A STUDY OF THE ULTRASTRUCTURAL LOCALIZATION OF HAIR KERATIN SYNTHESIS UTILIZING ELECTRON MICROSCOPIC AUTORADIOGRAPHY IN A MAGNETIC FIELD

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ABSTRACT

The sites of the incorporation of labeled cystine into keratinizing structures were studied in electron microscopic autoradiographs. The tracer used was cystine labeled with S^{35} emitting long-range ionizing particles. During exposure for 1 to 2 months, according to our method of electron microscopic autoradiography, emulsion-coated specimens were exposed to a static magnetic field which appeared to result in a marked increase in the number of reacted silver grains. In young Swiss mice receiving intraperitoneal injections at 1, 3, and 6 hours before biopsy, conventional autoradiography demonstrated that S^{35}-cystine was intensely localized in the keratogenous zone of anagen hair follicles, and that the radioactivity there increased in intensity progressively with time while the radioactivity in the hair bulb always remained very low. Our observations with electron microscopic autoradiography in a magnetic field appeared to indicate that at 3 and 6 hours after injection the S^{35}-cystine was directly and specifically incorporated into tonofibrils in the hair cortex and into amorphous keratin granules of the hair cuticle layer, possibly without any particular concentration of this substance in the other cellular components. There seemed to be an appreciable concentration of cystine in tonofibrils of the cuticle of the inner root sheath. However, trichohyalin granules in the hair medulla and inner root sheath failed to show any evidence of cystine concentration. The improved sensitivity of the electron microscopic autoradiography with S^{35}-cystine appeared to be partly due to the application of a static magnetic field. However, the reason for this could not be explained theoretically.

INTRODUCTION

Recent progress in the application of autoradiographic techniques to electron microscopy has made it possible to relate the fine structure of cells to their metabolic processes. Most of the studies utilizing this technique are concerned with the ultrastructural localization of DNA synthesis because of certain favorable conditions; i.e., tritiated thymidine is specifically incorporated in DNA synthesized by cells prior to division (15, 17, 19, 24–26). Materials labeled with tritium, a low-energy beta emitter, are considered most suitable for obtaining high resolutions with this technique, provided that ultrathin sections of tissue as well as ultrathin layers of photographic emulsion are used. Recently, extensive studies have been carried out by Caro and his associates to establish reliable standard procedures for this technique using tritiated materials (8–10). Although it is theoretically ideal to use tritium emitting low-energy beta particles, either insufficient knowledge of certain metabolic pathways or unavailability of certain tritiated compounds from commercial

sources occasionally make it necessary to select materials labeled with medium-energy beta emitters such as C^{14} and S^{35}. This situation existed in our present study of the keratinization process.

Ultrastructural studies of keratinization in mammalian hair follicles have previously been carried out in great detail by Birbeck and Mercer (4–6), Mercer (22, 23), and other investigators (11, 14, 27, 28). Their morphological observations lead to the conclusion that tonofibrils and trichohyalin are the early cornification substances in cells of hair follicles.

On the other hand, light-microscope studies of mouse hair follicles utilizing conventional autoradiography (2, 3, 16) revealed that radioactive cystine is taken up directly by the keratogenous zone and does not pass up into it from the bulb. Bélanger observed that labeled methionine is also taken up by the hair follicles of the rat, since methionine is the most efficient substitute for cystine and can be converted to cystine in the body (1). Ryder demonstrated, by sequential biopsies of mouse skin shortly after intraperitoneal injection of labeled cystine, that the time taken for cystine to diffuse into the hair follicle from the blood stream appears to be only a few seconds, and that an appreciable radioactivity begins to extend into the prekeratinization region about 2 hours after injection (29). The presence of an appreciable activity in the bulb and the lack of activity in the prekeratinization region and in the keratogenous zone after 2 and 20 minutes suggests that sulfur enters above the bulb but not at such a high level (30). It was, therefore, interpreted by Ryder that the enrichment of sulfur on keratinization is a concentration effect (30). The amount of activity in the keratogenous zone increases remarkably during the first few hours after injection and a peak is formed after 3 hours, whereas the activity in the bulb begins to decrease (30).

The results of these autoradiographic studies appeared to be partly explained by the concept that keratin is a complex made up of fine filaments (alpha keratin) embedded in an amorphous substance (gamma keratin) (4, 22, 27). The filamentous component having a lower cystine content first appears in the mid-bulb, and, at the level of the prekeratinization region and above, gamma keratin which has a higher cystine content is added to the interfilamentous space of alpha keratin (4, 22). Therefore, the localization and the amount of activity of labeled cystine in the autoradiographic

studies (2, 3, 16, 29, 30) appear to coincide with the distribution of alpha and gamma keratin in the hair follicle revealed by electron microscope studies (4, 22, 27).

The purpose of this study is to demonstrate precise ultrastructural sites of the incorporation of S^{35}-labeled cystine into keratin precursors in the hair follicles. In the work presented here, we have attempted to apply a static magnetic field to our method of electron microscopic autoradiography using the medium-energy beta emitter.

