nucleus (*NS*), with two nucleoli (*N*). Yolk granules (*Y*) and endoplasmic reticulum profiles (*ER*) are also indicated. Marker represents 1 μm. Scanning electron microscopy.

Fig. 2. Control pigment cell, two hours after renewal of BSS. Orientation of cisternae of rough endoplasmic reticulum (*RER*) is at right angles to substrate surface. Yolk, mitochondria (*M*) and melanin granules (*ML*) are present. Marker represents 0·5 μm.

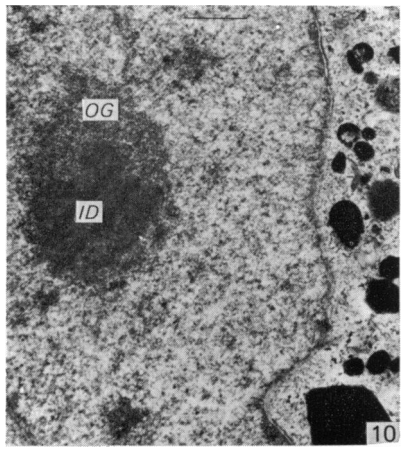
Fig. 3. Cell of control culture, two hours after renewal of BSS. Note early premelanosome (*EP*) containing fine laminae. Marker represents 0·1 μm.

Fig. 4. Cell of control culture, two hours after renewal of BSS. The intermediate premelanosome (*IP*) is characterised by the electron-dense nature of its laminae. *M*, mitochondrion; *ML*, melanin granule. Marker represents 0·1 μm.

Fig. 5. Nucleolus of pigment cell, prior to treatment. Note inner fibrillar (*IF*) and outer granular (*OG*) components of nucleolus. Marker represents 0·5 μm.

Figs 6–8. Nucleoli of cells treated for 2 hours with AMD. Large condensed inner masses have segregated from the peripheral granular 'blebs'. Markers represent 0·25 μm.

Fig. 9. Nucleolus with small central condensed area and undivided, surrounding granular mass. Marker represents 0·25 μm.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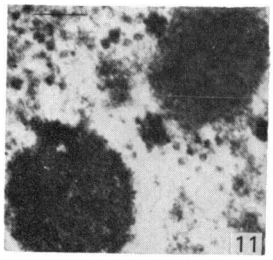
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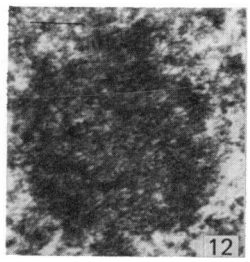
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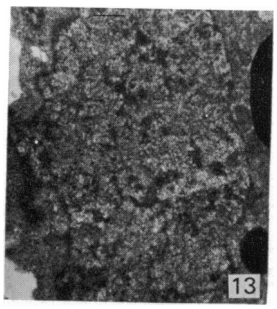
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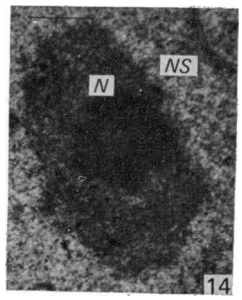
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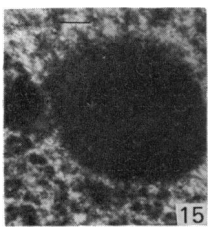
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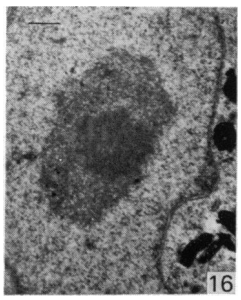
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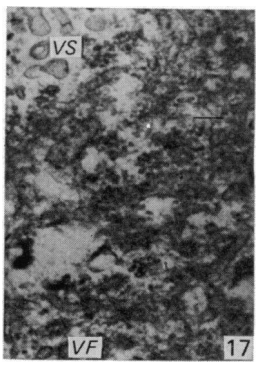
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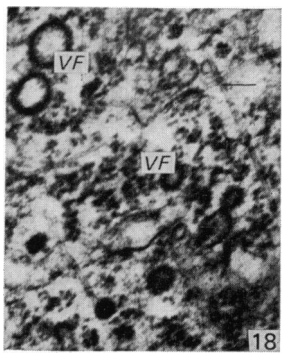
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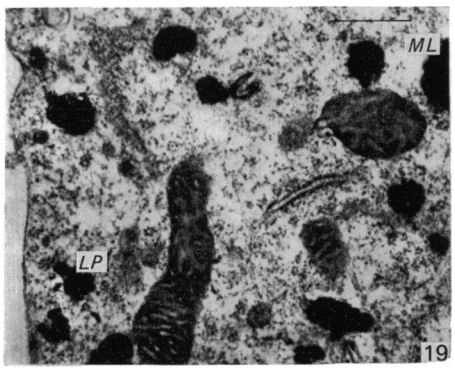
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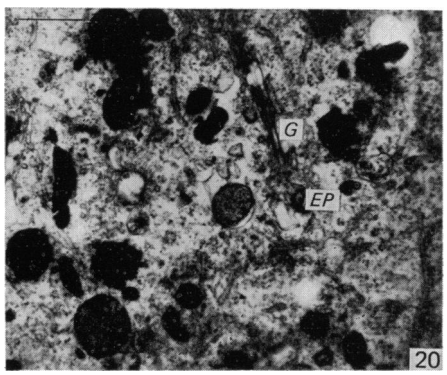
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after treatment with AMD. These authors concluded that the "morphology of an active (chromosomal) site is not only closely related to its capacity to synthesize RNA, but is dependent upon it." The results of the present study support this conclusion as return to 'normal' nucleolar morphology was followed by continued differentiation of the pigment cells (Kramer, 1978).

