it is taken into account [at about one-fifth of the present lunar dissipation (*3*)]. The observation of 31.56 ± 0.74 days for the Upper Cambrian (*1*) is much easier to explain with solar dissipation included in the dynamics of the earth-moon system.

Runcorn (*3*) suggested that the moment of inertia of the earth has decreased in geologic time—he expects a value of about 4 percent greater at 0.5×10^9 years ago. Such increases are difficult to reconcile with the data of Pannella *et al.* (*1*), in view of the maximum.

The measurements of Pannella *et al.* (*1*) show a tendency to remain at about 30 days. If real, this could be a resonance effect. The 30-day resonance is double; it comes at about 390 days per year, and so is nearly coincident with a resonance of 13 (synodic) months per year. Resonances are treated by Goldreich and Peale (*4*); an extension of their methods is required for this case. Such resonances complicate the dynamics substantially, but will probably not vitiate my arguments here.

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Polyoma Virus Gene Activity during Lytic Infection and in Transformed Animal Cells

Abstract. *Randomly labeled RNA's from animal cells either productively infected or transformed by polyoma virus were used to measure virus DNA transcription. During lytic infection, 50 percent of polyoma virus DNA was expressed. In two different polyoma-transformed hamster cell lines, the same 20 percent of the virus genome was transcribed. About 10 percent of polyoma DNA was expressed in the one mouse transformed cell line examined.*

Virus specific RNA's have been detected in animal cells transformed by polyoma, SV40, and several of the adenoviruses (*1–4*). Several reports have compared the virus-specific RNA's found in transformed cells with the RNA's present in cells productively infected with these agents (*3, 5*). In the present study, RNA of known specific activity was prepared from mouse cells undergoing productive infection with polyoma virus. This RNA was used to measure transcription of polyoma virus DNA during the course of lytic infection as well as in cells transformed by this agent.

Polyoma DNA, labeled with C^{14}-thymidine, was prepared from C-57 mouse embryo fibroblasts infected with SE wild-type polyoma virus. Virus was purified by zone sedimentation onto a CsCl cushion followed by two cycles of CsCl equilibrium density centrifugation. Virus DNA was extracted with a mixture of chloroform and isoamyl alcohol (24:1, by volume) in the presence of 1 percent sodium dodecyl sulfate and 1*M* sodium perchlorate (*6, 7*). The specific activity of the C^{14}-DNA was 6100 count min^{-1} μg^{-1}. Double-stranded, twisted, circular DNA (DNA I) was separated from nicked circular (DNA II) and linear DNA's by equilibrium density centrifugation in CsCl containing ethidium bromide (130 μg/ml) (*8*).

Randomly labeled RNA from cells productively infected with polyoma was prepared in the following manner. Monolayers of C-57 mouse embryo cells, growing in 20 Blake bottles in the presence of phosphate-free Eagle's minimal essential medium (MEM) and 2 percent fetal bovine serum, were exposed to carrier-free P^{32}-orthophosphate (10 μc/ml) and allowed to grow to confluency over a 96-hour period. Polyoma virus (SE wild type), previously dialyzed against phosphate-free MEM, was added to the mouse embryo cells at a multiplicity of 15 to 20 infectious units per cell. After a 2-hour adsorption period, infection was allowed to proceed in the presence of the same P^{32}-MEM used during the preceding 96 hours. At 7, 14, 21, 30, and 41 hours after infection, the cells from four Blake bottles were harvested and lysed by 0.35 percent sodium dodecyl sulfate in 0.1*M* NaCl, 0.01*M* sodium acetate (*p*H 5.3) and Bentonite. The preparation was deproteinized with phenol at 60°C, treated with deoxyribonuclease (Worthington Biochemical), 50 μg/ml, and then exposed to Pronase (Calbiochem), 50 μg/ml. After a second phenol extraction, the RNA was applied to a G-100 Sephadex column and the material appearing in the excluded volume was collected. The specific activities of the RNA's isolated from lytically infected cells at the five indicated time periods ranged from 55,000 to 59,000 count min^{-1} μg^{-1}. The RNA's were then pooled to ensure that representatives of all classes of virus-specific RNA transcribed during lytic infection were present in one preparation. This mixture of P^{32}-RNA's will be referred to as "lytic" RNA.