MATERIALS AND METHODS

Swiss albino mice of both sexes from the colony of this laboratory were used. They were 8 days old and weighed approximately 3 gm each. At this age, the hair follicles were found to be either at substage anagen V or at the beginning of anagen VI when the actual hair proliferation phase started. Each of six animals received, by intraperitoneal injection, 0.1 ml of physiological saline containing 15 μc. of L-cystine labeled with S^{35} (Schwarz BioResearch, Inc., Orangeburg, New York; specific activity of 50 μc/mg). Punch and surgical biopsies of the skin were taken, under light ether anesthesia, from the upper middorsum of two animals at each time, at 1, 3, and 6 hours after the injection.

Surgically removed pieces of skin for conventional autoradiography were fixed in Carnoy's fixative for 1 hour, dehydrated in 100 per cent ethanol, and embedded in paraffin. An effort was made to section the tissues in such a way that longitudinal sections of hair follicles could be observed. Under safe light illumination (Kodak Wratten AO filter), the sections were coated by dipping into a solution containing 20 gm of Ilford G-5 nuclear research emulsion per 20 ml of distilled water, and drying in a vertical position. They were stored in a light-tight container with a small amount of Drierite at 4° C. After an appropriate period of exposure, they were developed in Kodak D-19 for 5 minutes at 20° C, fixed for 10 minutes in Kodak acid fixer, washed in running water, and then stained with hematoxylin.

Punch-biopsied tissues about 1 mm in diameter or less were immersed in freshly prepared Palade's fixative (1 per cent osmium tetroxide solution in acetate-Veronal buffer, pH 7.4) at 0° C as quickly as possible after removal. Fixation was continued for 1 hour. The tissues were rinsed in cold distilled water for a few minutes. They were thereafter dehydrated and embedded in Epon epoxy resin according to Luft's method (21). Fairly large sections, ranging from 800 to 1000 A in thickness, were cut with glass knives on an LKB ultrotome and picked up on titanium grids (E. Fullam, Inc., Schenectady, New York) with a Formvar film and a thin carbon

layer as recommended by Przybylski (25). Under safe light illumination (Kodak Wratten AO filter), 10 gm of Ilford L-4, an electron-sensitive emulsion of finest grain, were melted in 20 ml of distilled water in a 300 ml beaker at 45°C for 15 minutes. After thorough but gentle stirring with a clean glass rod, the beaker was cooled in an ice bath for 4 minutes and then placed at

were outside of the adhesive. In order to apply a static magnetic field to these coated grids in a proper direction, they had to be placed within two limited areas corresponding in size and relative position to the two poles of a horseshoe magnet (Fig. 1 a); they were not displaced during further treatments and storage. The glass slide with the emulsion-coated grids was
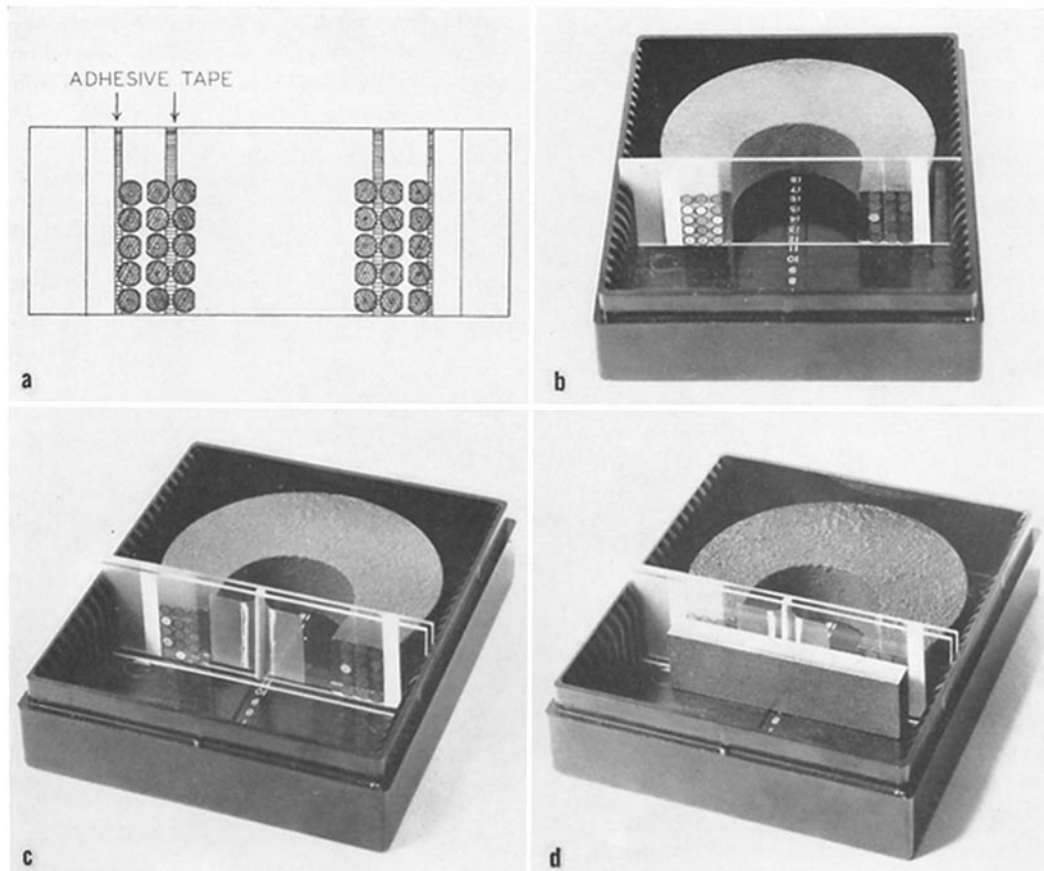

