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DURATION OF DNA SYNTHESIS IN THE GINGIVAL EPITHELIAL CELLS OF MARMOSETS*

by

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Considerable interest has been focused on a detailed study of the generative cycle of mammalian cell systems. The conventional subdivision of this cycle is,

$$M — G_1 — S — G_2 — M$$

where M = mitosis, G_1 = postmitotic, presynthetic phase, S = DNA synthesis phase and G_2 = postsynthetic, premitotic phase.

Most information concerning these phases has become available through the use of autoradiography following injection of tritiated thymidine (H^3-TDR). H^3-TDR injection results in a tagging of the cells in S-phase at the time of injection. Therefore, it is clearly of importance for the interpretation of H^3-TDR autoradiographs to know the duration of the phases in the generative cycle, particularly the duration of the S-phase.

Methods for the determination of S-phase

The length of the S-phase can be estimated by following the labeling of the mitotic figures as a function of time after H^3-TDR injection (*Quastler* 1960). For the intestinal crypt cells of mice the S-phase was found to be 7.5 hours (*Quastler & Sherman*

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1959; *Koburg & Maurer* 1962). The method has been applied to oral mucosa by *Toto & Ojha* (1961) who suggested a 10 hour S-phase in tongue epithelium of mice and by *Skougaard & Beagrie* (1962) who found the S-phase in the gingival epithelium of marmosets to be 7 hours. The method is, however, laborious and the accuracy is dependent on the regular onset and termination of mitosis. The findings of *Skougaard* (1965) seem to indicate that this is not always the case in the oral epithelia of the marmoset.

Wimber & Quastler (1963) introduced a method for the determination of S-phase, based on double labeling with H^3-TDR and C^{14}-TDR. The method may be summarized as follows: An asynchronous cell population in a steady state is "pulse-labeled" with H^3-TDR. This results in tritium labeling of the DNA in those cells which are in S-phase at the time of injection; t_a minutes later an injection of C^{14}-TDR is given, labeling the cells in S-phase at the time of the second injection with C^{14}. During the time interval between the two injections (t_a) a number of cells will have moved out of S, and will, therefore, be labeled with H^3 only. An equal number of cells will have moved into S and only be C^{14}-labeled. The following categories of labeling will occur:

H^3-labeled — the number is proportional to t_a

C^{14}-labeled — the number is proportional to t_a

$C^{14} + H^3$-labeled — the number is proportional to $T_S - t_a$

(T_S = duration of S-phase.)

On the autoradiographs the cells labeled with H^3 only can be distinguished from those labeled with C^{14} because of the longer range of the C^{14}-rays. After suitable exposure the C^{14}-labeled cells will show a halo of grains around the nuclei, whereas the H^3 labeling only produces grains directly over the nuclei. The C^{14} and the $C^{14} + H^3$ labeled cells cannot be distinguished; however, since the sum of these categories will be proportional to $T_S - t_a + t_a = T_S$, the following equation will apply,

$$\frac{H}{C} = \frac{t_a}{T_S}$$

where H = the number of cells labeled with H^3 only and C = the C^{14}-labeled cells.

MATERIAL AND METHODS

Three marmosets were injected intraperitoneally with 2 μCi H^3-TDR per g body weight; 1 or 1.5 hours later a second injection of 0.3 μCi C^{14}-TDR was given and 1 hour after the last injection the animals were sacrificed. Tissue samples were taken from the following areas: the attached gingiva, the marginal gingiva including the epithelial attachment, the palate, and the tongue. The tissues were fixed in 1:3 acetic acid-alcohol, stained according to Feulgen, whereafter the epithelium was squashed. During the preparation under the dissecting microscope of the tissue samples from the marginal gingiva, attempts were made to scrape off cells from the epithelial attachment only. The slides were coated with a double layer of NTB emulsion as suggested by *Wimber & Quastler* (1963) in order to obtain a thicker layer of emulsion for easier recognition of the C^{14} labeling. The slides were exposed for 30 days at 4° C. After photographical processing the slides were analyzed under the microscope and the number of H^3- and C^{14}-labeled cells recorded.

RESULTS

The findings from the three double injected marmosets are listed in Tables 1 and 2, and the labeling picture demonstrated in Fig. 1.

Table 1

The results from the three H^3-TDR and C^{14}-TDR injected marmosets.

