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

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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

Table II. MuLV production following SV40 infection of MuLV-producing cells

	MuLV-producing cells				SV40-infected MuLV-producing cells			
Passage in vitro	MuLV-specific fluorescence	Interference units per 60 mm dish	Cells per 60 mm dish	Interference units per cell	MuLV-specific fluorescence	Interference units per 60 mm dish	Cells per 60 mm dish	Interference units per cell
2	+	10^6	2×10^6	0.5				
7	+	10^5	6×10^5	0.2				
10	+	10^6	1.2×10^6	0.8	+	10^3	1.5×10^6	0.001
14	+	n.d.[a]	n.d.	n.d.	+	10^3	1.5×10^6	0.001

[a] n.d., not determined.

The sources of wild-type SV40 and MSV (Moloney) have been described[1,6]. Moloney leukaemia virus was obtained from Dr. R. J. HUEBNER as infectious tissue culture fluids. The virus was subsequently passaged in mouse embryo cells and infectious 24 h culture fluids were stored at −70 °C.

Moloney leukaemia virus was assayed by interference[5] with MSV (Moloney). Tissue culture fluids from MuLV-infected cultures were inoculated onto BALB/c primary cultures at dilutions ranging down to 10^{-6}. After 21 days incubation the cells were trypsinized and challenged by infection with MSV. Duplicate cultures were inspected 3 days later for evidence of MSV transformation. An interference unit is defined as the greatest dilution of infected tissue culture fluids able to prevent MSV transformation.

Anti-MuLV antiserum prepared in C57BL mice (by Dr. J. MOLONEY) was used in a sandwich technique with fluorescein-conjugated rabbit anti-mouse IgG. Simian virus 40 nuclear T antigen was detected with a fluorescein-conjugated antiserum produced in hamsters bearing an SV40-induced tumour (Microbiological Associates).

Results and discussion. Mouse cells have been previously shown to undergo senescence in vitro[4]. In this report, a similar curtailment of division is demonstrated in MuLV-infected cells by passages 6–7 (Table I). Although control cells remained quiescent up to passage 14, cells infected with MuLV recommenced growth as an established line by passages 9–12. The introduction of SV40 to uninfected senescent cultures resulted in even more rapid cell division and emergence of permanent cell lines. Superinfection by SV40 of an MuLV-producing line failed to enhance their growth to the same extent, although T antigen fluorescence was detected in their nuclei. Table II shows that MuLV production remained almost constant throughout the in vitro life-span of MuLV-infected cells but SV40 infection caused a marked reduction in MuLV output.

The fact that MuLV-producing cells continue extruding virus during senescence contrasts with the failure of MSV to productively infect cells already in senescence[2]. This confirms that senescent cells, once infected, are capable of supporting RNA tumour virus replication, without further cell division.

The decline in production of MuLV after infection with SV40 virus is at variance with other reports. SIMONS[7] showed that cells infected with both MSV and SV40 grew rapidly, assumed an MSV-transformed morphology and liberated increased amounts of MSV into the culture medium. Neither has interference been demonstrated between SV40 and RNA tumour viruses in other cell systems[8–10].

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Ultrastructure of Synapses of the Metacestode of *Hymenolepis microstoma*

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Summary. The ultrastructure of the synapses of the metacestode of *Hymenolepsis microstoma* is described. Many features observed are similar to those of many invertebrate and vertebrate synapses where mechanical strength is of importance. These observations indicate an early phylogenetic origin for this type of synapse.

Numerous studies have centred on the fine structure of the nervous tissue of platyhelminths (see[1] for references). While synaptic contacts have been observed, little attention has been given to the detailed morphology of the synapse. In the present study, the numerous synapses of the metacestode of *Hymenolepis microstoma* are shown to contain many of the morphological features observed in vertebrate synapses following specialized fixation and staining techniques[2–4].

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