be repaired nonhomologously (Fig. 2, e–j) yielding the various types of deletion we have discussed.

These considerations show that a network of branched DNA has topological features which can cause single exchanges within it to lead to the breaking of DNA molecules at nonhomologous places. The available sequence data from immunoglobulins with large deletions are compatible with the repair of such breaks. The data cause us to raise but do not permit us to answer the question of whether DNA breakage and repair occurs normally during the generation of antibody variability.

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Actinomycin D: Renewed RNA Synthesis after Removal from Mammalian Cells

Abstract. *Suppressed RNA synthesis exceeded control rates 2 hours after removal of actinomycin from serial lines of green monkey kidney cells in exponentially dividing monolayer cultures. Enhanced synthesis was directly related to the dose of actinomycin, and release from inhibition was temperature dependent. A broad range of RNA sizes was made after removal of drug, but cytoplasmic and nucleolar sedimentation profiles were abnormal for at least 20 hours.*

Stability of actinomycin-DNA complexes (*1*) and persistence of drug-induced effects in cells in the absence of drug (*2*) have lead to the widely accepted conclusion that suppression of DNA-dependent RNA synthesis by actinomycin in vivo is irreversible. We were surprised to find that suppressed RNA synthesis in two lines of green monkey kidney cells and in HeLa cells increased after withdrawal of drug. In fact, rates of synthesis sometimes exceeded that of control.

Drug toxicity in the monkey kidney cell lines Vero (*3*) and JR (*4*) is low, and renewed RNA synthesis continued for days. Cultures treated with up to 1 μg of actinomycin per milliliter often could be serially passaged. On the other hand, studies with HeLa cells were limited because of toxicity.

Using Vero cells for our test system, we looked at some of the factors affecting rates of RNA synthesis after drug treatment and at the kinds of RNA that were made. Monolayer cultures grown in plastic flasks were nour-

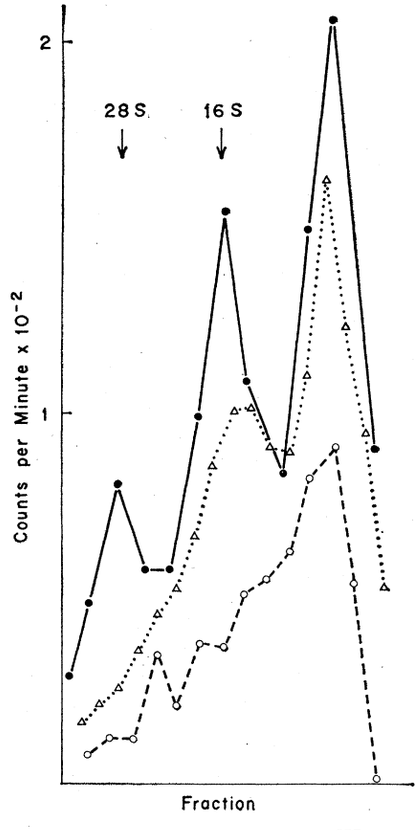

Fig. 1. Sedimentation profiles of cytoplasmic RNA from cells labeled for 1 hour with [5-H^3]uridine (4 μc/ml) during a 1½-hour exposure to 1 μg of actinomycin per milliliter (dashed line); 19 hours after removal of drug (dotted line); or in the absence of treatment (solid line). Sedimentation at 50,000 rev/min, 2¼ hours, SW 50.1 rotor.

Table 1. The effect of growth phase of culture and dose of actinomycin on RNA synthesis after removal of drug.

Growth phase culture	Exposure to actinomycin		Uridine incorporation (% of control)* after removal of actinomycin:				
	Amount (μg/ml)	Time (hr)	0 hr†	2 hr	19 hr	26 hr	43 hr
			Experiment 1				
Stationary	1.0	2	18	35	54		67
Exponential	1.0	2	15	130	80		70
			Experiment 2				
Exponential	0.3	½		112	107	122	91
Exponential	1.0	½		160	242	266	155
Exponential	3.0	½		126	377	298	230

* Counts per minute per 100,000 treated cells × 100/counts per minute per 100,000 untreated cells. † Label added during last ½ hour of exposure to actinomycin.

ished with Eagle medium (*5*) supplemented with calf serum (5 percent).

Concentrated solutions of actinomycin D (Merck Sharp & Dohme) were standardized according to absorbance at 440 nm. Determinations of DNA binding and subsequent spectral analyses revealed no irregularities in the lots used. Cultures were exposed to drug in medium warmed to 37°C. After treatment, cultures were drained, washed three times with warm medium, refed, and returned to the incubator.