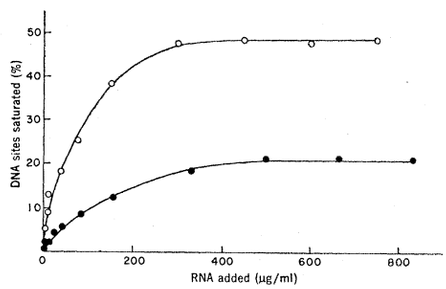

Fig. 1. Saturation of C^{14}-labeled polyoma DNA (6.1×10^3 count min^{-1} μg^{-1}) with P^{32}-labeled lytic RNA (○) (55×10^3 count min^{-1} μg^{-1}) and P^{32}-labeled RNA from the T-54 line of polyoma-transformed hamster cells (●) (88×10^3 count min^{-1} μg^{-1}).

Hamster cells transformed by polyoma virus, derived from a polyoma-induced tumor (T-54), were labeled in tissue culture with P^{32}-orthophosphate (10 μc/ml) for 96 hours. Radioactive RNA from transformed cells was extracted as outlined above. Unlabeled RNA was also prepared from polyoma-induced hamster (T-54) and mouse (1923) tumors. One hamster tumor, induced by a strain of polyoma that replicates relatively efficiently at 30°C (30°C–polyoma tumor) was also a source of unlabeled RNA.

Polyoma virus DNA II, randomly labeled with C^{14}, was immobilized on 50-mm nitrocellulose filters as described by Gillespie and Spiegelman (*9*). Smaller 7-mm filters, containing approximately 0.015 μg of DNA, were punched out of the 50-mm filter and added to a test tube (10 by 25 mm) containing 0.8*M* NaCl, 0.002*M* TES [*N*-tris(hydroxymethyl)methyl-2-amino-

ethane sulfonic acid] buffer (*p*H 7.2), 0.1 percent sodium dodecyl sulfate, and labeled RNA in a final volume of 0.25 ml. After a 16-hour incubation at 67°C, the filters were extensively washed in quadruple-strength standard saline citrate (*10*) at 67°C, dried, and counted. Less than 0.004 percent of the input P^{32}-RNA reacted with filters containing no DNA.

In the first series of experiments, increasing amounts of lytic RNA randomly labeled with P^{32} (55×10^3 count min^{-1} μg^{-1}) were added to nitrocellulose filters containing about 0.015 μg of polyoma DNA labeled with C^{14} in the standard hybridization reaction mixture. Filters containing no DNA were also included in each reaction mixture. In each case, any of the labeled RNA reacting nonspecifically with these filters was subtracted from the RNA reacting with the filters containing immobilized C^{14}–polyoma DNA. Figure 1 shows that an excess of P^{32}–lytic RNA saturated approximately 50 percent of polyoma DNA. Under conditions of maximum gene activity, the equivalent of one strand of double-stranded DNA is transcribed. This 50 percent saturation value obtained with P^{32}–lytic RNA implies that during productive infection, all regions of polyoma DNA capable of transcribing RNA are expressed.

Polyoma DNA (*11*) and virus-specific RNA (*1*) have been detected in cells transformed by this agent. However, unlike SV40 virus, polyoma has not been rescued from such transformed cells. Consequently, it is not known whether the entire polyoma genome or a selected portion thereof is present in cells transformed by this virus. The following DNA-RNA hybridization experiments were monitored with polyoma DNA prepared from purified virus, and the results are presented in terms of the saturation of the entire polyoma genome, whether or not it is actually present in the transformed cell. Figure 1 shows that when increasing amounts of randomly labeled P^{32}-RNA (88×10^3 count min^{-1} μg^{-1}), prepared from hamster cells transformed by polyoma (T-54), are added to a series of filters containing immobilized polyoma virus DNA labeled with C^{14}, about 20 percent of the DNA sites are saturated. This saturation value corresponds to 40 percent (20/50) of the polyoma gene activity during lytic infection and indicates that less than half of the polyoma genome is expressed in the T-54 line of transformed cells.

Table 1. Reduction of polyoma DNA sites available for reaction with P^{32}–lytic RNA after exposure to an excess of unlabeled polyoma-induced tumor RNA.

