AChE activity in amphibian optic tectum appears prominent in superficial layers, where sensitive (mainly retinic) afferents discharge. In comparison with previous observations[2], we noticed a more differentiated AChE distribution in anuran optic tectum. This kind of distribution corresponds to different bands of retinic terminals revealed with different experimental methods[12,18,19]. AChE rich bands in superficial tectal layers might correspond to sites of action of the cholinergic inhibitory system described by STEVENS[20]. In the anuran telencephalon, AChE activity is prominent in sub-pallial regions, in agreement with observations made with a histochemical semi-quantitative method[17]. A characteristic difference in AChE localization between *Rana* and *Bufo* is the positive reaction corresponding to the lateral olfactory tract, observed only in the telencephalon of *Rana*.

Regarding AChE reaction on capillary walls, the use of two different histochemical methods, two different substrates and selective inhibitors permits us to draw some conclusions. In anurans the complete absence of histochemical reaction achieved by either eserine inhibition or the use of butyrylthiocholine as substrate, permits exclusion of interferences of non-specific esterases or pseudocholinesterases. This finding is confirmed by the lack of any remarkable inhibitory effect using the selective pseudocholinesterase inhibitors, iso-OMPA and DFP. Thus, from histochemical controls, it clearly results that the histochemical reaction is only due to true AChE. However, some of our observations suggest that AChE localization on capillary walls may be actually a false localization. In fact, this kind of localization is more or less evident using different histochemical methods. Furthermore, in nervous areas devoid of AChE activity, as for example the telencephalon of *Triturus* and some pallial regions of anurans, one cannot find any positive reaction on the capillary walls; this last finding might indicate a diffusion process from areas rich in enzyme activity and subsequent absorption of the enzyme on capillary walls. In *Triturus* the situation is little different, and it appears that also pseudocholinesterases and non-specific esterases may partly contribute to reaction on capillary walls; however histochemical controls do not confirm that this kind of reaction is only or prevailingly due to non-specific esterases in *Triturus*[3,4].

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Incorporation of Tritiated Uridine During Pachytene and Diplotene Stages in the Oocytes of the Japanese Quail (*Coturnix coturnix japonica*)

M. HARTUNG and A. STAHL

Laboratoire d'Histologie et Embryologie II, Faculté de Médecine, 27, boulevard J. Moulin F-13385 Marseille Cedex 4 (France), 11 July 1975.

Summary. Incorporation of ^{3}H-uridine was studied during pachytene and diplotene stages of quail oocytes. No labelling could be detected during early pachytene. During advanced and late pachytene, labelling simultaneously appeared on the macrochromosomes and on certain michromosomes in the zone where they emerge from the chromocentric surface periphery. The latter localization corresponds to the region of ribosomal RNA synthesis. At diplotene the same localizations were labelled with a considerably increased intensity.

Incorporation of RNA precursors during pachytene and diplotene in the oocyte has been studied by autoradiography only in Amphibians[1,2] and Invertebrates[3-6].

In a previous study, we have shown that the heterochromatic regions of quail microchromosomes fuse during pachytene to constitute the chromocenters. Euchromatic regions of the microchromosomes remain free, radiating around each chromocenter. During diplotene, nucleoli appear and develop in contact with the chromocenters and in strict relation with euchromatic segments of certain microchromosomes. These segments either encircle or penetrate the nucleolus[7], Since labelled nucleosides are incorporated early by precursors of ribosomal RNA, administration of tritiated uridine may contribute to the identification of the sites of nucleolar organization[8].

Material and methods. Cortex from the ovaries of hatching quails aged 12 and 24 h were sectioned into fragments of about 1 mm. Fragments were incubated in a medium of 80% Hanks solution and 20% fetal calf serum containing 100 µCi of H^3-uridine/ml (specific activity 25 Ci/m*M*, CEA France).

Incubation times were 5, 15, 20, 30, 45 and 60 min. Incubation was followed by a 5 min chase using the same medium containing non-radioactive uridine at a concentration of 1 mg/ml. Specimens were immediately fixed in methanol acetic acid (3/1) and treated by the method of LUCIANI et al.[9] which permits one to obtain isolated and well-spread germinal cells. Preparations were stained by acetic orcein and covered by Ilford K2 emulsion. Exposure times were 3 weeks, 1 month and 45 days.