Marmoset no.	t_a	H	C	T_S
1	1.5	82	459	8.4
2	1.5	71	412	8.7
3	1	362	2498	6.9

t_a = hours between the two injections; H = the number of H^3-labeled cells; C = the number of C^{14}-labeled cells; T_S = duration of S-phase calculated by:

$$T_S = \frac{t_a \times C}{H}$$

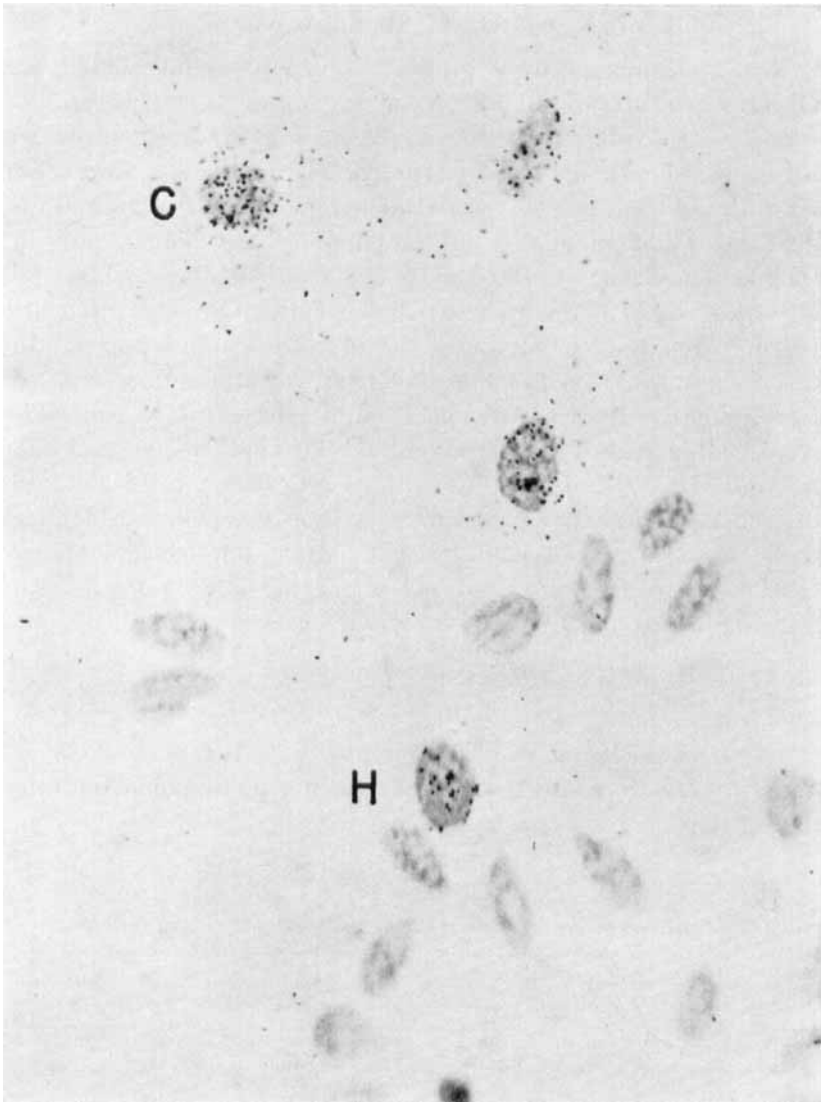

Fig. 1. Autoradiograph of squash preparation from a double injected animal. Note the difference between the C^{14}-labeled cell (C) and the H^3-labeled cell (H).

The squash autoradiographs from marmosets nos. 1 and 2 were prepared from biopsies taken from the attached gingiva whereas the data from marmoset no. 3 were compiled from autoradio-

Table 2

The H^3- and C^{14}-labeled cells observed in four different oral epithelia from marmoset no. 3.

Epithelium	t_a	H	C	T_S
Attached gingiva	1	89	605	6.8
Epithelial attachment	1	93	660	7.1
Tongue	1	90	621	6.9
Palate	1	90	612	6.8

graphs of squash preparations from attached gingiva, epithelial attachment, tongue and palate. The figures from these epithelia are seen in Table 2.

The results suggest a DNA synthesis phase in the oral epithelia of the marmoset of 8 ± 1 hours' duration.

DISCUSSION

The double labeling method for determination of S-phase in the mammalian organism is based on the following assumptions,

1) the cell population must be asynchronous,

2) the cell population must be in a steady state with equal number of cells being removed and generated,

3) the duration of the S-phase must undergo only minor variations,

4) the time interval between the first injection and sacrifice must be less than the duration of $G_2 + M$. Otherwise some of the labeled cells will have divided.