Unlabeled RNA added	Amount added (μg)	Polyoma DNA sites saturated (%)
None		50.2
Hamster T-54 tumor	210	31.2
Hamster T-54 tumor	420	30.7
Hamster 30°C–polyoma tumor	345	30.5
Mouse 1923 tumor	325	40.3
Hamster Ad-12 tumor	416	49.7

The ability to saturate the equivalent of one strand of polyoma DNA with the labeled lytic RNA (Fig. 1) provides a means for comparing transcription of the viral genome in several cell lines transformed by this agent. Saturating amounts of unlabeled RNA from cells transformed by polyoma were incubated for 16 hours in the standard reaction mixture with filters containing C^{14}-labeled polyoma DNA. At this point, saturating amounts of P^{32}–lytic RNA (Fig. 1) in 0.8*M* NaCl, 0.002*M* TES buffer (*p*H 7.2), and 0.1 percent sodium dodecyl sulfate were added to the reaction mixture. After a second 12-hour incubation at 68°C, the filters were removed, washed, dried, and assayed for radioactivity. The extent of polyoma DNA expression in each transformed cell line could be determined from the fraction of polyoma DNA sites still available for reaction with the P^{32}-RNA.

Table 1 summarizes the results obtained from such a series of competition-saturation experiments. First, it can be seen that saturating amounts of unlabeled RNA from the T-54 line of polyoma-transformed hamster cells reduced the DNA sites available for reaction with P^{32}–lytic RNA from 50 to 30 percent. This 20 percent reduction is very similar to the saturation value achieved when randomly labeled RNA from this transformed cell line was assayed directly (Fig. 1). Virus-specific RNA from "30°C–polyoma tumor" cells was also examined in this system. As was the case with RNA from the T-54 line of polyoma-transformed hamster cells, only 30 percent of the polyoma DNA was still available for reaction with P^{32}–lytic RNA. Table 1 shows that virus-specific RNA from polyoma-transformed mouse cells (1923) reduces the polyoma DNA sites available for reaction with P^{32}–lytic RNA from 50 to 40 percent, not from 50 to 30 percent observed with RNA's from hamster transformed cells. This result suggests that about 10 percent of the polyoma genome is transcribed in this mouse transformed cell. This is only one-half of the virus-specific gene activity found in the two hamster transformed cell lines. To conserve the P^{32}–lytic RNA used in the competition-saturation experiments, the nitrocellulose filters, which had been exposed to unlabeled transformed-cell RNA for 12 hours, were thoroughly blotted and transferred to a second reaction mixture (total volume of 0.25 ml) containing only P^{32}–lytic RNA. The results obtained were indistinguishable from those reported in Table 1 where no filter transfer occurred.

Table 2. Reduction of polyoma DNA sites available for reaction with P^{32}–lytic RNA after exposure to combinations of unlabeled polyoma-induced tumor RNA.

Unlabeled RNA added	Amount added (μg)	Polyoma DNA sites saturated (%)
None		50.7
Hamster T-54 + hamster 30°C–polyoma tumor	210 225	30.1
Hamster T-54 + mouse 1923 tumor	210 220	29.8

In order to determine whether similar regions of polyoma DNA were expressed in each of the transformed cells described above, additive-saturation-competition experiments were performed. Saturating levels of two of the unlabeled transformed-cell RNA's were simultaneously added to the standard reaction mixture. The reduction in the DNA sites saturated by the P^{32}–lytic RNA could be compared to the value obtained when the unlabeled RNA's were added separately (Table 1). The results presented in Table 2 suggest that similar regions of polyoma DNA are expressed in both types of polyoma-transformed hamster cells. Table 2 also indicates that the region of the polyoma DNA transcribed in mouse transformed cells is also expressed in polyoma-transformed hamster cells. Additional regions of the polyoma genome, however, are also transcribed in hamster cells transformed by polyoma.

It is not yet known whether the entire polyoma genome is present in transformed animal cells. The results described above, therefore, can be interpreted in either of two ways. If

polyoma DNA is present in its entirety in transformed cells, our data suggest that nearly 60 percent of the DNA transcribed during lytic infection is repressed. Alternatively, if only a selected portion (for example, 40 percent) of the polyoma genome is integrated in these cells, the saturation values reported above may represent complete expression of a segment of virus DNA.

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Endocrine Control of Adrenal Progesterone Secretion in the Ovariectomized Rat

Abstract. *In the ovariectomized rat, the adrenal gland secretes progesterone. Adrenocorticotropin treatment elevates concentrations of this steroid in plasma, and inhibitors of adrenocorticotropin or progesterone biosynthesis lower the concentration. Adrenocorticotropin controls progesterone secretion by the adrenal but not by the ovary. Adrenal progesterone and its mode of control may have important influences on reproductive processes.*

Progesterone occupies a key position in the biosynthesis of the adrenal corticoids in vitro (*1*). In addition to its role as precursor, progesterone has been isolated in considerable quantities from adrenal venous blood of cattle, sheep, and pigs (*2*) and of humans (*3*) and dogs (*4*) treated with corticotropin (ACTH). Recently, progesterone has been found in systemic plasma of rats after ovariectomy. The disappearance of progesterone from systemic plasma 8 hours after removal of the ovary and the adrenal suggests an adrenal origin for this compound (*5*). The experiments described in this report were efforts to understand control of adrenal progesterone secretion in the rat.