FIGURE 1 a to d   Procedure for exposing specimen grids coated with Ilford nuclear emulsion L-4 to a magnetic field (see details in text).

room temperature for 30 minutes according to Caro's method (10). The grids were coated with emulsion picked up with a copper loop. Subsequently the grids were exposed to a static magnetic field. For this purpose, they were mounted on a microscope slide placed in contact with the poles of a horseshoe magnet as shown in Fig. 1 a to d. Narrow strips of cellophane tape with adhesive on both sides were applied to the microscope slide in appropriate positions (Fig. 1 a). The titanium grids, having a wider edge than ordinary copper grids, could be affixed by the edge to the tape and remained parallel to the slide, so that sections mounted on the carbon-coated Formvar film

placed in a light-tight slide box with a small amount of Drierite, in the same way that histology slides are usually stored. Both poles of a strong horseshoe magnet made from an alloy of aluminum, nickel, and cobalt (Fisher Scientific Co.) were then brought in contact with the reverse side of the slide (Fig. 1 b). Another plain slide was inserted in the slit next to the grid-carrying slide. To insure a constant space between the two slides, a short wooden rod was taped to the middle of the second slide (Fig. 1 c). Finally, a thick rectangular iron plate was carefully applied to the outer side of the second slide (Fig. 1 d). By means of the strong magnetic

action, these two glass slides were sandwiched between the magnetic poles and the iron plate, yet a very narrow space between them was maintained to store the coated grids without any damage. Under these conditions, the lines of force in the static magnetic field were practically perpendicular to the emulsion-coated sections on the grids. The light-tight box was sealed with an electric black tape and kept in a refrigerator (0 to 4°C) for 1 to 2 months. Similar sections from the same blocks were also prepared by the same method without exposing them to a magnetic field.

were done with an RCA EMU 3F operating at 50 KV.

OBSERVATIONS AND RESULTS

Light Microscopic Autoradiography

In the mice that received injections 6 hours before biopsy, an intense localization of S^{35}-L-cystine was demonstrated in the keratogenous zone of all anagen hair follicles (Fig. 2). The hair bulb showed a slightly higher activity than the epidermis but
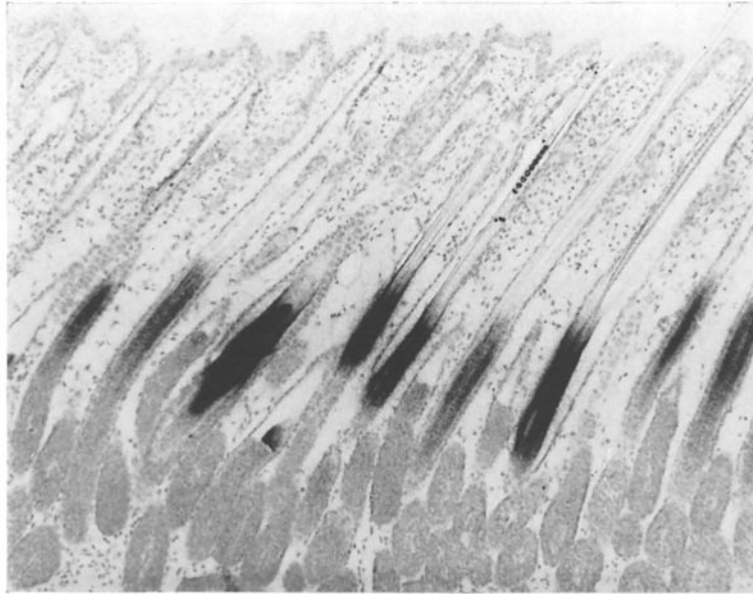

FIGURE 2   Photomicrograph of a light microscopic autoradiograph of skin with anagen hair follicles of 8-day-old mouse 6 hours after injection of S^{35}-L-cystine. Most of the radioactivity is in the keratogenous zone. Counterstained with hematoxylin. ×100.

After appropriate exposure, the coated grids were developed in undiluted and filtered Kodak D-19 developer for 2 minutes at 20°C, rinsed briefly in distilled water, and fixed in Kodak rapid fixer for 5 minutes. They were then washed for 10 minutes in several changes of distilled water and dried. They were lifted from the glass slides, and most of them were stained in a saturated solution of uranyl acetate in distilled water for 30 to 60 minutes, followed by a wash in three changes of distilled water. Some of the developed grids were treated for 15 minutes with the Karnovsky lead stain at high pH (18), which not only stained sections but also removed the gelatin to improve the quality of images as described by Hay and Revel (17). Observation and microphotographic recording of the processed grids

much less activity than the keratogenous zone. Sections biopsied 3 hours after injection recorded an appreciable radioactivity also in the keratogenous zone, but the grain density was markedly less than that of the 6 hour group. At 1 hour after the injection, the autoradiographs already indicated the incorporation of S^{35}-L-cystine at the level of keratogenous zone and prekeratinization region, although the activity was very weak. Therefore, it was noted that the concentration of S^{35}-L-cystine in the keratogenous zone increased progressively with time. Regardless of the time interval after injection, however, the localization was almost the same in all anagen hair follicles, and the radioactivity in the hair bulb always remained very low.

