isoenzyme is much less than that of the LDH-5 isoenzyme in normal mice. This suggests that clearance of the two isoenzymes takes place by different mechanisms, but it may not be valid to compare clearance rates of isoenzymes derived from different animal species. However, Fleisher and Wakim have reported wide differences in the clearance rates of homologous glutamic-oxaloacetic transaminase isoenzymes in the dog (*9*).

Blockade of the reticuloendothelial system with thorotrast or cholesterol oleate causes increases in plasma enzyme of a pattern similar to those found in mice infected with Riley virus (*10*). Thus in blockaded, as in infected, mice LDH and phosphoglucose isomerase increased, whereas aldolase and alanine transaminase did not. Probably some plasma enzymes, or isoenzymes, are cleared by the reticuloendothelial system, whereas others are not.

Our results provide an explanation of why only the LDH-5 isoenzyme activity is elevated in the plasma of mice infected with Riley virus.

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8. Starch-gel electrophoresis of the isoenzyme preparations showed that the LDH-1 isoenzyme, although contaminated with an appreciable amount of LDH-2, contained no detectable LDH-3, LDH-4, or LDH-5. The LDH-5 isoenzyme, although contaminated with an appreciable amount of LDH-4 and a visible trace of LDH-3, contained no detectable LDH-1 or LDH-2.
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Transformation by Rous Sarcoma Virus: A Requirement for DNA Synthesis

Abstract. *Transient inhibition of DNA synthesis immediately after infection of cells with Rous sarcoma virus prevents subsequent morphological transformation of the cells. Inhibition of DNA synthesis for an identical period before infection, or later in the course of infection, has only slight effect on transformation. Thus DNA synthesis is a specific requirement in the transformation of cells by Rous sarcoma virus.*

The conversion of a cell from a normal to malignant form must be accompanied by specific biochemical changes. The changes are deemed hereditary since tumor cells confer upon their progeny the continuing capacity for tumorigenesis.

One might suspect, therefore, that the conversion of a cell to the malignant form is dependent in some way upon DNA. The observation that actinomycin inhibits chemical carcinogenesis in animals (*1*), or conversion of cells by simian virus 40 (SV40) (*2*), has led these investigators to suggest that carcinogenesis requires DNA-dependent RNA synthesis, although a requirement for DNA synthesis was not excluded in the experiments.

Rous sarcoma virus (RSV) has a specific requirement for DNA synthesis early in its infectious cycle (*3–5*), although the virus presumably contains only RNA (*6*). Whether the requirement is for new cellular DNA synthesis or for a virus-primed DNA synthesis has not been resolved. This requirement for DNA synthesis is transitory since the growth of virus is unaffected if specific inhibitors of DNA synthesis are added later than 12 to 16 hours after infection (*5*). However, the completion of the virus as an infectious entity is unnecessary for transformation of chick-embryo cells by RSV (*7*). The following experiments demonstrate that DNA synthesis is necessary during the early stages of infection by RSV in order for cells to be transformed.

The methods for the growth of chick-embryo cells and for the propagation and assay of the Bryan strain of RSV have been described (*3, 8*). The test medium consisted of Eagle's medium No. 2 with 10 percent calf serum and antibiotics. Antiserum to RSV was induced in turkeys and prepared by University Laboratories, Highland Park, New Jersey. A dilution of 1 : 100 of this serum, as used in these experiments, was capable of inactivating more than 99 percent of a suspension of RSV containing 10^5 focus-forming units of RSV per milliliter during incubation for 30 minutes at 37°C.

Cells transformed by RSV are easily recognized if they occur in a mass culture or, more quantitatively, as a group (focus) against a background of normal cells, but they cannot be recognized with confidence if they occur singly. Cellular divisions are necessary for focus formation, and DNA synthesis is a continuous requirement for dividing chick-embryo cells. In order to test the effect of reduced DNA synthesis on transformation, it was necessary to use experimental conditions in which DNA synthesis resumed after a period of interrupted activity.

Cytosine arabinoside [1-β-D-arabinosylcytosine (ara-C)] effectively blocks

Table 1. Reversal of inhibition of DNA synthesis. Chick-embryo cell cultures were exposed to ara-C ($10^{-3.5}M$) for 4 hours at 37°C. The medium on some cultures was then replaced with medium containing deoxycytidine (dC, $10^{-3}M$). Cultures were exposed to H^3-thymidine (10 μc in 3 ml) for exactly 1 hour, and incorporation of H^3 into material precipitable by cold $HClO_4$ was measured (*5*).

Treatment	Addition of H^3 (hr after exposure to ara-C)	H^3 (count/min)
None	0	26,618
ara-C 5 hr	0	896
ara-C 4 hr, then dC	0	2,296
ara-C 4 hr, then dC	1	4,763
ara-C 4 hr, then dC	3	20,390
ara-C 4 hr, then dC	5	41,386

Table 2. Effect on virus yield of limited exposure of cells to ara-C. Cells were infected with RSV (about two focus-forming units per cell), washed, and exposed to ara-C ($10^{-3.5}M$) for 8 to 12 hours. Ara-C was removed, deoxycytidine ($10^{-3}M$) added, and culture fluids were removed at various later times.

Exposure to ara-C (hr)	Focus-forming units per culture at intervals after infection		
	24 hr	32 hr	36 hr
None	69,000	255,000	1,650,000
8	1,635	6,300	
12	<15		375

Table 3. Inhibition of RSV-transformation by ara-C. Various concentrations of ara-C were added for 16-hour intervals before or after exposure of cells to dilutions of RSV stock. Antiserum to RSV was added in all media used after infection, and deoxycytidine ($10^{3}M$) was added to all mediums after removal of ara-C. Cultures were incubated for 1 week at 40°C under nutrient agar, and characteristic focuses of Rous sarcoma cells were counted. The numbers indicate resulting focuses. When no ara-C was added, there were 56,900.

