Binding parameters of (^{3}H) spiroperidol in the brains of VEE infected rats

Brain regions	N*	B_{max} (fmol/mg protein) Control	Infected	K_d (nM) Control	Infected
Striatum	12	474.3 ± 40.6	342.0 ± 23.6**	0.69 ± 0.06	0.85 ± 0.10
Midbrain	7	142.5 ± 12.3	109.1 ± 8.1**	2.80 ± 0.40	2.37 ± 0.15
Frontal cortex	6	197.2 ± 14.3	110.0 ± 32.1**	1.35 ± 0.39	0.82 ± 0.30

Results represent the means and SE. * Number of assays, ** difference is significant at $p < 0.05$.

and 3.00 nM (specific activity, 35.9 Ci/mmol). Specific binding was defined as the total binding minus that which occurred in the presence of 1 µM d-butaclamol. Scatchard graphs of data for binding of (^{3}H) spiroperidol were used for calculating the density of receptor sites (B_{max}) and the dissociation constants (K_d). In order to make each Scatchard plot the brain regions of 4 rats were pooled after homogenization. Student's t-test was used for statistical analysis. P-values less than 0.05 were considered significant. As shown in the table the Bmax's found in neostriatum, midbrain, and frontal cortex of VEE infected rats were significantly lower than those observed in controls. The decrease was more accentuated in frontal cortex (44%).

In the past few years we have accumulated some information regarding the neurochemical abnormalities of VEE virus infection[2-4,12]. As a result of these studies, it looks probable that the dysfunctions are due to degeneration of neurons, especially those located within the substantia nigra and basal ganglia which utilize dopamine, GABA and acetylcholine as their neurotransmitters. Because of the neuronal loss and cavitary necrosis observed in the brain of VEE infected rats[13], it was considered possible that the receptors for these neurotransmitters were to some extent damaged during the infection.

The reduction in specific receptor binding shown by this report appears then to be due to neuronal loss. It could also result from a decreased receptor affinity rather than from a fall in the density of receptors. However, the K_D's obtained from the VEE infected rats were not significantly different from the K_D's found in controls.

The significant reduction produced in the number of dopamine and/or serotonin receptor sites in different brain regions as a result of VEE viral infection might explain some of the neurological symptoms and behavioral responses (irritability, hyperthermia, tremor, ataxia, paralysis, and coma) observed in infected rats.

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Differential sensitivity to ethidium bromide of replicative DNA synthesis and bleomycin-induced unscheduled DNA synthesis in permeable mouse sarcoma cells[1]

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Summary. Replicative DNA synthesis in permeable mouse sarcoma cells was more sensitive to ethidium bromide (EtBr) than bleomycin-induced unscheduled DNA synthesis (UDS). A similar difference in sensitivity to EtBr was observed between DNA polymerases α and β. The difference in sensitivity to EtBr of replicative DNA synthesis and UDS in the present system seems to reflect mainly the sensitivity difference between DNA polymerases α and β.

Ethidium bomide (EtBr) which intercalates in double stranded regions of DNA and RNA has been shown to affect the activities of various enzymes involved in DNA and RNA metabolism[2-11]. Of eukaryotic DNA polymerases, DNA polymerase γ (mitochondrial DNA polymerase) has been shown to be highly sensitive to the drug[2,5,7,9]. There have been only a few reports on the effects of EtBr on eukaryotic replicative DNA synthesis, unscheduled DNA synthesis (UDS) and activities of DNA polymerases α and β. Mattern and Painter[10] reported that EtBr gave a biphasic dose effect, stimulation at low concentrations and inhibition at higher concentrations, on replicative DNA synthesis in permeable Chinese hamster ovary cells. The present experiments were performed to see whether or not a biphasic effect of EtBr on replicative DNA synthesis and UDS in permeable mouse sarcoma cells exists, and whether or not there is a difference in sensitivity to EtBr be-

tween replicative DNA syntheses and UDS. Following Kaufman et al.[12], the term 'unscheduled' DNA synthesis is used in the present paper to indicate non-replicative DNA synthesis occurring in vitro in permeable cells or in nuclei.

Materials and methods. EtBr was obtained from Sigma Chemical Co., St. Louis. Aphidicolin was kindly provided by Dr A.H. Todd of Imperial Chemical Industries, Macclesfield, Cheshire. The other reagents used were obtained as described previously[13]. Mouse ascites sarcoma (SR-C3H/He) cells were permeabilized by treatment with buffer B (0.25 M sucrose, 10 mM Tris-Cl, 4 mM $MgCl_2$, 1 mM EDTA and 2-mercaptoethanol, pH 8.0) supplemented with Triton X-100 at 0.0175% (Triton-buffer B)[13]. DNA polymerase α was partially purified from SR-C3H/He cell homogenate as described previously[14]. DNA polymerase β was purified essentially according to the method of Chang[15]. Partially purified DNA polymerase α was highly sensitive to aphidicolin and N-ethylmaleimide, and was resistant to 2',3'-dideoxythymidine-5'-triphosphate (ddTTP). Partially purified DNA polymerase β was highly sensitive to ddTTP, and was resistant to aphidicolin and N-ethylmaleimide. The cross contamination of DNA polymerases α and β and contamination of DNA polymerase γ were not detected in these preparations under the present assay conditions.