These observations made on 8-day old Swiss mice not only confirmed the previous experiments by Harkness and Bern (16), who used adult C57 black mice, but also indicated to us that the keratogenous zone of anagen hair follicles taken 6 hours following administration of the radioisotope would be most suitable for detecting an ultrastructural localization of S^{35}-L-cystine under the electron microscope because of the presence of the most intense radioactivity.

Electron Microscopic Autoradiography

Electron microscopic observation of the keratogenous zone of anagen hair follicles in Swiss mice injected with S^{35}-L-cystine 6 hours before biopsy showed that adequate amounts of the incorporated radioisotope were present in the ultrathin sections used for exposure to the coated emulsion. Photographic silver grains usually appeared as simple, coiled filaments or rods of complete, high electron opacity. Most of the grains present were seen to be associated with keratinizing structures in the hair and inner root sheath.

Fig. 3 shows an electron micrograph of a portion of the keratogenous zone of an anagen hair follicle which is cut longitudinally. All layers and main structures of the hair follicle in mice are indicated by lettering in Fig. 3.

In the cortex of the keratogenous zone, the tonofibril system was extremely well developed and the fine parallel tonofibrils formed distinct bundles with an increased electron opacity. Many silver grains were located over these bundles (Fig. 4). A much smaller number of grains was found to be located over tonofibrils in the cuticle of the inner root sheath. In the upper portion of the keratogenous zone, trichohyalin granules of the medulla appeared to change into a fibrous form where very few grains were seen. Cells of the hair cuticle layer in the keratogenous zone contained a number of small amorphous keratin granules or droplets, particularly in the peripheral portions of these cells. Grains were located quite often over these structures in spite of the fact that a very small space was occupied by these amorphous keratin granules (Fig. 5).

Trichohyalin granules were exclusively observed in the medulla and inner root sheath of hair follicles. Silver grains were rarely seen over these granules.

As expected from the results of light microscopic autoradiography, developed silver grains were rarely found over regions other than the keratogenous zone. Because of their random distribution and infrequent appearance, most of them were considered to be background.

In a survey of longitudinal sections of the keratogenous zone of many hair follicles, the number of grains over each cellular structure was counted. The detailed results are given in Table I. Out of a total of 708 grains counted, 452 grains (63.8 per cent) were found over tonofibrils of the hair cortex, 64 grains (9.1 per cent) over amorphous keratin granules of the hair cuticle, and 43 grains (6.1 per cent) over tonofibrils of the cuticle of the inner root sheath. Only 24 grains (3.4 per cent) were seen over the basic cytoplasm of cells in the hair cortex. The grain counting was done in materials stained with uranyl acetate, since it was felt that some loss of grains might be caused by Karnovsky's lead staining at high pH (18).

In the specimens examined, grains exposed in the emulsion outside of the keratin structures were few, and some of these were considered to be the normally present background. There were some grains located a few tenths of a micron away from tonofibril bundles (Fig. 4). We arbitrarily listed, in the column "vicinity of tonofibril," a small number of grains which were located within a distance of 0.1 μ from the keratinizing structures (Table I). The reason for this and the significance of these grains will be discussed later.

Electron microscopic autoradiographs of sections biopsied 3 hours after the injection of S^{35}-L-cystine revealed almost identical results, except that much fewer grains were recorded in these materials than in the 6 hour series (Fig. 6). The total number of silver grains in electron microscopic autoradiographs of hair follicles biopsied 1 hour after the injection was originally so small that their significance could not be positively evaluated, although some grains were found over tonofibrils in the prekeratinization region and in the lower part of the keratogenous zone.

In the identical preparations for electron microscopic autoradiography that were not exposed to the static magnetic field, the over-all number of exposed grains was almost negligibly small regardless of the time interval after injection, even though the exposure time was longer than 2 months for some preparations. For a rough estimation of the relative sensitivity of these two methods, the grain counting was also performed on identical preparations with and without exposure to the

magnetic field. The exposure time was 1 month in both specimens. In an area 100 μ^2 over the hair cortex, 698 grains were counted from five grids, each prepared from a different block and exposed to the magnetic field, whereas in the identical area in five similar grids prepared from the same blocks but without the application of the magnetic field only 48 grains were counted.

DISCUSSION

Part of the success of the electron microscopic autoradiography using S^{35}-L-cystine, a medium-energy beta emitter, appeared to be obtained by introducing a static magnetic field into this technique. Actually, Harford and Hamlin (15) were the first to apply a magnetic field to their method of electron microscopic autoradiography. They studied with this method the intranuclear localization of thymidine labeled with tritium atoms which are known to emit beta particles with energies varying from 0 to 18 kev. With this method, they were able to demonstrate a marked increase in number of reacted grains in the nuclei of cells (15), although many other investigators have obtained quite satisfactory electron microscopic autoradiographs showing intranuclear localization of tritiated thymidine without the application of a magnetic field (17, 19, 24-26). However, the radioactive tracer used in the present experiments emits beta particles with energies ranging from 0 to 167 kev, almost ten times greater than those of tritium. According to computations by Pelc et al. (24) based on data given by Lea (20), it is shown that in an emulsion with an average grain size of 0.01 μ the energy loss of an electron with an energy of 10 kev is approximately 150 ev per grain, while a 100 kev electron will lose only 17 ev. It is indicated by the experience of Pelc et al. (24) with exposures to visible light that an absorption