In the presence of the larger dose of AMD, the nucleolus went through remarkable morphological alterations before it ultimately disintegrated and disappeared. The diminution and condensation of nucleolar material that has been observed in this experiment are comparable to the changes seen with the light microscope in HeLa cells (Goldstein, Slotnick & Journey, 1960) and in connective tissue cells of the human ovary (Bierling, cited in Hackman, 1960) following treatment with actinomycins C, D, F_1 and F_3. The most striking effect of AMD on nucleoli in the present system was the distinct separating out of the two zones. Schoefl (1964) described a similar sorting out of pre-existing nucleolar components in baboon kidney cells treated with AMD. She, however, included a third component (amorphous material found near the nucleolonema) not seen either in her control cultures or in any cells in the present study.

As the granular component of the nucleolus seemed to undergo more marked changes in size and arrangement than the fibrillar portion in the present study, it appears that the former may be the source of ribosomal RNA precursors. Stevens (1964) found that treatment of dipteran salivary glands with AMD caused disappearance of RNA from the particulate (granular) but not from the fibrillar nucleolar component. Phillips & Phillips (1971) suggested that the particulate zones of the segregated nucleoli of fetal Chinese hamster and fetal mouse fibroblastic-type cells contain most or all of the RNA and that the fibrillar zones are principally proteinaceous. Sameshina, Shiokawa & Kawakam (1970), in the first study of the effect of AMD on the formation of nucleolar structure in cells of amphibian embryos, described a comparable situation. They found that the nucleoli of very early gastrula cells contained only a fibrous component, but that after two to five hours of culture a

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Fig. 10. Cell of control culture, two hours after renewal of BSS. Note inner dense (*ID*) and outer, less dense, granular portion (*OG*) of the nucleolus. Marker represents 1 μm.

Figs 11–12. Small nucleoli composed of a granular substance of variable density, in cells treated with AMD for 2 hours. Markers represent 0·25 μm.

Fig. 13. Nucleus of treated cell, two hours after the start of treatment. Note the apparent absence of a nucleolus. Marker represents 1 μm.