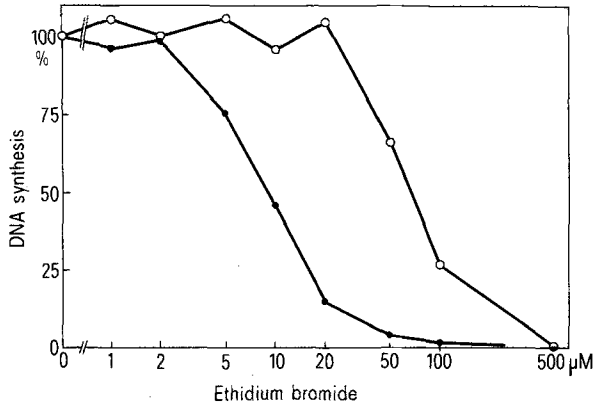

Figure 1. Effects of increasing EtBr concentration on replicative DNA synthesis and bleomycin-induced UDS. Replicative DNA synthesis (●) and bleomycin-induced UDS (○) were measured as described in Materials and methods. Results are expressed as a percentage of the activity measured in the absence of EtBr.

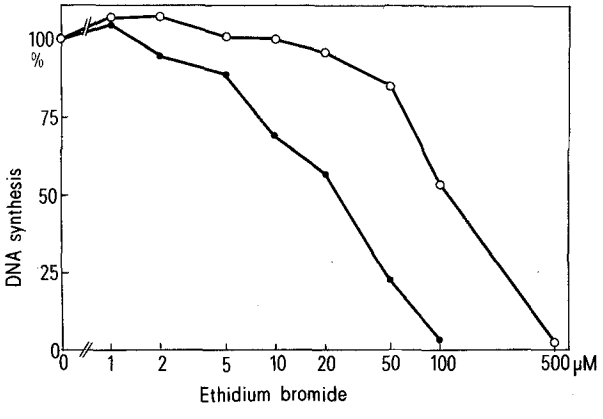

Figure 2. Effects of increasing EtBr concentration on DNA polymerase activity. Activities of DNA polymerases α (●) and β (○) were measured as described in Materials and methods. Results are expressed as a percentage of the activity measured in the absence of EtBr.

Permeable cell suspension was distributed in assay tubes at 2×10^6 cells (0.38 ml) per tube. For replicative DNA synthesis, 0.2 ml of substrate mixture (0.1 M Tris-Cl, 7 mM $MgCl_2$, 0.24 M NaCl, 7.5 mM ATP, 0.15 mM dATP, 30 μM dCTP, 0.15 mM dGTP, and 7.5 μM [^{3}H] dTTP, 0.5 Ci/mmol, pH 8.0) was added to the suspension[13]. For UDS, ATP was omitted from the above substrate mixture, and bleomycin A_2 was added at 0.22 mM[13]. After addition of EtBr and other inhibitors, the final volume of the assay mixture was adjusted to 0.6 ml by adding Triton-buffer B. The reaction mixture was incubated at 37 °C for 10 min for replicative DNA synthesis and for 60 min for UDS. For assaying DNA polymerase activity, partially purified DNA polymerase α (or DNA polymerase β) and activated calf thymus DNA (30 μg final concentration) dissolved in buffer B were added to the ATP-free assay mixture in place of the permeable cell suspension[16]. The volume of the assay mixture was reduced to one fourth of that for the assay of DNA synthesis in permeable cells i.e. to 0.15 ml. The reaction mixtures were incubated at 37 °C for 30 min. The reaction was terminated by rapidly chilling to 0 °C. The radioactivity incorporated into acid insoluble materials was measured by a filter disc method.

Results and discussion. DNA synthesis of permeable SR-C3H/He cells, measured in the presence of ATP, 4 deoxynucleoside triphosphates and other necessary components, was shown to be replicative[13]. UDS was induced by bleomycin in permeable SR-C3H/He cells. The unscheduled nature of the DNA synthesis was determined by autoradiography[17] and by isopycnic centrifugation of BrdUrd-labeled DNA. We estimated that in bleomycin-treated cells UDS accounted for over 90% of DNA synthesis and that interference by replicative DNA synthesis, which is inhibited by bleomycin[17], could be neglected.

Both replicative DNA synthesis and bleomycin-induced UDS were inhibited by EtBr, but the sensitivity of the 2 types was different (fig. 1). The concentrations of EtBr resulting in 50% inhibition (IC_{50}) of DNA synthesis were approximately 10 μM for replicative DNA synthesis and 70 μM for bleomycin-induced UDS. The relatively high resistance to EtBr of UDS compared to replicative DNA synthesis was not limited to bleomycin-induced UDS. UDS measured without bleomycin in isolated rat liver nuclei[13,16] showed a resistance to EtBr similar to that of bleomycin-induced UDS. The IC_{50} of UDS in isolated liver nuclei was approximately 60 μM.

