

## The synthesis and migration of nuclear proteins during mitosis and differentiation of cells in rats

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### Summary

Hooded rats were given an intraperitoneal injection of  $^3\text{H}$ -tyrosine, and killed in pairs 10 min, 30 min, 12 h, 36 h, 7 days, and 30 days later. A piece of skin with white growing hair, and the tongue, were taken from each animal and radioautographs were prepared. Silver grains were counted over whole nuclei and whole mitotic figures of the germinal cells and whole nuclei of differentiating cells of both tissues. It was found that the interphase nuclei have significantly more silver grains over them than the chromosomes at all stages of mitosis and there are virtually no grains over metaphase, anaphase, and early telophase chromosomes in both tissues of all the animals killed up to 36 h after the injection. The difference between the grain counts over the interphase nuclei and the chromosomes of dividing cells is at least 20-fold at 30 min in the hair matrix, at least 5-fold at 30 min in the tongue and at 36 h in both tissues. It was established that the differences observed between the radioactivities of the nuclei and chromosomes of mitotic figures are real from estimates of: the radioactivity of the cell cytoplasm, volumes of the metaphase chromosomes and interphase nuclei within  $1\ \mu$  of the photographic emulsion, and the volumes of cytoplasm separating the photographic emulsion and these structures. No protein synthesis was demonstrable in the chromosomes during metaphase, anaphase, and early telophase. Nuclear proteins leave the chromosomes during prophase and prometaphase and return to the nucleus during late telophase. The cells in the matrix and upper bulb of the growing hair follicle and those in the germinal, prickle, and granular cell layers of the tongue are in different functional states; 30 min after injection of  $^3\text{H}$ -tyrosine they have different amounts of it in their nuclear proteins. It is suggested that the amount incorporated into each nucleus is related to the rate at which proteins are being synthesized by the cell.

### Introduction

At present the structure of the interphase nucleus of a cell is considered to be a lipoprotein envelope in which the chromosomal material (DNA and protein) and nucleoli (RNA, DNA, and protein) are supported by an amorphous protein medium (Fell and Hughes, 1949). Most experiments on the incorporation of radioactive amino-acids into nuclear proteins by radioautography do not discriminate between the proteins of the four main structural components of the nucleus. A number of such experiments on *Amoeba* have demonstrated the functional characteristics of some of the proteins associated with the nucleus of this animal (Goldstein, 1958, 1963; Byers, Platt, and Goldstein, 1963; Prescott, 1963). Two types of protein are synthesized in the cytoplasm and rapidly enter the nucleus. One moves backwards and forwards between the nucleus and the cytoplasm while the other remains in the nucleus.

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Both types of protein leave the nucleus when its interphase form is demolished at the beginning of mitosis and they both return to the nucleus at the end of mitosis.

Experiments with nuclear proteins of *Vicia faba* labelled with  $^3\text{H}$ -arginine by Prensky and Smith (1964) show that some nuclear proteins of this plant behave in a different way. The proteins remain with the chromosomes during the first mitosis after labelling but are not with the chromosomes during the second mitosis. The demonstration of this type of behaviour might depend on the experimental method, the type of nucleus, or the amino-acid that they used.

Evidence of a different type which shows that protein leaves the nucleus during mitosis is the loss of mass suffered by the nucleus during prophase. This has been demonstrated in endosperms by Richards and Bajer (1961) and in cultures of embryonic mouse heart, ascites tumour, and plant cells by Richards (1960). All the observations on the flight of proteins from the nucleus during mitosis available at present have been made on protozoa, plants, or tissue cultures; no experiments on intact mammals have been reported.

Although Byers *et al.* (1963) demonstrated that some nuclear proteins are synthesized in the cytoplasm their experiments do not exclude the possibility of protein synthesis by the *Amoeba* nucleus. Leblond, Pinheiro, Droz, Amano, and Warshawsky (1963) review quantitative experiments carried out by themselves, and the relevant literature, and conclude that protein synthesis does take place in mammalian interphase nuclei. No observations have been reported on the relationship between the functional state of cells and the quantity of labelled amino-acids incorporated into their nuclei.

The incorporation of radioactive amino-acids into the nuclei of *Vicia faba* has been studied by Mattingly (1963). She claims to have shown that the nuclei incorporate labelled amino-acid at all stages of mitosis as well as interphase, but I think that her observations do not warrant this conclusion. No similar investigation has been made on intact mammals.