Fig. 14. Nucleus of untreated pigment cell, prior to treatment. Note prominent nucleolus (*N*) in homogeneously granular nucleus (*NS*). Marker represents 1 μm.

Fig. 15. Very small, condensed nucleolus of cell treated with AMD for three hours. Marker represents 0·1 μm.

Fig. 16. Nucleolus of untreated pigment cells, three hours after renewal of BSS. Note large size of nucleolus and presence of two components. Marker represents 1 μm.

Fig. 17. Pigment cell of culture treated with AMD for 2 hours. Note numerous smooth (*VS*) and fuzzy (*VF*) membraned vesicles. Marker represents 0·1 μm.

Fig. 18. Untreated pigment cell, two hours after renewal of BSS. Note the fuzzy (*VF*) membraned vesicles. Marker represents 0·1 μm.

Fig. 19. Cell of culture treated with AMD for 2 hours. This cell contains numerous very late premelanosomes (*LP*) and melanosomes (*ML*), but few, if any, early premelanosomes. Marker represents 0·5 μm.

Fig. 20. Untreated cell, two hours after renewal of BSS. Note Golgi complexes (*G*) and also very early premelanosome (*EP*). Marker represents 0·5 μm.

Table 1. *Comparison of features in untreated and actinomycin D-treated differentiating pigment cells as seen with the transmission electron microscope*

	Untreated cells			Treated cells			
	Prior to treatment	During treatment	2–3 hours after the end of 'treatment'	2 hours after the start of treatment	3 hours after the start of treatment	3½ hours after the start of treatment	2–3 hours after the end of treatment
Nucleolus	Distinct inner fibrillar and outer granular portions		Distinct zones not evident	Disappearance or blebbing and rearrangement of components	Smaller than nucleoli of 2-hour treated cell		Reappearance and/or increase in size. Distinct zones not evident
Mitochondria	+++++ Not dense	+++++ Not dense		++++ Dense	++++ Dense	++++ Dense	++++ Dense
Golgi complex	Well developed	Well developed	Well developed	Well developed and distended			Well developed, *not* distended
Vesicles							
Fuzzy-membraned	—	+++++	+	++	++	+++	+++
Smooth-membraned	+++++	+++	+	++++	++++	++++	+++
Rough endoplasmic reticulum	+++++	+++++	+	++	+	+	+++
Premelanosomes	+++	++++	+++++ late stages	++ Mainly late stages occasional intermediate	+ Mainly later stages		+++ All stages
Melanosomes	+++	++++	+++++	++	++	++	++
Free ribosomes	+++++	+++++	+++++	+	++	++	+++++
Polyribosomes	+++++	+++++	+++	+−¼	¼	¼	+++
Yolk platelets	+++++	++	¼	++++	++++	++++	++

¼, +−+++++ refers to the *number* of organelles at a particular stage, from extremely few at ¼ to very many at +++++.

granular component appeared. If AMD was added prior to the appearance of the granular component, this component failed to appear. They attribute the failure of the granular portion to appear to the inhibition of rRNA synthesis by AMD.

The reduction in the amount of rough endoplasmic reticulum during treatment of the pigment cells with AMD no doubt reflects a reduction in protein synthesis. The reduction in the number of ribosomes is evidence for repression by the antibiotic of ribosome formation in the nucleus. Girard *et al.* (1964) showed that the incorporation of rRNA into whole ribosomes involves a process which is AMD-sensitive. Smuckler & Benditt (1965) stated that AMD, if not given in very high doses, does affect finished cytoplasmic ribosomes. As fewer ribosomes were seen in AMD-treated cells in the present study than in control cells, and fewer than in cells prior to treatment, and since the dose of AMD was high, it is possible that the AMD not only inhibited the formation of the ribosomes, but also had an effect on the finished cytoplasmic ribosomes.