RNA was labeled with [5-^{3}H]uridine (100 mc/mg, New England Nuclear). Cells were lysed with 2 percent sodium dodecyl sulfate, and duplicate samples from replicate cultures were dried on filter paper disks for the determination of acid-insoluble radioactivity (Table 1) (*6*). Sucrose-gradient fractions were processed in the same way or precipitated in the presence of carrier RNA and collected on Millipore disks by filtration. Radioactivity was counted in a Beckman model LS-150 to an accuracy of 5 percent.

Uridine incorporation was higher in actively growing cultures than in fully sheeted monolayers after treatment with actinomycin (Table 1). In the first experiment, a cell suspension was divided so that one group of culture flasks was lightly seeded with cells, and another group was seeded with ten times that amount. At 48 hours, when half the cultures were in stationary growth phase and half were exponentially dividing, cells were exposed to 1 μg of actinomycin per milliliter for 2 hours. At intervals thereafter, cultures were exposed for 30 minutes to [5-^{3}H]uridine (4 μc/ml). During the first 20 hours after the drug was removed, RNA synthesis was greater in rapidly growing cells than in stationary cells. The data from the second experiment indicate that enhancement of uridine incorporation is a function of drug concentration. Because the data were calculated on the basis of the number of cells per culture and, of greater importance, since only 2 hours after removal of actinomycin RNA synthesis exceeded that of controls, the findings cannot be attributed to outgrowth of drug-resistant cells from a heterogeneous population.

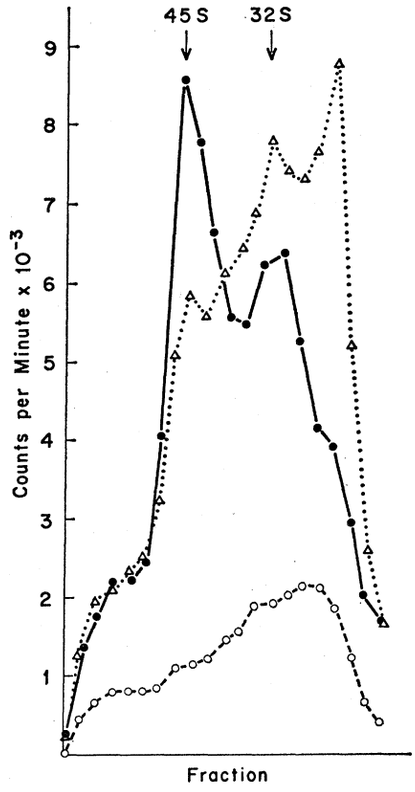

Fig. 2. Sedimentation profiles of nucleolar RNA from cells labeled for 20 minutes with [5-^{3}H]uridine (10 μc/ml) during a 45-minute exposure to actinomycin (1 μg/ml) (dashed line), 19 hours after removal of drug (dotted line), or in the absence of treatment (solid line). Sedimentation at 21,000 rev/min, 16 hours, SW 27 rotor.

To find out what kinds of RNA were being made after withdrawal of actinomycin, we fractionated cells labeled during and after exposure to actinomycin (*7*). RNA was extracted and separated on sucrose gradients (15 to 30 percent, by weight) containing 0.5 percent sodium dodecyl sulfate. Sedimentation profiles of cytoplasmic RNA (Fig. 1) show that ribosomal RNA was the most sensitive to the action of actinomycin as was previously reported (*8*). Nineteen hours after treatment there was an increase in RNA in the 14*S* to 16*S* region and in the 4*S* region, but there was no indication of renewed synthesis of 28*S* RNA. This indicates that there is little recovery of ribosomal RNA synthesis at this time.

This conclusion is supported by data obtained from nucleolar RNA gradients. Optical density tracings showed a complete elimination of the 45*S* ribosomal precursor peak after a 45-minute exposure to 1 μg of actinomycin per milliliter. A 45*S* peak again was seen 19 hours after drug withdrawal, but was only 18 percent of the control peak. The distribution of pulse-labeled RNA in these gradients is shown in Fig. 2. In the presence of drug, a marked overall suppression in synthesis can be seen and the 45*S* and 32*S* peaks have been leveled. After 19 hours, incorporation was extensive throughout the gradient but was not separated into 45*S* and 32*S* peaks. The highest activity was in low-molecular-weight RNA.

Thus, whereas there is extensive RNA synthesis after removal of actinomycin, reversal is not complete. That is, RNA synthesis does not qualitatively return to normal during the 20-hour interval examined in spite of the fact that synthesis may be considerably greater in treated cells than in control cells. When cells were examined after longer intervals—48 hours or more—cells treated with 1 μg concentrations showed typical cytoplasmic RNA profiles, while cells treated with 10 μg had abnormal distributions of radioactive RNA.

Our findings are consistent with and help to explain earlier reports of reversal of actinomycin-induced effects on virus replication in other cell systems (*9*). They also emphasize the need for controls and careful interpretation of studies in which actinomycin is used as a preliminary treatment to block cellular RNA synthesis.