The purpose of the experiment reported here was to obtain evidence from the intact mammal on the migration of nuclear proteins during mitosis and the incorporation of  $^3\text{H}$ -tyrosine into chromosomal material during mitosis and into nuclei at different functional states of the cells. The cortex of growing hair and the stratified squamous epithelium of the tongue were chosen as suitable tissues for the investigation because their germinal cells exhibit a high mitotic rate and are clearly separated from differentiating cells which do not divide.

### Material and methods

The material was obtained from 12 hooded rats whose weights ranged from 60 to 80 g at the beginning of the experiment. Each rat was given an intraperitoneal injection of radioactive DL-tyrosine, 10  $\mu\text{C/g}$  body weight. The tyrosine was generally labelled with tritium and had a specific activity of 434 mc/mM. The rats were killed in pairs 10 min, 30 min, 12 h, 36 h, 7 days, and 30 days after injection. A piece of skin with white hair which was growing

at the time of injection and the tongue were taken immediately after death from all the animals.

The tissues were fixed in Bouin's solution and embedded in paraffin wax. Then radioautographs of  $5\ \mu$  sections stained with haematoxylin and eosin were prepared by the coating technique of Kopriwa and Leblond (1962). The photographic emulsion was exposed to the sections for 7, 22, and 44 days before it was developed.

The main observations were made on the material exposed for 22 days. The silver grains were counted over the chromosomes of whole mitotic figures of 20 cells at each stage of mitosis and over the interphase nuclei that were close to but not cut by the emulsion surface of the section and were nearest to the cells in early telophase. It is possible to establish that the upper surface of a nucleus is close to but not cut by the emulsion surface of the section if the chromatin network is close to the silver grains in the emulsion and is intact. For the counts the two sets of chromosomes in the anaphase and telophase cells were regarded as the contents of one cell. The dimensions of the chromosome groups and the nuclei were measured with an ocular micrometer that had a range of error of  $0.5\ \mu$ . When observations had been made on a cell its position on the slide was charted to ensure that observations on each cell were recorded only once.

The criteria used to identify the stages of mitosis in this work were as follows:

*Prophase*—nuclear membrane intact, chromatin resembles a tangled thread, nucleolus absent.

*Prometaphase*—nuclear membrane absent, thick chromatin threads arranged as a sphere.

*Metaphase*—chromosomes arranged as a single plate.

*Early anaphase*—chromosomes arranged as two parallel plates; the distance between them is less than the diameter of the plates.

*Late anaphase*—as in early anaphase except that the distance between the plates is greater than their diameter.

*Early telophase*—chromosomes arranged as two homogeneous dark masses; the cell boundary is constricted at the equator of the division.

*Late telophase*—chromatin resembles a tangled thread; cell boundary constricted at the equator of the division.

When the plane of section through a mitotic cell did not allow its stage to be recognized it was excluded from the observations. No cell was diagnosed as being in anaphase or telophase unless both sets of chromosomes were visible.

## Observations

### *Mitotic nuclei*

Two sets of observations were made on dividing cells. First a survey was made to discover whether there are fewer grains over the chromosomes of

mitotic cells than over interphase nuclei. This was done by comparing the number of grains over the chromosomes of the mitotic cells with the number over the nearest whole interphase nucleus. If more grains were found over the chromosomes the result was recorded as positive and if fewer were found the result was recorded as negative. This was done on the 10 min, 30 min, 12 h, and 36 h material for 20 pairs of cells at each stage of mitosis except late telophase. The late telophase cells were excluded because less than 10 of them

TABLE I

*The number of cells out of 20 with more grains over the chromosomes than over the nearest whole interphase nucleus*

Animal no.	Time after injection of $^3\text{H}$ -tyrosine	Site	Stage of Mitosis					
			Pro-phase	Prometa-phase	Meta-phase	Early ana-phase	Late ana-phase	Early telo-phase
1	10 min	hair	5*	0	0	0	0	0
		tongue	6*	0	0	0	0	0
2	10 min	hair	0	0	0	0	0	0
		tongue	5*	0	0	0	0	0
3	30 min	hair	0	0	0	0	0	0
		tongue	3	2	0	0	0	0
4	30 min	hair	0	0	0	0	0	0
		tongue	6*	5*	0	0	0	0
5	12 hr	hair	3	0	0	0	0	0
		tongue	2	1	0	0	0	0
6	12 h	hair	3	0	0	0	0	0
		tongue	3	1	0	0	0	0
7	36 h	hair	2	0	0	0	0	0
		tongue	1	0	0	0	0	0
8	36 h	hair	3	0	0	0	0	0
		tongue	2	3	0	0	0	0

The symbol \* indicates no significant difference at the 1% level of probability between mitotic and interphase cells.

were identified in each of the preparations. The results of this survey are presented in table 1. For both tissues of all the rats the number of mitotic cells with more grains over the chromosomes than over the nearest interphase nucleus is greatest for prophase, intermediate for metaphase, and zero for metaphase, anaphase, and early telophase.