Penman, Sherrer, Becker & Darnell (1963) demonstrated a 50 % reduction in the number of functioning cytoplasmic polyribosomes after three hours of treatment of HeLa cells with AMD. As polyribosomes disappeared from treated cells in the present study, it seems probable that the antibiotic not only interferes with the formation of ribosomes, but inhibits also the formation of polyribosomes from those few ribosomes which are present. As the formation of polyribosomes is dependent on the presence of mRNA (Penman *et al.* 1963; Wettstein, Staehelin & Noll, 1963), the lack of polyribosomes despite the presence of some free ribosomes, may indicate that mRNA synthesis has been inhibited. Thus it appears that AMD treatment caused a reduction in newly produced rRNA and perhaps in mRNA.

In the present study, smooth- and fuzzy-membraned vesicles were seen associated with the Golgi apparatus before and during treatment with AMD. There were, however, far fewer fuzzy-membraned vesicles during treatment. It is not possible to decide whether or not the fuzzy-membraned vesicles have been 'pinched off' from the Golgi apparatus or whether they play a role in tyrosinase transport.

Few melanosomes were present in the AMD-treated cells. These are no doubt melanosomes which were formed prior to the onset of treatment. Some may have been derived from the oocyte and subsequently incorporated into the cells of the neural crest, as Piatt (1966) has shown that the entire neural axis contains egg melanin. Eppig (1970*a*, *b*) and Eppig & Dumont (1971) have shown the existence of pre-existing egg melanosomes in amphibian pigment epithelium of the eye.

It is clear from an autoradiographical study (Kramer, 1978) that RNA synthesis in these differentiating pigment cells is significantly inhibited by exposure to AMD. The reduction in number of ribosomes and rough endoplasmic reticulum and the disappearance of the granular component of the nucleolus occur concurrently with an inhibition of pigment formation indicating the functional correlation of the former with the latter.

As there were very many fewer premelanosomes of all stages in treated cells, it is evident that the formation of the structural part of the premelanosome depends on DNA-dependent RNA synthesis. Soon after removal of the antibiotic the number of premelanosomes increased slightly; this is thought to be indirectly due to the demonstrated increase in RNA synthesis which occurred during the latter part of the treatment (Kramer, 1978). The major reappearance of premelanosomes occurred 14–16 hours after the end of treatment. This indicates availability of additional sites

for RNA synthesis, and therefore the possibility of renewed pigment synthesis. These additional sites for RNA synthesis must have been freed on removal of the AMD (Kramer, 1979). As already suggested (Kramer, 1978) the delay in renewed pigment synthesis (14–16 hours) might be because the RNA synthesis after the end of treatment may not be used immediately or solely in the production of pigment.

Certainly structural premelanosome formation, and therefore melanosome formation, was inhibited by AMD. Thus, the synthesis of the structural protein component of melanin, and perhaps of tyrosinase, which is involved in melanin synthesis, was inhibited by AMD. It is believed that the AMD inhibits more than the synthesis of tyrosinase in the present study as then at least the structural premelanosomes would form. This is what occurs in the human albino who lacks tyrosinase – cytoplasmic structures mature to form apparent melanin granules but no melanin is deposited on them (Mishima & Loud, 1963). Fitzpatrick *et al.* (1966) and Hearing, Phillips & Lutzner (1973) report that premelanosomes are present in mouse albinos but these granules never mature. Thus non-melanized premelanosomes do occur in organisms of certain genotypes.

The synthesis of tyrosinase almost certainly occurs on ribosomes and since it may well be transported through the rough endoplasmic reticulum to the Golgi apparatus (Seiji & Iwashita, 1965; Maul & Brumbaugh, 1971), a decrease in the amount of rough endoplasmic reticulum, which in general suggests decreasing protein synthesis, may here reflect a decrease in the synthesis of tyrosinase as well as of structural and other proteins.

SUMMARY

The effect of AMD on the nucleolus and on melanogenesis in differentiating pigment cells of *Xenopus laevis* was investigated in cultured neural crest cells. Cultures were treated with either 2 or 10 μg/ml AMD for 4½ hours. Following treatment the antibiotic was removed. Observations of the cells were made with both scanning and transmission electron microscopes. Actinomycin D almost entirely stopped pigment formation in neural crest cultures during treatment.