of approximately 35 ev per grain is needed to make the grain developable. They also suggested that if insufficient energy is lost during the passage of one particle through a grain, a second or third hit would be necessary (24). Since S^{35} produces long-range ionizing particles, it is likely that most of the emitted particles do not lose sufficient energies to make silver halide grains developable particularly at their first hits, although the size of the grains in Ilford L-4 emulsion is larger (approximately 0.12 μ in diameter) than that assumed in the computations by Pelc et al. (24). Because of an extremely thin layer (possibly 0.1 to 0.2 μ in thickness) of the photographic emulsion in combination with ultrathin sections (0.08 to 0.1 μ in thickness), the chances of two or three hits being made by a single ionizing particle appeared to be very rare. This may account for the low efficiency obtained in our electron microscopic autoradiographs without the application of the static magnetic field.

When the specimens were exposed to the magnetic field in our electron microscopic autoradiography a highly increased number of silver grains was observed but the resolution did not seem to be improved. This result appeared to be consistent with that obtained by Harford and Hamlin (15). Their explanation for the improved sensitivity in this method was that the magnetic field used had a demonstrable effect on the path of emitted beta particles from tritium and that these particles by taking an altered path would have a greater chance to meet silver grains in the emulsion (15).

However, Caro has presented his opinion that a static magnetic field of a strength currently available cannot modify autoradiographic results (7). First, he theoretically calculated that a commonly available static magnetic field of 10,000 gauss bends the path of a 10 kev beta particle into

Key to Abbreviations

ak, amorphous keratin granules	*irsc,* inner root sheath cuticle
c, hair cortex	*m,* mitochondrion
cu, hair cuticle	*med,* medulla
g, Golgi zone	*n,* nucleus
gr, reacted silver grains	*ors,* outer root sheath
he, Henle's layer	*tf,* tonofibrils
hu, Huxley's layer	*th,* trichohyalin