Observations. (A) *Pachytene stage.* During early pachytene, prior to fusion of the heterochromatic zones of the microchromosomes, no radioactivity can be detected. Incorporation of tritiated uridine can only be detected in

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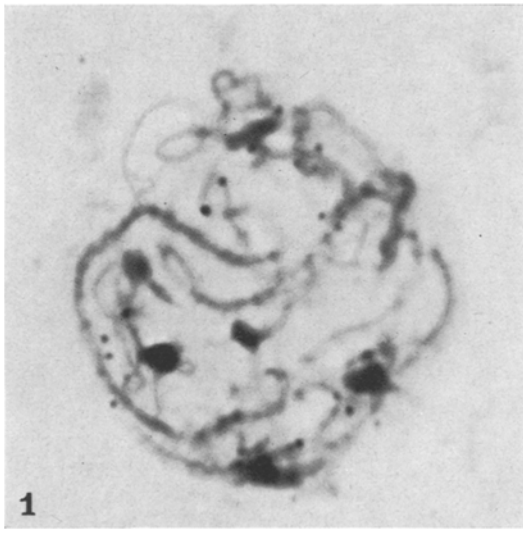

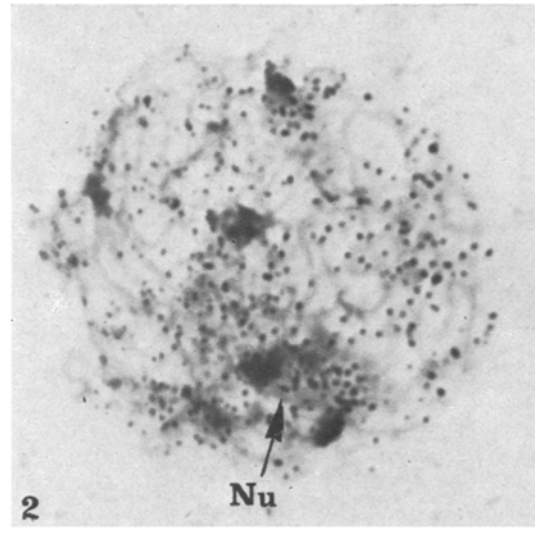

Fig. 1. Autoradiography during pachytene of a quail oocyte aged 12 h. Labelling is localized at the periphery of several chromocenters and along a few macrochromosomes. Tritiated uridine incorporation during 15 min. Exposure: 30 days. × 2200.

Fig. 2. Autoradiography during diplotene of a quail oocyte aged 12 h. Labelling is much greater than during pachytene and particularly abundant at the periphery of several chromocenters and in the nucleolus (Nu). Grains mark out the outline of chromosomes. Tritiated uridine incorporation during 30 min. Exposure: 30 days. × 1800.

oocytes during late pachytene. After 5 min incubation, it is exceptional to find labelled oocytes. Following incorporation of 15 to 20 min, silver grains are observed in two distinct areas: 1. at the periphery of most chromocenters; 2. along the macrochromosomes. (Figure 1).

Grains concentrated at the chromocentric periphery are situated on the microchromosomes, at the site where their euchromatic region emerges from the chromocenter. A few grains are more rarely observed along the microchromosomal euchromatin, more or less near the telomeric region. Grains observed on the macrochromosomes do not present any particular localization. Following incorporation of 30 min to 1 h, the same localizations are observed, but the grains are more numerous.

(B) *Diplotene stage.* On the whole, radioactivity is much greater than during pachytene (Table), with the condition that incubation lasted at least 15 min.

The increased number of grains involves both localizations observed during pachytene. After 20 to 30 min incorporation, the contours of certain chromocenters are dotted with grains. In certain cases, it is possible to localize grains on the juxta-chromocentric portion of the microchromosomes. Preparations in which staining intensity permits visualization of the nucleolus verify that grains are superposed on the latter; however, grain concentration does not exceed that observed at the periphery of certain chromocenters (Figure 2). Moreover, grains are present along the entire length of the macrochromosomes. Grains are not observed between the chromosomes, thus indicating in our experimental conditions that the nuclear sap is non-labelled. Following incorporation from 45 min to 1 h, grain number is increased along the macrochromosomes but not at the periphery of the chromocenters nor in the nucleolus.

Discussion. Early appearance of radioactivity at the periphery of the chromocenters, where the microchromosomes emerge, indicates the site of ribosomal RNA synthesis. It is interesting to note that such radioactivity is detectable during late pachytene whereas staining does not reveal the nucleolus before diplotene. This fact indicates that ribonucleic constituents of the nucleolus are elaborated well before the latter becomes visible in the light microscope using basic staining. Presence of radioactivity along the macrochromosomes from the 15th min on should essentially correspond to formation of messenger RNA precursors which are known to label rapidly[10].

No type of RNA seems to be formed at early pachytene, at which time no radioactivity can be detected. Grains appear during advanced or late pachytene at a stage when macrochromosomes take on lampbrush form; yet labelling remains relatively weak, becoming very intense during diplotene.

Comparing our results with those of MONESI[11,12] using mouse spermatocytes, a few analogies can be drawn, while certain important differences also exist. With both cell types incorporation is very weak during the beginning of pachytene. In mouse spermatocytes incorporation reaches maximum intensity during mid-pachytene and thereafter diminishes; whereas in quail oocytes incorporation continually increases until end-pachytene, reaching a great intensity at diplotene.