To ensure that the results of the first set of observations were not produced by the geometrical relationships of the photographic emulsion with the nuclei of the interphase and dividing cells, a second set of observations were made on the 30 min and 36 h material. The silver grains were counted over the chromosomes of mitotic cells and the whole interphase nuclei nearest to the cells in telophase. The results of the grain counts are presented in table 2. The counts are greatest for the interphase nuclei, decrease through prophase and prometaphase and are negligible for the other stages of mitosis. When the grain counts were made the diameter and thickness of the metaphase plates were measured and the longest and shortest diameters of the interphase

nuclei. At the degree of accuracy with which the measurements were made these dimensions are constant for all the cells examined in both tissues of the four animals. The diameter of the metaphase plates is  $5\ \mu$ , and their thickness  $2.5\ \mu$ , the mean of the longest and shortest diameters of the interphase nuclei is  $6.5\ \mu$ . In addition, the grains were counted over whole cells for the telophase and interphase cells in the 30 min tongue sections. The means and standard deviations of the number of grains over the telophase and interphase cells are  $19\pm 3$  and  $20\pm 4$  respectively for animal number 3, and  $21\pm 4$  and

TABLE 2

*The means and standard deviations of 20 observations on the number of grains over whole nuclei or chromosomes of cells at each stage of mitosis*

Animal no.	Time after injection of $^3\text{H}$ -tyrosine	Site	Stage of mitosis						
			Inter-phase	Pro-phase	Prometa-phase	Meta-phase	Early ana-phase	Late ana-phase	Early telo-phase
3	30 min	hair matrix	$21\pm 4$	$7\pm 3$	$7\pm 3$	$1\pm 1$	$0\pm 0$	$0\pm 0$	$0\pm 0$
3	30 min	tongue	$10\pm 2$	$7\pm 2$	$5\pm 2$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$
4	30 min	hair matrix	$23\pm 3$	$8\pm 3$	$7\pm 2$	$1\pm 1$	$0\pm 0$	$0\pm 0$	$1\pm 1$
4	30 min	tongue	$10\pm 2$	$6\pm 3$	$8\pm 3$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$
7	36 h	hair matrix	$5\pm 1$	$2\pm 2$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$
7	36 h	tongue	$8\pm 2$	$5\pm 1$	$2\pm 2$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$
8	36 h	hair matrix	$5\pm 2$	$2\pm 1$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$
8	36 h	tongue	$6\pm 3$	$3\pm 1$	$2\pm 2$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$

$21\pm 4$  for animal number 4. An attempt to make similar observations on the cells in the hair matrix from the same animals was defeated because the cell boundaries were not visible.

#### *Nuclei of cells at different functional states*

On the 30 min and 36 h material, the grains were counted over 20 whole nuclei of cortical cells at three positions along the upper bulb of growing hair follicles. The observations were restricted to the region of the upper bulb where the cell nuclei were not elongated and where the number of silver grains per unit area of the radioautograph was similar to that of the matrix (Sims, 1964). The results are presented in table 3. The counts show that in the 30 min sections the radioactivity of the nuclei  $70$  to  $140\ \mu$  from the bottom of the follicle is about half that of the interphase nuclei in the adjacent matrix (table 2). The radioactivity increases again as the upper bulb is ascended. At 36 h there is a gradual increase in the radioactivity of the nuclei from the matrix to the top of the upper bulb.

Grain counts over the nuclei of cells in different layers of the tongue epithelium were made only on the 30 min material. The means and standard deviations of the number of grains over whole nuclei of prickle and granular

cells are  $23 \pm 7$  and  $6 \pm 3$  respectively for animal number 3, and  $20 \pm 7$  and  $5 \pm 2$  respectively for animal number 4.