FIGURE 3   Electron micrograph of a longitudinal section of the keratogenous zone of anagen hair follicle. All layers of a hair follicle are illustrated. Stained with uranyl acetate. × 3,500.
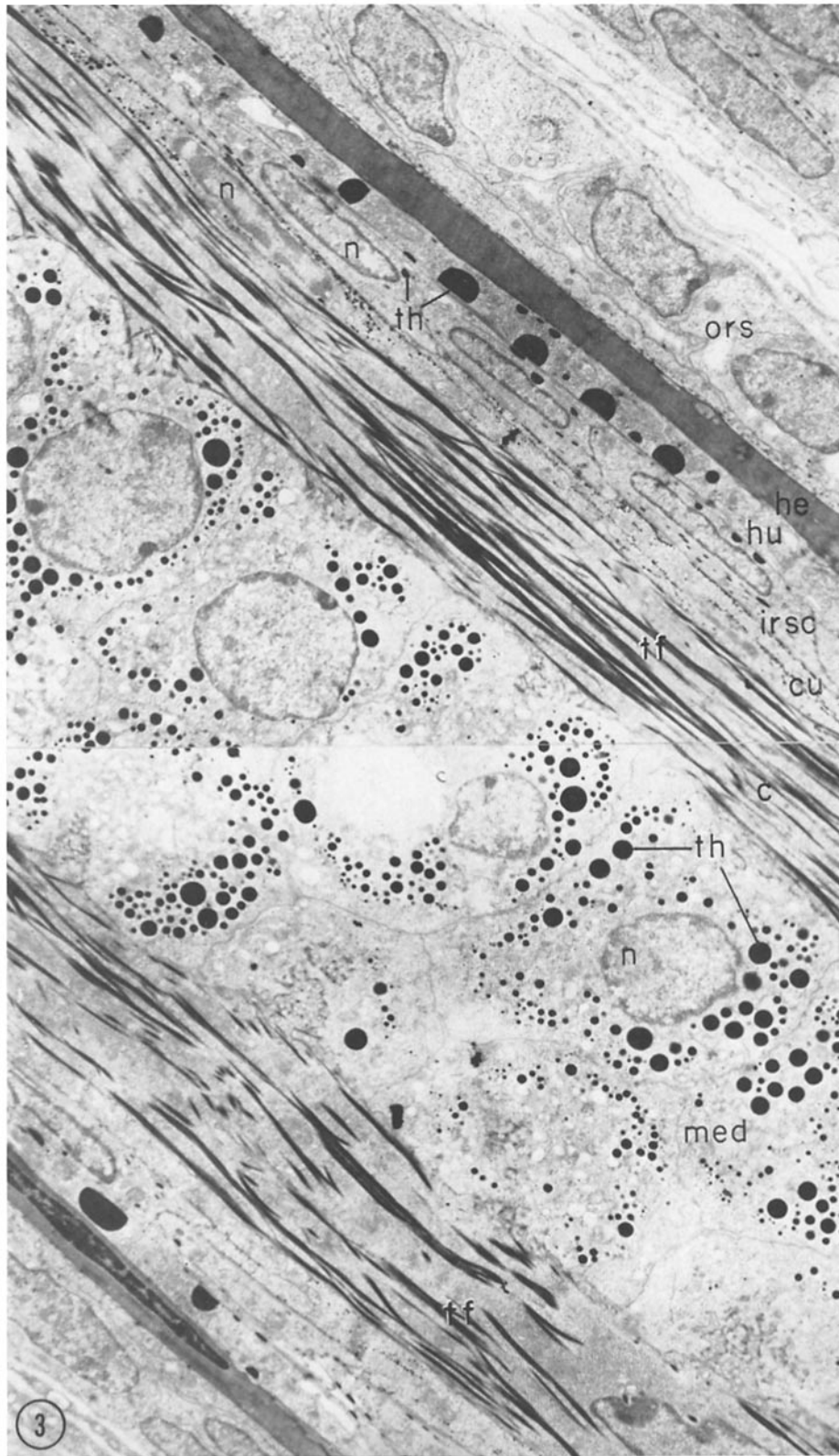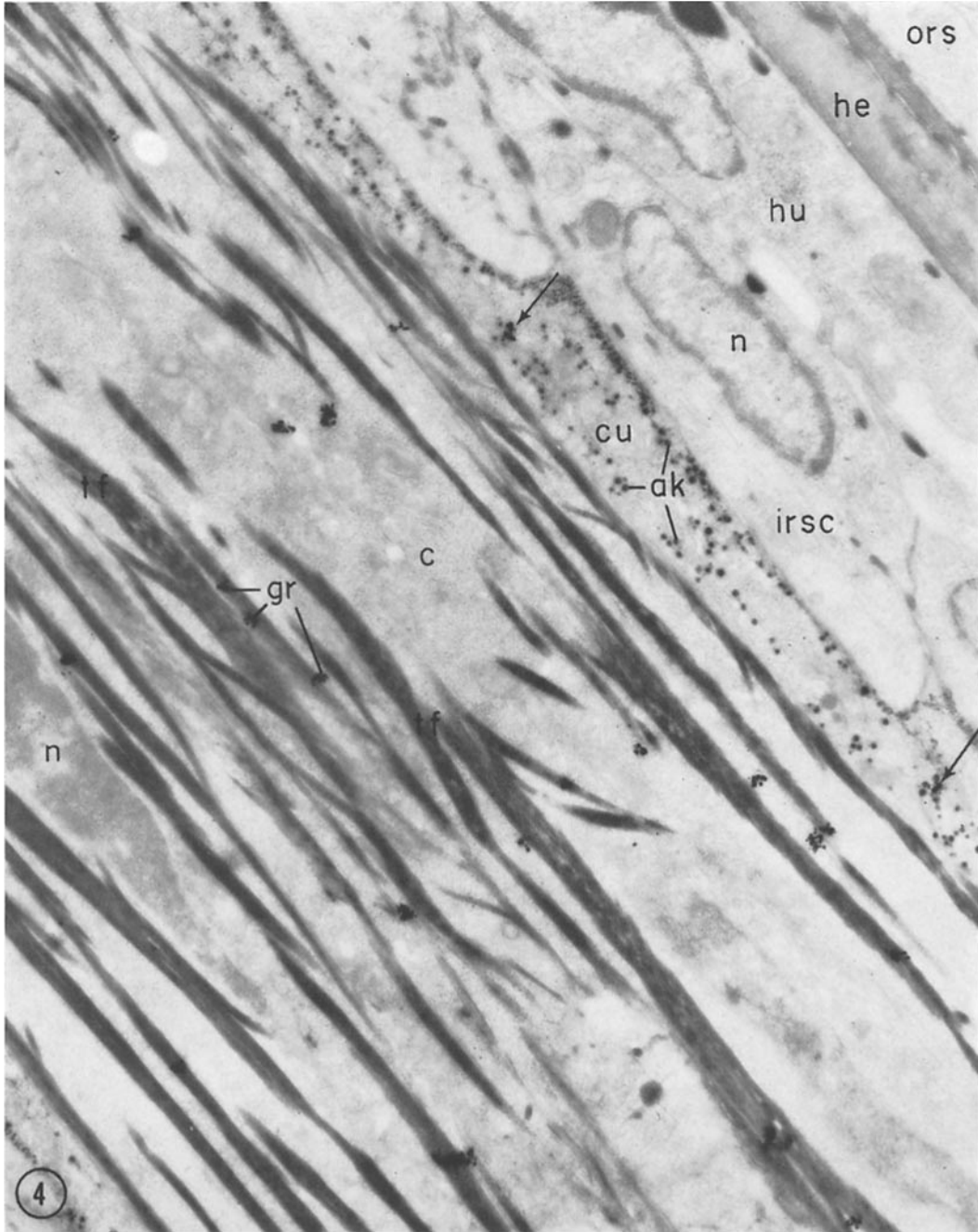
TABLE I