Mattern and Painter[10] reported that replicative DNA synthesis was enhanced up to twofold by 1–10 μg/ml (2.5–25 μM) of EtBr, and inhibited by concentrations greater than 10 μg/ml. In our experiments neither replicative DNA synthesis nor UDS was enhanced at any concentration of EtBr. The reason why our results were different in this regard from those of Mattern and Painter is unclear, but the cells and assay systems were different.

It seemed possible that the difference in sensitivity to EtBr between replicative DNA synthesis and UDS was due to the difference in the DNA polymerases involved. Previous studies showed that DNA polymerase α plays a major role in replicative DNA synthesis[18], and that DNA polymerase β plays a major role in bleomycin-induced UDS in permeable mouse sarcoma cells and UDS in isolated rat liver nuclei[13]. EtBr effects on DNA polymerases were studied under the same conditions of ionic composition and concentration as were used in the assay mixtures for replicative DNA synthesis and UDS, because the interaction of DNA with EtBr has been shown to be influenced by the ionic composition and concentration of the medium[19]. DNA polymerase activities measured with the DNA polymerase α-activated DNA system and with the DNA polymerase β-activated DNA system were both sensitive to EtBr (fig. 2). The IC_{50}s were approximately 20 μM for DNA polymerase α and 120 μM for DNA polymerase β. Although the IC_{50} for replicative DNA synthesis was not identical with that of DNA polymerase α, and the IC_{50} for UDS was not

identical with that of DNA polymerase β, comparing the results shown in figures 1 and 2, it is suggested that the differential sensitivity to EtBr in replicative DNA synthesis and UDS was due mainly to the difference in the DNA polymerases involved and the differential sensitivity to EtBr of DNA polymerases α and β. The difference in the IC_{50} values between DNA synthesis in permeable cells and DNA synthesis in the DNA polymerase-activated DNA system may be due to the difference in the physical properties between chromatin DNA and activated DNA[20].

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Chromosome stabilizing structures in mitotic Indian muntjac *(Muntiacus muntjak)* cells[1]

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Summary. A new technique which removes all membranes, cytoskeletal elements, organelles, but preserves intact metaphase, anaphase and telophase configurations is combined with scanning electron microscopy (SEM) as an approach for direct visualization of chromosomal behavior in late mitosis. With this approach we are able to confirm the presence of a centromeric ring which stabilizes the centromeres during the cell cycle and present evidence for a lattice-like sheet of interchromatidic fibers in late mitosis.

The radial arrangement of chromosomes on the mitotic spindle of plant and animal cells has led to the agreement that the telophase arrangement of chromosomes is maintained throughout interphase[2-6]. The radial arrangement of the metaphase chromosomes of the Indian muntjac and the low diploid number (7 in the male) of this species is ideal for investigation of the centromere clustering. By using a cell synchrony procedure, large numbers of metaphase, anaphase and telophase configurations were obtained for analysis without the use of spindle-disrupting drugs. The configurations, collected at various stages of mitosis were stripped of their membranes, organelles and cytoskeletal elements by a newly developed technique which permitted direct visualization of the intact mitotic structures. A large percentage of the mitotic configurations retain the radial array of chromosomes in spite of the treatment with hypotonic solution, fixation with methanol:acetic acid, spreading onto glass coverslides, hot acetic acid treatment and critical point drying. The radial arrangement of metaphase chromosomes is maintained because all chromosomes are associated at the centromere region, by a filamentous ring which interconnects the adjacent centromeres.

Also in this study it was revealed by 3-dimensional visualization of critically point-dried, organelle-free anaphase and telophase configurations that a lattice-like network of fibers bind the chromatids and their telomeres into a late mitotic configuration. It is with these data and that obtained from the the arrangement of chromatin in interphase cells[2,4-7] that we support the concept of centromere clustering throughout the muntjac cell cycle.

Materials and methods. Fibroblast-like cells of the Indian muntjac *(Muntiacus muntjak)* were obtained from the American Type Tissue Culture Collection. Cells were cultured in Ham's F-12 media supplemented with 20% fetal calf serum. Cells were synchronized using a double-thymidine block[8] and mitotic cells were removed by selective detachment.

Collected cells were hypotonically shocked with 0.075 M KCl for 6 min at 37 °C. Cells were recollected and fixed in 3:1/methanol:acetic acid for 24 h. After several washings in 3:1 fixative cells were concentrated in fixative. One drop of cell concentrate was dropped on a 22-mm diameter No. 2 coverglass and permitted to dry for 30 sec. The coverglass was then immersed in 50% acetic acid at 101 °C for 1–2 sec. Rapidly, so as to prevent drying, the coverglass was transferred to 50% ethanol at room temperature. The coverglasses were then transferred through graded alcohols to acetone for dehydration. The cells were critically point-dried in liquid-gaseous CO_2 and sputter-coated with gold palladium, about 50 Å thickness. An AMR-1000A scanning electron microscope was used for observation of the surface topography of the pre-nuclear structures and records of selective configuations were collected on Polaroid N/P 55 film.

Results. Cells harvested after mitotic selection yielded largely metaphase and anaphase with the remaining telophase and interphase cells. Metaphase were difficult to find because the ma-