How the cell deals with drugs like actinomycin is not known. We did find that when treated cells were washed with cold medium and then fed with warm medium containing labeled uridine immediately or at intervals up to 6 hours after being held at 8°C, RNA synthesis was related to the time at 37°C, not to the total time after withdrawal of drug. Therefore, "reversal" cannot be attributed solely to simple diffusion which is only twofold slower at the lower temperature. The data suggest that there is a previously unknown temperature-dependent cell process involved.

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Detoxication Enzymes in the Guts of Caterpillars: An Evolutionary Answer to Plant Defenses?

Abstract. *Higher activity of midgut microsomal oxidase enzymes in polyphagous than in monophagous species indicates that the natural function of these enzymes is to detoxify natural insecticides present in the larval food plants. Differing strategies of adaptation to plant defenses may partly account for the great diversity of insect herbivores.*

Many plant species are chemically defended. Their defensive toxins or inhibitors belong mostly to the large class of secondary substances (*1, 2*), and include such compounds as alkaloids, some terpenoids and steroids, rotenoids, and organic cyanides. Herbivores and pathogens exploiting a defended plant species are presumably the relatively few species that have evolved appropriate countermeasures against the particular toxins present in their hosts (*2, 3*). Sometimes these toxins may even contribute to the behavioral clues which help such an herbivore to find its chosen food plants (*4*).

In the livers of mammals, birds, and fish there are enzymes that promote metabolic attack on a variety of drugs and pesticides (*5, 6*). The remarkable diversity of reactions catalyzed by these microsomal mixed-function oxidases includes oxidation of thioethers; aromatic, aliphatic, and alicyclic hydroxylation; *O*-, *S*-, and *N*-dealkylation; and epoxidation. These reactions affect a wide variety of substrates; they serve both to speed excretion of a foreign compound from the body and generally, also, to decrease its toxicity. Similar enzymes are known in invertebrates (*7*), and recent work in our department has revealed their presence in lepidopterous insect larvae, where their activity is concentrated in the midgut tissues (*8*).

Microsomal detoxication enzymes certainly existed long before the advent of modern drugs and pesticides, yet their natural role and substrates have received surprisingly little attention. Here we describe experimental results which support the hypothesis that these enzymes exist in lepidopterous caterpillars for metabolizing potential toxins present in the natural food. Such a function for the equivalent enzymes in vertebrates was suggested in 1922 by Sherwin (*9*), and more recently by Williams, Brodie, and others (*6, 10*). Gordon (*11*) has proposed a similar hypothesis for insects.

Different species of phytophagous Lepidoptera have widely differing ranges of larval food plants, varying from strict monophagy in some species to wide polyphagy in others. Biosynthesis and metabolic action of their microsomal detoxication enzymes must require expenditure both of energy and of nutrients. If metabolism of plant secondary substances is the chief natural function of these enzymes, and if natural selection acts to reduce energetic and nutritional waste, we would expect to find a correlation between enzyme activity and degree of feeding specialization. In our experiment, the microsomal oxidase activities in the larval midguts of 35 species of Lepidoptera were estimated in terms of the rate at which aldrin was epoxidized to dieldrin (*8*).

Midguts from last instar larvae were cleared of gut contents (which contain an inhibitor of oxidase) (*8, 12*) and homogenized in ice-cold 0.15*M* KCl with a Ten Broeck tissue grinder. Samples (0.5 ml) of this homogenate, containing 1 to 10 mg of protein, were added to a medium (total volume 5.0 ml) containing tris-HCl buffer (50 mmole/liter, *p*H 7.8), 2.4 m*M* glucose-6-phosphate, 51 μM nicotinamide-adenine dinucleotide phosphate, glucose-6-phosphate dehydrogenase (1.6 enzyme units), and 2.7 m*M* KCl. After addition of aldrin (100 μg) in ethanol (25 μl), mixtures were incubated at 30°C for 10 or 15 minutes, after which the reactions were terminated by the addition of acetone (4.0 ml). The chlorohydrocarbons were then extracted quantitatively into petroleum ether, and dieldrin production was measured by electron-capture gas chromatography (*8*). The protein content of each homogenate was estimated by the Biuret method of Fincham (*13*).

Epoxidase activities are listed in three categories (Table 1) according to the range of host plants used by the larvae (*14*): (i) species normally confined to plants of one family, or two closely related families, (ii) species known to feed on between two and ten families of plants, and (iii) species which normally feed on plants of more than ten families. These categories represent positions along the traditional scale from monophagy, through oligophagy, to polyphagy; they depict the scale of host plant range in the absence of more precise knowledge of larval feeding habits. Epoxidase activity was found to be higher in the polyphagous group than in the more oligophagous group ($P < .01$) and higher in the oligophagous group than in the mo-