The two first assumptions have been discussed elsewhere for the oral epithelia of marmosets (*Skougaard* 1965) and found to be sufficiently fulfilled. The majority of reports dealing with DNA synthesis phase indicate that the S-phase for renewing cell populations in mammals shows only small variations even for different species and tissues. There seems to be no reason to expect that the S-phase in cells within a tissue should vary significantly. Also the last assumption is met for the present material since no labeled mitoses were observed.

Table 3

Duration of S-phase in different mammalian tissues determined by various investigators.

Investigators	Year	Species	Tissue	Duration of S-phase (hours)
Quastler & Sherman	1959	mouse	gut epithelium	7.5
Lajtha	1959	mouse	bone marrow	5—10
Sherman *et al.*	1961	mouse	ear epidermis	32
Toto & Ojha	1961	mouse	oral epithelium	10
Koburg & Maurer	1961	mouse	gut epithelium	7—8
			cornea	—
			pancreas	—
			forestomach	—
			oesophagus epithelium	—
Skougaard & Beagrie	1962	marmoset	gingival epithelium	7
Cameron	1964	mouse	embryonic	6.5—7.1
Present investigation	1965	marmoset	oral epithelium	8

The 8 hour S-phase in the oral epithelia of marmosets found in the present study is in agreement with the results of most other investigators (Table 3). The 32 hour S-phase for the mouse ear epidermis reported by *Sherman et al.* (1961) differs drastically from the other S-phase durations listed in Table 3. This result was, however, believed to be related to the lower temperature in the outer ear, resulting in an extraordinarily slow metabolism for these cells compared to the rest of the organism (*Bullough* 1949; *Sherman et al.* 1961). The S-phase found in the present investigation supported the concept that the DNA synthesis phase in the mammalian organism is relatively constant for different types of cell populations even in different species (*Koburg & Maurer* 1961; *Lajtha* 1963; *Cameron* 1964).

It is of interest to note that there appears to be no appreciable difference between the S-phase of the four different epithelial

areas examined in marmoset no. 3. Even if it cannot be ruled out that the squash preparations from the epithelial attachment may have included some cells from the free marginal gingiva, the majority of the cells on these slides belonged to the epithelial attachment. If a significant difference existed between the duration of S-phase in the epithelial attachment and the rest of the gingiva, such difference would manifest itself in the ratio of H^3- and C^{14}-labeled cells recorded on the autoradiographs.

SUMMARY

The DNA synthesis phase (S-phase) in marmosets was determined by the double labeling method (H^3-TDR + C^{14}-TDR) suggested by *Wimber & Quastler* (1963). The ratio of H^3- and C^{14}-labeled cells recorded on the autoradiographs showed that the S-phase in the oral epithelia of marmosets lasted 8 hours. The result corroborates the concept that the S-phase in the mammalian organism is relatively constant.

The present investigation further showed that the S-phase in the epithelial attachment of marmosets does not differ from that found in other oral epithelia.

RÉSUMÉ

LA DURÉE DE LA SYNTHÈSE DU DNA DANS L'EPITHÉLIUM GINGIVAL DU OUISTITI

La période de synthèse du DNA (phase S) fut déterminée chez le ouistiti par la méthode de double marquage (H^3-TDR et C^{14}-TDR) suggérée par Wimber et Quastler (1963). Le rapport des cellules marquées par H^3 ou C^{14} comptées sur des préparations autoradiographiques a montré que la phase S dans l'épithélium buccal du ouistiti dure 8 heures. Le résultat renforce la conception d'une phase S relativement constante chez les mammifères.

ZUSAMMENFASSUNG

DIE DAUER DER DNA-SYNTHESE IM GINGIVALEPITHEL DER MARMOSETS

Die DNA-Synthesephase (S-Phase) bei Marmosets wurde mit Hilfe der Methode der doppelten Markierung (H^3-TDR + C^{14}-TDR) nach *Wimber & Quastler* (1963) bestimmt. Das autoradio-

graphisch registrierte Verhältnis der H^3- und C^{14}-markierten Zellen zeigte, dass die S-Phase im Mundepithel der Marmosets 8 Stunden dauert. Das Ergebnis bestätigt die Vorstellung, dass die S-Phase in Säugetierorganismen relativ konstant ist.

Weiterhin zeigte die vorliegende Untersuchung, dass die S-Phase im Epithelansatz der Marmosets sich nicht von der im übrigen Mundepithel gefundenen unterscheidet.

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