The morphological sequence in the formation of melanin granules in the untreated pigment cells appears to be as follows: The earliest identifiable premelanosome is membrane-bound and contains very thin laminae and/or small vesicles. The premelanosomes become elongated until they are approximately the size of mature melanin granules and there is thickening of the laminae, which appear to have a periodic substructure. The cells eventually become packed with electron-dense melanin granules.

Compared with controls, the cytoplasm of treated cells showed a greater abundance of smooth- than of fuzzy-membraned vesicles, less rough endoplasmic reticulum, dilatation of the Golgi cisternae, and a much smaller number of premelanosomes. The nucleolus showed segregation and blebbing of its components, decrease in size and even disappearance; sometimes confluence of the components occurred. The most consistent morphological effect of AMD on the nucleolus was the separation of the fibrillar and granular areas.

The granular component appeared to undergo marked changes in size and arrangement and is thought to be the source of ribosomal RNA precursors. The alteration in size of the outer granular component of the nucleolus went hand in

hand with disappearance of free ribosomes from the cytoplasm of treated cells and inhibition of pigment synthesis.

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REFERENCES

ANDREW, A. & GABIE, V. (1969). Hanging drop culture of *Xenopus laevis* neural crest. *Acta embryologica experimentalis* **2–3**, 123–136.

BIERLING, R. See Hackman (1960).

BOYD, A., WEISS, R. A. & VESELY, P. (1972). Scanning electron microscopy of cells in culture. *Experimental Cell Research* **71**, 313–324.

EPPIG, J. J. (1970*a*). Melanogenesis in amphibians. I. A study of the fine structure of the normal and phenylthiourea-treated pigmented epithelium in *Rana pipiens* tadpole eyes. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **103**, 238–246.

EPPIG, J. J. (1970*b*). Melanogenesis in amphibians. III. The bouyant density of oocyte and larval *Xenopus laevis* melanosomes and the isolation of oocyte melanosomes from the eyes of PTU-treated larvae. *Journal of Experimental Zoology* **175**, 467–475.

EPPIG, J. J. & DUMONT, J. N. (1971). The distribution of melanosomes in larvae reared from normal and from pigmentless eggs of *Xenopus laevis*. *Journal of Experimental Zoology* **177**, 79–88.

FITZPATRICK, T. B., QUEVEDO, W. C. JR., LEVENE, A. V., MCGOVERN, V. J., MISHIMA, Y. & OETTLE, A. G. (1966). Terminology of vertebrate melanin-containing cells: 1965. *Science* **152**, 88–89.

GIRARD, M., PENMAN, S. & DARNELL, J. E. (1964). The effect of actinomycin on ribosome formation in HeLa cells. *Proceedings of the National Academy of Sciences* **51**, 205–211.

GOLDSTEIN, M. N., SLOTNICK, I. J. & JOURNEY, J. L. (1960). *In vitro* studies with HeLa cell lines sensitive and resistant to actionomycin D. *Annals of the New York Academy of Sciences* **89**, 474–483.

HACKMANN, C. (1960). The effect of actinomycins on experimental tumors. *Annals of the New York Academy of Sciences* **89**, 361–372.

HEARING, V. J., PHILLIPS, P. & LUTZNER, M. A. (1973). The fine structure of melanogenesis in coat colour mutants of the mouse. *Journal of Ultrastructure Research* **43**, 88–106.

IZAWA, M., ALLFREY, V. G. & MIRSKY, A. E. (1963). The relationship between RNA synthesis and loop structure in lampbrush chromosomes. *Proceedings of the National Academy of Sciences* **49**, 544–551.

JACOB, J. & SIRLIN, J. L. (1964). Electron microscope studies on salivary gland cells. IV. The nucleus of *Smittia parthenogenetica* (chironimidae) with special reference to the nucleolus and the effects of actinomycin thereon. *Journal of Ultrastructure Research* **11**, 315–328.