Adult, cycling female rats were ovariectomized 1 week before treatment. Adrenalectomies were performed on intact animals (day 1 or 2 of the cycle) with two consecutive 4-day cycles prior to the operation (*6*). The only special postoperative care given the animals was an ample supply of 0.9 percent saline as drinking water. Blood was collected by cardiac puncture into heparinized glass syringes and centrifuged shortly after removal. Samples, of the plasma were stored at −16°C until steroid analyses were made. Within 3 weeks after collection, about 4 ml of plasma was used for progesterone determination. Gas-liquid chromatography with electron-capture detection was used for steroid analysis (*7*). This technique had been used in my laboratory for the estimation of progesterone in rat plasma (*5*).

Aminoglutethimide (AGT) (Ciba) [30 mg in 0.5 ml of acetate buffer, *p*H 4.0 (*8*)] was injected subcutaneously (*9*). Acetate buffer injection without AGT was used as a control. Three hours after injection animals were anesthetized with Nembutal (35 mg per kilogram of body weight) and the blood was removed by cardiac puncture. Dexamethasone (DM) (Merck Sharp and Dohme) (100 mg/kg) was injected subcutaneously in 0.5 ml of propylene glycol. The animals were anesthetized with Nembutal 3 hours after treatment, and blood was collected. Corticotropin (5 U.S.P. units, Armour "Acthar") was injected via the tail vein in 0.3 ml (total volume) of saline. Another group of animals was treated with luteinizing hormone (100 μg; Calbiochem equine). Animals treated with saline served as controls. Animals were anesthetized with Nembutal at the time of injection, and anesthesia persisted until blood was collected 1 hour later.

Table 1. The effects of ACTH, dexamethasone, and aminoglutethimide on progesterone concentrations in systemic plasma in the ovariectomized rat.

Treatment	*N*	Progesterone (ng/ml ± S.E.)
Buffer	10	5.0 ± 2.2
Aminoglutethimide	10	0.5 ± 0.2*
Dexamethasone	10	ND†
Saline	9	3.9 ± 2.6
ACTH	9	24.1 ± 2.9‡

* Aminoglutethimide-treated compared to buffer-treated rats, $P < .05$. † ND, not detectable. ‡ ACTH-treated compared to saline-treated, $P < .01$.

Analysis of variance was used to determine the general effects of treatment on the concentration of progesterone in systemic rat plasma. Two groups of analyses were made. The animals in group 1 were treated with saline (control), luteinizing hormone, and ACTH; those in group 2 were treated with buffer (control), AGT, and DM. In both cases a significant *F* value was obtained for the effect of treatment (group 1, $P < .01$; group 2, $P < .05$). A *t*-test was used to compare treatment means with control.

In the ovariectomized rat, progesterone remains in the systemic blood for as long as 25 days after ovariectomy, whereas combined adrenalectomy and ovariectomy reduce the concentrations of this hormone in plasma to undetectable amounts in 8 hours (*5*). It is shown here that adrenal progesterone secretion in the rat is under ACTH control.

The quantitative effects of ACTH, AGT, and DM are shown in Table 1. One hour after injection of a corticotropin preparation, significant increases (ACTH versus saline) in progesterone concentrations in systemic plasma were noted ($P < .01$). Animals treated with dexamethasone were without circulating progesterone in their systemic plasma. Aminoglutethimide, a biosynthetic inhibitor that blocks the conversion of cholesterol to pregnenolone (*9*), significantly reduced systemic quantities of progesterone compared with those in buffer-injected controls ($P < .05$).

Adrenocorticotropin does not seem to affect systemic progesterone concentrations when the adrenals are absent but the ovaries left intact. Animals treated with saline showed a progesterone concentration of 2.2 ± 0.9 (S.E.) ng per 100 ml of plasma ($N = 4$), and those treated with ACTH showed a concentration of 2.6 ± 1.1 (S.E.) ng per 100 ml of plasma ($N = 4$). These observations limit the action of ACTH on the biosynthesis of progesterone to