Layers of hair follicle	Cellular components	No. of grains	Percentage
Hair medulla	Tonofibril	3	
	Trichohyalin granule	4	
	Nucleus	1	
	Basic cytoplasm	3	
	Mitochondrion	1	
	Golgi zone	0	
Hair cortex	Tonofibril	452	63.8
	Vicinity of tonofibril	37	5.2
	Nucleus	10	1.4
	Basic cytoplasm	24	3.4
	Mitochondrion	6	
	Golgi zone	0	
Hair cuticle	Amorphous keratin granule	64	9.1
	Nucleus	3	
	Basic cytoplasm	3	
	Mitochondrion	1	
Cuticle of inner root sheath	Tonofibril	43	6.1
	Vicinity of tonofibril	3	
	Trichohyalin granule	3	
	Nucleus	6	
	Basic cytoplasm	6	
	Mitochondrion	1	
	Golgi zone	0	
Huxley layer	Tonofibril	1	
	Trichohyalin granule	7	
	Nucleus	2	
	Basic cytoplasm	4	
	Mitochondrion	1	
	Golgi zone	0	
Henle layer		13	1.8
Outer root sheath	Keratinizing structure	0	
	Other cellular component	6	
Total		708	

Figure 4   Electron micrograph of an autoradiograph of a longitudinal section passing almost tangentially through the cortex, showing a portion of the keratogenous zone of a hair follicle in a mouse that received intraperitoneal injection of S^{35}-cystine 6 hours before biopsy. The developed silver grains appear as dense, irregularly shaped particles or filaments. Sixteen grains are found over or associated with tonofibril bundles of the cortex. Two grains (arrows) are associated with amorphous keratin granules of the hair cuticle. Three grains lie outside of the tonofibril bundles but within the distance of 0.1 μ from them. The significance of these grains is discussed in the text. Only one grain is found over the basic cytoplasm of a cell in the cortex. Stained with uranyl acetate. × 10,000.

a curvature of radius 340 μ, while the maximum range of the particle in emulsion is less than 2.5 μ. A curvature of radius 340 μ on a path of 2.5 μ is actually too large to show any significant effect. Secondly, he stated that, even if the path of the beta particles is altered in stronger magnetic fields, a field perpendicular to the sections would not affect the vertical component of velocity of emitted particles according to the theory of inclined planes in mechanics. Since the vertical component determines the time taken by the particles in emulsion, the length of the path in the emulsion would not be modified. A similar explanation appears to apply to the cases of C^{14} and S^{35} (7).

While the author is inclined to agree with the theoretical explanation of Caro (7), the improved sensitivity in the static magnetic field observed in the present experiment and in Harford and Hamlin's work cannot be explained theoretically. It should be noted that the rate of the increased sensitivity determined by our grain counting appears to vary from one specimen to the other because of the great difficulty of producing constant preparations in this autoradiographic technique. Although we have had no intention of determining an accurate increase of the sensitivity, it was recognized that the specimens exposed to the magnetic field tend to demonstrate a significantly increased number of reacted grains. Further studies to confirm the differences in the sensitivity under various conditions are in progress.

In an attempt to increase resolution in electron microscopic autoradiography with the use of strong magnetic fields, Harford and Hamlin failed to demonstrate any significant improvement (15). Moreover, Caro has theoretically proven that such an improvement cannot be achieved by the practically attainable magnetic fields (7). It was noted also in our electron microscopic autoradiographs that a small number of reacted silver grains was located a few tenths of a micron away from tonofibrils. Some of the grains could be interpreted as background, but most appeared to be

caused by the phenomenon of scatter. However, it was difficult to decide whence they originated, although they had possibly recorded S^{35} concentrated in keratinizing structures. According to his theoretical calculation and experiments, Caro (9) concluded that an autoradiographic resolution of 0.1 μ could be achieved with a tritiated material and a currently available emulsion. Even though beta particles with greater energies emitted from S^{35} would be expected to produce more scatter, we have arbitrarily interpreted occasional grains located more than 0.1 μ away from tonofibrils as those recorded by S^{35} in the basic cytoplasm (Table I). A small number of grains found within a distance of 0.1 μ from tonofibrils was counted separately (Table I) and it was interpreted that these grains had possibly originated from S^{35} concentrated in the tonofibrils.

The findings from electron microscopic autoradiographs of the keratogenous zone of anagen hair follicles in mice receiving injections of S^{35}-L-cystine have made it possible to identify the tonofibrils and tonofibril bundles of the hair cortex as the site of high concentration of this labeled amino acid.

A relatively large number of silver grains was counted over small amorphous keratin granules and droplets in the hair cuticle layer. In view of the much smaller space occupied by this layer as compared with the cortex, the radioactivity appeared to be significantly high. Tonofibrils in the cuticle of the inner root sheath were also shown to possess an appreciable level of radioactivity.

However, other cellular components, such as the nucleus, Golgi zone, mitochondrion, and basic cytoplasm including endoplasmic reticulum, did not show any significant radioactivity by grain count, although a few grains possibly attributable to background were observed over these structures. This was also true in the hair follicles of mice injected 1 or 3 hours before biopsy. It has been demonstrated by Fell *et al.* that, after injection of radioactive cystine, a radioactivity is also localized

FIGURE 5   Material similar to that in Fig. 4, showing two grains over tonofibril bundles of the cortex and three grains (arrows) associated with amorphous granules of the hair cuticle. Stained with uranyl acetate. $\times$ 24,000.