TABLE 3

*The means and standard deviations of 20 observations on the number of grains over whole nuclei at three positions along the hair cortex of the upper bulb*

Animal no.	Time after injection of $^3\text{H}$ -tyrosine	Number of microns from bottom of follicle		
		70 to 140	140 to 210	210 to 280
3	30 min	$12 \pm 4$	$20 \pm 5$	$23 \pm 6$
4	30 min	$11 \pm 4$	$17 \pm 5$	$24 \pm 7$
7	36 h	$9 \pm 3$	$13 \pm 4$	$17 \pm 5$
8	36 h	$10 \pm 3$	$12 \pm 6$	$18 \pm 6$

### Discussion

#### *Mitotic nuclei*

If proteins leave the nuclear apparatus during mitosis in this experiment, then the radioactivity of the chromosomes in mitotic cells ought to be less than that of the interphase nuclei. This should be true for both tissues of all the animals regardless of the interval between injection of the radioactive amino-acid and death. The situation can be tested by consideration of the null hypothesis that there is no difference between the radioactivity of the chromosomes of the mitotic cells and the interphase nuclei. If the null hypothesis is correct the probability of there being more, or fewer, grains over the chromosomes of a mitotic cell than over the nearest interphase nucleus is 0.5. It follows that the probability of obtaining  $n$  out of 20 mitotic cells with more grains over the chromosomes than over the nearest interphase nucleus is given by summation of the first  $(n+1)$  terms of the expansion of the binomial expression  $(\frac{1}{2} + \frac{1}{2})^{20}$ . As there is reason to believe that the null hypothesis does not hold, a probability of 1% was selected as the level for a significant difference. The results (table 1) show that there are significantly fewer grains over the chromosomes of most prophase, most prometaphase, all metaphase, all anaphase, and all early telophase cells, than over the interphase nuclei.

It is essential to consider the possibility that the radioactivities of the mitotic chromosomes and interphase nuclei are similar and the results of the first set of observations are produced by the geometric relationships of the photographic emulsion and these structures. The effect could be produced in three ways by the cytoplasm which separates photographic emulsion from the interphase nuclei and mitotic chromosomes (fig. 1): first, by the radioactivity of this cytoplasm being less in mitotic cells than in interphase cells; second, by the distance through this cytoplasm being greater for the chromosomes than for the nuclei; third, by the volume of this cytoplasm being greater for the nuclei than the chromosomes.

Prescott and Bender (1962) and Konrad (1963) have shown that the incorporation of radioactive amino-acids into the cytoplasm of some types of cells in tissue culture is greatly reduced during mitosis. In the present experiment the mitotic cells at the stage of telophase would be expected to show the greatest response to such an effect in the animals killed 30 min after the injection of the radioactive amino-acid, because they would have been in the process of division longest while the amino-acid was available. In fact, the estimates of the mean number of grains over the whole of the cell are

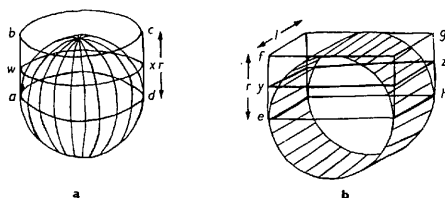


FIG. 1. (a) The volume of a nucleus within  $1 \mu$  of the photographic emulsion at the plane  $bc$  = the volume of the cap of the sphere between the planes  $bc$  and  $wx$ . The volume of the cytoplasm between a nucleus and within  $1 \mu$  of the photographic emulsion = the volume of the cylinder  $wbcx$  minus the volume of the cap of the sphere.

(b) The volume of a metaphase plate within  $1 \mu$  of the photographic emulsion at the plane  $fg$  = the volume of the segment between the planes  $fg$  and  $yz$ . The volume of the cytoplasm between a metaphase plate and within  $1 \mu$  of the photographic emulsion = the volume of the cuboid  $yfgz$  minus the volume of the segment.

$r$  = radius of nucleus or metaphase plate,  $l$  = thickness of metaphase plate. A distance of  $1 \mu$  separates the plane  $bc$  from the plane  $wx$  and the plane  $fg$  from the plane  $yz$ .

similar for the interphase and telophase cells of these animals. Therefore the difference between the radioactivity of the interphase and mitotic nuclei demonstrated by the first observations is not produced by a difference in the radioactivity of their cytoplasm.

Caro (1962) has calculated that if a tritium source is more than  $1 \mu$  away from an emulsion more than 80% of its beta emission is filtered off before it reaches the emulsion. The proportions of the interphase nucleus and metaphase chromosomes less than  $1 \mu$  away from the emulsion is an indication of the extent that filtration is affecting the emissions from the two structures. The proportions were calculated by simple geometry for the ideal situations in the manner indicated in fig. 1, with the observed measurements of their dimensions. The calculations show that one fourteenth of the interphase nuclei and one seventh of the metaphase chromosomes are within  $1 \mu$  of the emulsion. It follows that if the radioactivity of the interphase nuclei and metaphase chromosomes are the same, the reduction of the number of grains observed over the chromosomes could not be produced by cytoplasmic filtration because a greater proportion of the chromosomes is within range of the emulsion.