Exposures to ara-C	Molarity of ara-C		
	$10^{-3.5}$	10^{-4}	$10^{-4.5}$
Before RSV	12,300	23,800	37,400
Immediately after RSV	360	550	1,120
16 hr after RSV	7,100	11,900	26,000

DNA synthesis in cultures of chick-embryo cells but has no effect on RNA synthesis over a 24-hour period (*5*). The inhibitory effect of ara-C can be prevented by deoxycytidine but not by cytidine (*5*), and cells exposed to ara-C resume DNA synthesis if ara-C is removed and deoxycytidine added (Table 1). Reversal of the effect of ara-C decreases if the interval between initial exposure and removal is extended beyond 8 to 10 hours and the capacity of cells to resume growth begins to decrease by 12 hours after initial exposure to ara-C.

Experiments dealing with transformation stemmed from the following observation on virus synthesis. Chick-embryo cells were exposed to RSV for 45 minutes, then washed with buffered saline. Cytosine arabinoside ($10^{-3.5}M$) was added, and 8 or 12 hours later the medium containing ara-C was replaced with medium containing deoxycytidine. The yield of virus 24 to 36 hours after infection was considerably reduced compared to that in untreated controls (Table 2). The reduction could not be attributed merely to an 8- or 12-hour delay in virus synthesis since virus production in untreated cultures after 24 hours was much greater than in treated cultures after 32 or 36 hours. This inhibition of DNA synthesis during infection with RSV may have resulted in an abortive infection, although the effect of a slight delay in resumption of DNA synthesis is difficult to assess. The effect of interruption of DNA synthesis on transformation of cells by RSV was examined.

Groups of chick-embryo cell cultures were exposed to various dilutions of RSV for 45 minutes and washed with buffered saline. After infection, antiserum to RSV was incorporated in all mediums to prevent elution and reinfection by superficially attached virus and to reduce complications of virus-yielding cells later in the course of incubation. Cultures were exposed to ara-C for 16-hour periods: 16 hours before infection, 0 to 16 hours after infection, or 16 to 32 hours after infection. These three intervals were necessary to assess the temporal effect of inhibition of DNA synthesis on subsequent morphological transformation of cells. After the removal of ara-C, cultures were washed with buffered saline, and growth medium containing deoxycytidine was added. At the 32nd hour, nutrient agar medium containing deoxycytidine was added, the cultures were incubated at 40°C for 1 week, and typical Rous sarcoma focuses were counted. The recorded focuses were calculated relative to an undiluted sample of infecting virus.

The presence of ara-C from 0 to 16 hours immediately after infection reduced the number of focuses to about 3 percent of cultures treated with ara-C before infection and to 5 percent of cultures treated 16 to 32 hours after infection (Table 3). The slight inhibition noted in cultures treated before infection compared to untreated controls can be attributed to the failure to reverse the effect of ara-C immediately upon addition of deoxycytidine (Table 1). The inhibition noted in cultures treated 16 to 32 hours after infection may be due in part to the asynchrony of the DNA-dependent phase of transformation among different cells. In all cases where ara-C is used, some inhibition of focus formation may be caused by the failure of a portion of the treated cells to resume growth. However, maximum inhibition of transformation occurred only in the group receiving ara-C immediately after infection. Therefore, the inhibition of DNA synthesis by ara-C has a specific effect on the transformation process, and the observation cannot be attributed to a generalized toxic effect on chick-embryo cells, or to an increased susceptibility of RSV-infected cells to the cytotoxic action of ara-C.

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Unique Sterol in the Ecology and Nutrition of Drosophila pachea

Abstract. Drosophila pachea, *which breeds only in the stems of senita cactus* (Lophocereus schottii) *throughout the Sonoran Desert, requires the cactus as a dietary supplement when reared on laboratory media.* Δ^{7}*-Stigmasten-3β-ol, isolated from the cactus or synthesized, can replace the cactus in the diet of flies reared nonaseptically or axenically.* Δ^{7}*-Cholesten-3β-ol and* $\Delta^{5,7}$*-cholestadien-3β-ol could be substituted for the cactus sterol;* $\Delta^{5,7}$*-stigmastadien-3β-ol produced infertile females. Cholesterol, 4α-methyl-*Δ^{7}*-cholesten-3β-ol, β-sitosterol, stigmasterol, ergosterol, and* Δ^{7}*-ergosten-3β-ol did not support larval growth.*

Drosophila pachea Patterson and Wheeler was described from two males and two females collected near Hermosillo, Sonora, Mexico, in August 1941 (*1*). The species would not breed on standard laboratory media for *Drosophila*, and therefore no culture was established at that time. The species was rediscovered in 1962 during a survey of the breeding sites of cactiphilic *Drosophila* in the Sonoran Desert and attracted our attention on two counts: first, *D. pachea* was bred only from stems of senita cactus, *Lophocereus schottii* (Engelmann) Britton and Rose, a columnar cactus abundant in the states of Sonora and Baja California, Mexico (Table 1); and second, no other species of *Drosophila* utilize the stems of this cactus for breeding purposes, even though two other species of local *Drosophila* have been reported to breed in the fruits of senita (*2*).

Our early tests showed that *D. pachea* could breed successfully on a standard medium composed of bananas, malt, corn syrup, and yeast only when the medium is supplemented with a cube of fresh or autoclaved senita cactus. Without the