In orthopteran spermatocytes the situation approaches that of quail oocytes, as incorporation continues until

H^3-Uridine incorporation during 30 min in the quail oocytes

Stage	Cell number	Average grains/cell
Pachytene	20	48
Diplotene	20	173

Autoradiographic exposure for 30 days.

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diplotene. Nevertheless the nucleolus, if it persists at this stage, cannot be labelled[13].

Our observations approach those of KIERSZENBAUM and TRES[14] who studied nucleolar formation in mouse spermatocytes during zygotene and pachytene. In effect, ribosomal RNA synthesis occurs in contact with the 'basal knobs' in a paracentromeric region corresponding to the nucleolar organizer of several autosomes.

Using brief incorporation times, grains are essentially localized on the chromosomes, few grains if any are visible between the chromosomes. This explains that during our conditions of incorporation the nuclear sap does not present any radioactivity, as opposed to the observations of GALL[15] on Amphibians using more prolonged labelling times.

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The Influence of MuLV and SV40 Viruses on Senescence in Mouse Fibroblasts in vitro

R. S. U. BAKER

Radiation Biology Laboratory, School of Public Health and Tropical Medicine, University of Sydney, Building A27, Sydney (N.S.W. 2006, Australia), 8 July 1975.

Summary. Mouse cells productively infected with Moloney leukaemia virus (MuLV) underwent senescence in a manner similar to control cells, although they recovered more readily as an established line. Rapidly growing cell lines were also obtained following simian virus 40 (SV40) infection of senescent cells. However, superinfection of senescent MuLV-producing cells by SV40 led to slower growing cells with a reduced output of infectious MuLV.

Simian virus 40 (SV40) has been shown to stimulate DNA synthesis in senescent mouse cells[1] which also regain their susceptibility to murine sarcoma virus (MSV). The enhancement of MSV replication by SV40 appears to be due to dependence of MSV on host cell DNA synthesis for successful initial infection[2,3]. Without SV40 infection senescent cells retain little DNA-synthetic or mitotic activity[4].

In the present investigation primary mouse cells were infected with Moloney leukaemia virus (MuLV) and serially subcultured through senescence until established as a permanent line. Studies were conducted on MuLV production during senescence and on the effect of SV40 superinfection on cell growth and leukaemia virus synthesis.

Materials and methods. Cell culture procedures have been described previously[2]. Briefly, BALB/c mouse embryo cells were trypsinized every 3 days and passed at a concentration of 3×10^4 cells per cm^2 of plastic dish. The growth medium was Eagle's Minimum Essential Medium (Gibco Powder Medium F15) supplemented with 10% inactivated calf serum. Cell growth was monitored by counting the cells on days 1 and 3.

Primary embryo cells were infected with MuLV and held, with accompanying control cells, for 21 days before subculturing[5]. A continuous line was derived from MuLV-infected cells, which were carried at reduced densities from passage 10 onwards, as described for the 'low line'[2]. Uninfected cells were carried through to passage 14 at the same time. The MuLV-producing and uninfected BALB/c cells to be treated with SV40, were infected 24 h after trypsinization, in their eighth in vitro passage. Cells at 1.56×10^6 per 100 mm Falcon plastic dish were inoculated with $10^{8.6}$ $TCID_{50}$ SV40 in 1 ml for 60 min at 37 °C. Growth medium was then added and the cells were incubated for a further 3 days before subculture.

Table I. Growth rates[a] of uninfected, MuLV- and SV40-infected cell lines

Passage in vitro	Control line 1	Control line 3	Control line 4	Control line 10	Control line 5 +SV40	MuLV-producing line 10	MuLV-producing line 10 +SV40
1	n.d.[b]	2.2	2.5	2.8		n.d.	
2	3.7	3.8	4.3	3.5		3.2	
3	2.4	3.1	2.1	1.7		2.1	
4	1.5	2.4	2.0	1.7		1.2	
5	0.9	2.4	2.0	1.1		1.0	
6	1.3	1.0	1.4	1.0		1.1	
7	0.9	1.0	0.9	0.9		0.9	
8	0.9	0.8	0.8	1.0	1.8	1.0	1.4
9	0.8	0.9	0.9	0.8	2.3	1.5	2.8
10	0.6	0.7	1.0	0.6	4.0	1.6	2.2
11	0.6	0.6	0.6	0.8	5.0	n.d.	2.0
12	0.9	1.0	0.9	0.8	5.0	2.0	1.7
13	0.8	0.7	1.1	1.0	n.d.	2.0	2.0
14	0.8	1.0	1.1	0.9	n.d.	3.8	1.6

[a] number of cells per dish on day 3 . [b] n.d., not determined.
number of cells per dish on day 1