The volumes of the cytoplasm separating the emulsion from the interphase nucleus and the metaphase chromosomes and situated within  $1 \mu$  of the emulsion were estimated in the ideal conditions illustrated in fig. 1. The volumes are  $23 \mu^3$  for the interphase nucleus and  $6 \mu^3$  for the metaphase chromosomes. Although there is 4 times as much cytoplasm within range of the emulsion over the interphase nucleus as over the metaphase chromosomes it is not sufficient to account for the differences in the grain counts. Table 2 shows that at 30 min there are 20 times more grains over interphase nuclei than over metaphase, anaphase, and telophase chromosomes in the hair matrix, and at least 10 times more grains over interphase nuclei than over metaphase, anaphase, and telophase chromosomes in the tongue. Also, at 36 h there are over 5 times more grains over the interphase nuclei than over the metaphase, anaphase, and telophase chromosomes in both tissues. In all cases the numbers of grains over the prophase and prometaphase chromosomes are intermediate between those of the interphase nuclei and the metaphase chromosomes. The observations were made so that nuclei cut by the emulsion surface of the section were excluded. If the interphase nuclei and metaphase chromosomes are below the surface of the section the difference between the volume of cytoplasm within  $1 \mu$  of the emulsion over them is reduced and will bias the observations against the result obtained. It is concluded that the observed difference between the radioactivity of the interphase nuclei and metaphase chromosomes must be real. Consideration of the results presented in table 2 and the similarity in the form of the chromosomes shows that it is reasonable to extend this conclusion to the other stages of mitosis.

Droz and Warshawsky (1963) have shown that radioautographs of animals injected with radioactive amino-acids demonstrate sites of radioactive proteins. The presence of such proteins in the interphase nuclei of the tongue and hair 10 and 30 min after injection of radioactive tyrosine is compatible with the hypotheses that protein synthesis occurs in nuclei (Carneiro and Leblond 1959) and/or that the protein is synthesized in the cytoplasm and rapidly transferred to the nucleus (Byers *et al.*, 1963). Because there were no grains over the anaphase chromosomes and almost none over the metaphase and early telophase chromosomes of the 30 min material the other material was re-examined. No grains were present over the metaphase, anaphase, and early telophase chromosomes of either tissue at any time. The observations on the 10 and 30 min material can be interpreted in two ways. First, the mitotic chromosomes did not contain labelled proteins so they are not a site of protein synthesis. Second, the mitotic chromosomes contained small amounts of labelled proteins that escaped detection under the conditions of this experiment and the chromosomes could be a site of protein synthesis. This is most improbable especially as it is known that the ribonucleic acids leave the nuclear apparatus during mitosis in mice (Monesi, 1964). It is concluded that proteins are not synthesized by the chromosomes during metaphase, anaphase, and early telophase.



The germinal cells of the tongue divide about once every five days (Bertalanffy, 1960) so those dividing when the 12 and 36 h animals were killed (table 1) had been labelled during interphase. The absence of radioactive protein from the metaphase, anaphase, and early telophase chromosomes of mitotic cells in the tongues of these animals shows that this protein has left the nuclear apparatus. The grain counts over the prophase and prometaphase nuclei are intermediate between those of the interphase and metaphase so it is reasonable to conclude that the proteins leave the nuclei during these stages. A similar argument establishes the loss of proteins from mitotic nuclei in hair, except that there the rate of cell division is greater.

Nothing is known about the movements of nuclear proteins in the interphase nucleus of mammalian cells. It is reasonable to suppose that some cannot leave the nucleus, some take part in a continuous exchange across the nuclear envelope between nuclear and cytoplasmic pools, and there may be others with more complicated patterns of behaviour. Only the simple cases of proteins confined to the nucleus and proteins in a continuous exchange across the nuclear envelope will be considered in this discussion.