FIGURE 6   A longitudinal section from the keratogenous zone of the hair follicle fixed 3 hours after injection of S^{35}-cystine. Four grains are found over tonofibril bundles of the cortex, and one grain (arrow) appears over an amorphous keratin granule in the hair cuticle. Stained with uranyl acetate. $\times$ 12,000.
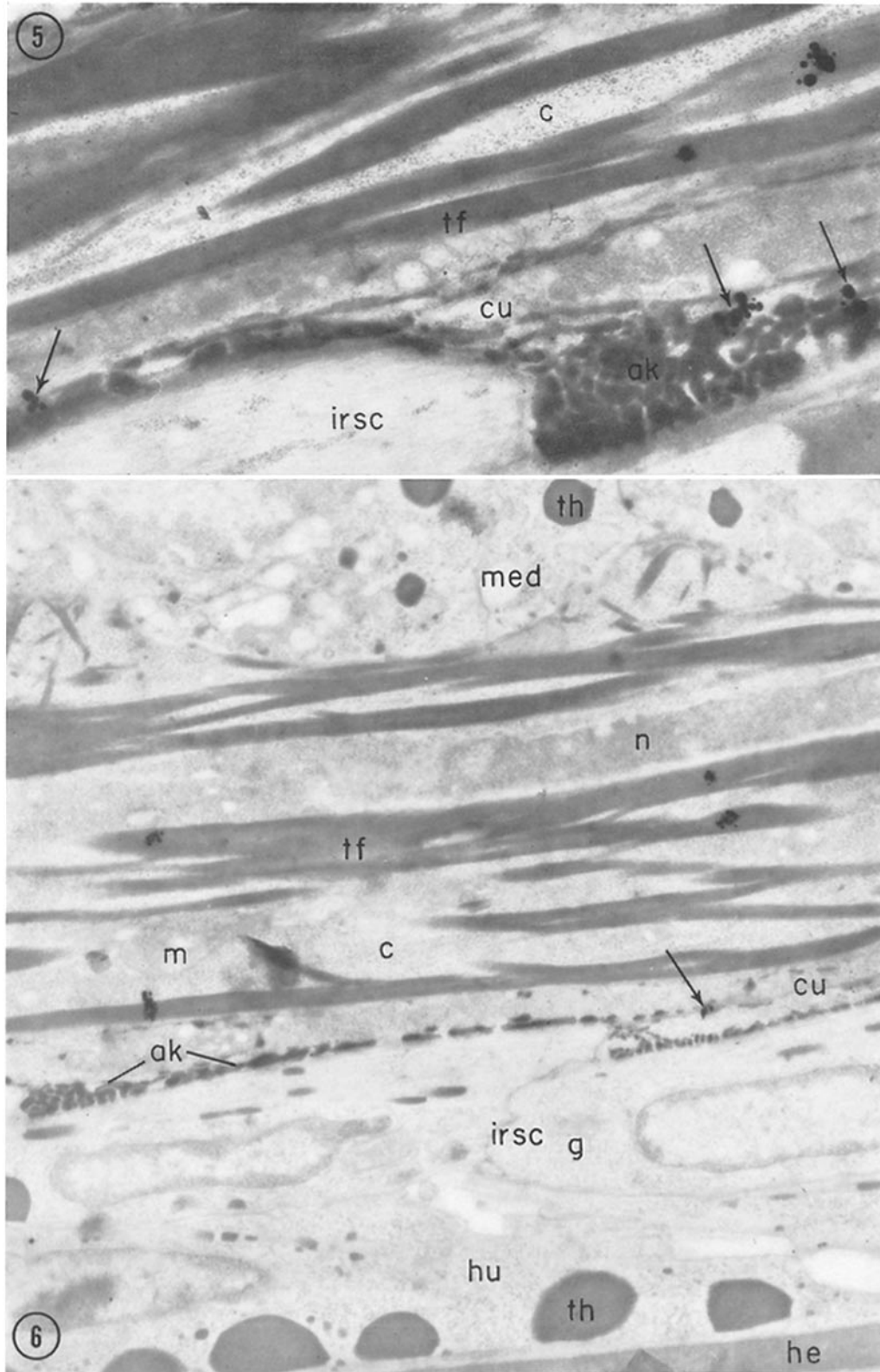
in nuclei of the epithelial cells of the esophagus (12, 13). According to an explanation of this phenomenon by Mercer, a sulfur-containing protein may well be associated with nuclear RNA (22). Since we were not able to demonstrate a significant number of reacted grains over the nuclei, it appeared that a more sensitive method for electron microscopic autoradiography is possibly needed for detecting sulfur incorporation in nuclear RNA.

The observations described above appear to indicate that cystine is directly and specifically incorporated into tonofibrils in the hair cortex and also into amorphous keratin granules of the hair cuticle layer, possibly without any particular concentration of this substance in the other cellular components. There seemed to be a certain degree of cystine concentration in tonofibrils of the cuticle of the inner root sheath.

On the other hand, trichohyalin granules in the hair medulla and inner root sheath did not show any significant evidence of concentration of S^{35}-L-cystine. These observations coincide with the results of chemical analyses by Rogers (28) showing that the medulla and inner root sheath, both of which are characterized by an abundance of trichohyalin, have high concentrations of citrulline (5.2 and 6.1 per cent by weight, respectively) while the cystine content is very low (0 and 1 per cent by weight, respectively). It was also mentioned by Rogers (28) that trichohyalin granules which have been extracted with 8 M urea contain little or no citrulline but that a conversion of arginine into citrulline may be accompanied by morphological alterations in which the granular trichohyalin changes to fibrous protein.

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