KRAMER, B. (1972). The effect of actinomycin D on developing pigment cells of *Xenopus laevis*. *Developmental Biology* **29**, 220–226.

KRAMER, B. (1978). The effect of actinomycin D on RNA synthesis in differentiating pigment cells of *Xenopus laevis*. *South African Journal of Science* **74** (12), 462–465.

KRAMER, B. (1979). Reversibility of the effect of actinomycin D on the nucleolus and pigment synthesis in pigment cells of *Xenopus laevis*. *South African Journal of Science* **57**, 130–132.

MAUL, G. G. & BRUMBAUGH, J. A. (1971). On the possible function of coated vesicles in melanogenesis of the regenerating fowl feather. *Journal of Cell Biology* **48**, 41–48.

MISHIMA, Y. & LOUD, A. V. (1963). The ultrastructure of unmelanized pigment cells in induced melanogenesis. *Annals of the New York Academy of Sciences* **100**, 600–617.

NIEUWKOOP, P. D. & FABER, J. (1967). *Normal Table of Xenopus laevis (Daudin)*, 168–169. Amsterdam: North Holland.

NIU, M. C. & TWITTY, V. C. (1953). The differentiation of gastrula ectoderm in medium conditioned by axial mesoderm. *Proceedings of the National Academy of Sciences* **39**, 985–989.

PENMAN, S. (1966). RNA metabolism in the HeLa cell nucleus. *Journal of Molecular Biology* **17**, 117–130.

PENMAN, S., SHERRER, K., BECKER, Y. & DARNELL, J. E. (1963). Polyribosomes in normal and poliovirus-infected HeLa cells and their relationship to messenger – RNA. *Proceedings of the National Academy of Sciences* **49**, 654–662.

PHILLIPS, S. G. & PHILLIPS, D. M. (1971). Nucleoli of diploid cell strains – their normal ultrastructure and the effects of toyocamycin and actinomycin D. *Journal of Cell Biology* **49**, 785–802.

PIATT, J. (1966). Origin and development of retinal melanin in *Ambystoma punctatum* devoid of ovarian pigment. *Journal of Experimental Zoology* **163**, 247–258.

RECHER, L., BRIGGS, L. G. & PARRY, N. T. (1971). A reevaluation of nuclear and nucleolar changes induced *in vitro* by actinomycin D. *Cancer Research* **31**, 140–151.

REYNOLDS, R. C., MONTGOMERY, P. O'B. & KARNEY, D. H. (1963). Nucleolar 'caps', a morphological entity produced by the carcinogen 4-nitroquinoline-N-oxide. *Cancer Research* **23**, 535–538.

SAMESHINA, M., SHIOKAWA, K. & KAWAKAM, I. (1970). The effect of actinomycin D on nucleolar formation in early *Xenopus laevis* gastrulae. *Journal of Experimental Zoology* **173**, 113–120.

SCHOEFL, G. L. (1964). The effect of actinomycin D on the fine structure of the nucleolus. *Journal of Ultrastructure Research* **10**, 224–243.

SEIJI, M. & IWASHITA, S. (1965). Intracellular localization of tyrosinase and site of melanin formation in melanocyte. *Journal of Investigative Dermatology* **45**, 305–315.

SMUCKLER, E. & BENDITT, E. (1965). The early effects of actinomycin on rat liver. *Laboratory Investigation* **14**, 1699–1709.

STEVENS, B. J. (1964). The effect of actinomycin D on nucleolar and nuclear fine structure in the salivary gland of *Chironomus thummi*. *Journal of Ultrastructure Research* **11**, 329–353.

WAGNER, L. & DREWS, J. (1970). The actinomycins and their importance. *Annals of the New York Academy of Sciences* **89**, 484–485.

WETTSTEIN, F. O., STAEHELIN, T. & NOLL, H. (1963). Ribosomal aggregate engaged in protein synthesis: characterization of the ergosome. *Nature* **197**, 430–435.