When the nucleus is demolished during the early stages of mitosis, proteins confined in it may either break down into their constituent amino-acids or migrate intact into the cytoplasm. Replacements are required when the nucleus is reconstructed only if the proteins are broken down. Similar possibilities exist for proteins moving between nuclear and cytoplasmic pools. During mitosis the nuclear pool is abolished, with or without protein breakdown, and all the molecules are restricted to the cytoplasmic pool. When the interphase form of the nucleus is reconstructed, molecules from the cytoplasmic pool enter again and re-establish the exchange. If both confined and mobile nuclear proteins are labelled by a pulse dose of radioactive amino-acid then their radioactivities will not be affected by mitosis if they are not broken down, but their radioactivities will be drastically reduced if they are broken down and replaced by fresh unlabelled proteins synthesized after the dose of radioactive amino-acid has been exhausted.

In mice the germinal cells in the hair matrix divide about once every 13 h (Bullough and Laurence, 1958). This value has not been determined for rats and is assumed to be similar to that for mice. The matrix cells in interphase when the 36 h animals were killed must have divided at least twice after their nuclear proteins were labelled by the dose of radioactive amino-acid. It follows that intact nuclear proteins of these cells have migrated from and returned to their nuclei at least twice. The protein cannot have been broken down into amino-acids because its radioactivity would have been diluted to virtually nothing by non-radioactive tyrosine in the cell amino-acid pool.

An attempt to discover the exact stage of division at which the radioactive proteins return to the nucleus was successful only for the hair matrix. In each of the 36 h preparations, 7 late telophase cells were found in which both daughter nuclei were present. The mean number of grains over the two nuclei together was  $4 \pm 2$  in one animal and  $3 \pm 1$  in the other animal. These

observations are sufficient to establish that radioactive proteins return to the nucleus during its reconstruction at late telophase.

#### *Nuclei of cells at different functional states*

Hair growth results from the concurrent migration up the hair follicle and differentiation of primordial cells which originate from the germinal cells in the matrix. The changes which take place in cells during their differentiation into one component of hair are represented in a histological section of a growing hair by the series of differences in the cells of that component from the matrix to the top of the follicle. It has been reported in an earlier paper (Sims, 1964) that the number of grains per unit area is constant over the matrix and the part of the upper bulb studied in the present work, so the differences between the grain counts over the nuclei (table 3) are real. The gradual increase in the number of grains over the nuclei as the matrix and upper bulb are ascended in the 36 h specimens (tables 2 and 3) can be expected if the amount of isotope in each matrix nucleus is halved when the cells divide. Thus, the nuclei of cells produced last at the proximal end of the upper bulb would contain less isotope than the nuclei of those produced earlier at the top of the upper bulb. If it is assumed that the cells in the matrix divide about once every 12 h and that there is no loss of isotope from the nuclei, then the mean number of grains over the matrix nuclei 12 h before a given value is reached can easily be estimated. The mean number of grains over the matrix nuclei at 36 h is 5, so the estimated mean number of grains over them at 24 and 12 h are 10 and 20 respectively. These estimated values are roughly the same as the actual counts for the nuclei which are 70 to 140  $\mu$  and 210 to 280  $\mu$  from the bottom of the follicle. Therefore, these nuclei have taken about 12 and 24 h respectively to move from the matrix to their present positions. These times agree well with previous estimates obtained from observations on grain counts over the whole cell (Sims, 1965) and thus indicate that observations on the number of grains over the nuclei of cells labelled with radioactive amino-acids are significant.

If the mean number of grains expected over the matrix nuclei at 36 h is calculated from the 30 min value a discrepancy is apparent. The expected value of 3 is about half the actual value of 5. The discrepancy could result from the assumed rate of cell division being too great or the incorporation of isotope into the nuclei after the dose injected has been cleared from the body fluids. It is known that an injection of a labelled amino-acid does not behave as an ideal pulse dose; small amounts of it continue to be available to the tissues after the pulse has gone (Shemin and Rittenberg, 1944).

The cells in the matrix of the hair bulb are constantly dividing and they have never been shown to contain keratin fibrils. The cells in the upper bulb never divide; they increase in volume and contain keratin fibrils. The cells in the two regions are clearly in different functional states and the figures in tables 2 and 3 show that they have different amounts of  $^3\text{H}$ -tyrosine in their nuclear proteins 30 min after its injection. Similarly, the cells in the germinal,

prickle, and granular cell layers of the tongue epithelium are in different functional states with regard to volume, incidence of division, and cell inclusions and it has been found that they also have different amounts of  $^3\text{H}$ -tyrosine in their nuclear proteins 30 min after its injection. At present there are no observations that indicate the changes in the cells with which the differences in radioactivity can be associated but one can speculate that the rate at which the cells are synthesizing proteins is an important factor.

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