THE DIFFERENTIAL EFFECT OF ACTINOMYCIN D
IN NORMAL AND VIRUS-TRANSFORMED CELLS

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ABSTRACT

Actinomycin D (AMD) at concentrations up to 0.25 μg/ml shows a differential effect on cell RNA synthesis and on the replication of an influenza virus in normal and virally transformed cells, both functions being more resistant to AMD in the transformed cell. A possible explanation for these differences in AMD sensitivity is provided by the observation that isotopically labeled AMD is maintained at a lower concentration in transformed BHK 21/13 (BHK) cells. There is evidence that the decreased sensitivity of the transformed cells to AMD is a result of maintenance of a lower internal concentration of the drug, since a correlation exists for a number of polyoma virus-transformed clones between sensitivity to and uptake of AMD.

INTRODUCTION

AMD is a potent inhibitor of RNA synthesis, exerting its effect by binding to DNA and preventing transcription (7). Several studies have been made of differences in response to AMD between various cell types, e.g. differential cytotoxicity (24) and differential rate of recovery of RNA synthesis after inhibition by AMD (3). Comparisons have also been made of AMD response between normal and transformed cells, e.g., differential cytotoxicity (26) and differential sensitivity of fowl plaque virus (FPV) replication (27, 17, 19). Using FPV replication inhibition and inhibition of RNA synthesis as functional tests for AMD sensitivity and determining uptake of AMD isotopically, an attempt has been made to analyze further the differences between normal and transformed BHK cells in the hope that this might reveal a more fundamental difference between them.

MATERIALS

Reagents

Reagents were British Drug Houses (BDH) "analar" grade, with the following exceptions.

AMD: AMD, from Sigma Chemical Co., St. Louis, Mo., was stored as a 50% methanolic solution at -70°C, in the dark.

PRONASE: Pronase was Calbiochem (San Diego, Calif.) B-grade.

ACRYLAMIDE AND BIS-ACRYLAMIDE: Acrylamide and bis-acrylamide were obtained from Eastman Kodak Co. (Eastman Organic Chemicals Div., Rochester, N. Y.).

ISOTOPES: [5-^{3}H]Uridine, 20,000 mCi/mmol, [2-^{14}C]uridine, 50 mCi/mmol, and [^{3}H]actinomycin D, 3,000 mCi/mmol, were obtained from The Radiochemical Center, Amersham, England.

BUFFERS: Buffers used were RSB, 0.1 M NaCl; 0.01 M Tris-HCl, pH 7.4; 0.0015 M MgCl$_2$; and JSK, 0.01 M EDTA; 0.05 M NaCl; 0.1 M Tris, pH 7.5; 1% sodium dodecyl sulfate (SDS); 4 μg/ml polyvinyl sulfate.

METHODS

Cell Culture

Cell lines used were BHK21/13 (BHK), a clone of baby hamster kidney cells (16), BHK21/13/ PyH6 (BHK-PV), a polyoma virus-transformed derivative of BHK, and BHK21/13/B4 (BHK-RSV), a Bryan strain Rous sarcoma virus-transformed

derivative of BHK. Cells were routinely passaged in Nunc plastic Petri dishes in modified Eagle's medium containing 10% calf serum (previously heated to 56°C for 30 min) and hereafter referred to as DC_{10}. Frozen replicates of each cell type were prepared and no culture was passed for longer than 1 mo (~60 generations) before replacement. Regular mycoplasma checks proved negative.

Transformation and cloning were performed on freshly recovered BHK as follows. Cells in suspension at 1×10^6/ml were infected with 10^3 plaque-forming units (PFU)/cell of small plaque polyoma virus at 37°C for 1 h, cultured in soft agar, and well separated large colonies were picked after 10 days. Mock infected controls contained no colonies. Untransformed BHK clones were obtained by picking well separated colonies from plates seeded with 100 cells.

Effect of AMD on FPV Replication

This was determined using 24 h cultures seeded with 5×10^5 cells/5 cm Petri dish; this seeding level gave a subconfluent monolayer with all the cell types used. The monolayer was washed once with phosphate-buffered saline (PBS) containing 0.5% calf serum (PBS-Ca) and infected with 0.5 ml of the same solution containing 10 PFU/cell of egg-grown FPV (PFU/hemagglutination unit = 10^6). Plates were left at room temperature for 30 min with a few agitations when this inoculum was removed and the plates were washed three times with 2 ml of PBS-Ca. Finally, cells were overlaid with 2 ml of DC_{10} containing AMD at levels from 0 to 0.25 μg/ml. (This infection procedure produces a single-cycle infection as monitored by hemadsorption at 6 h when > 95% of the cells adsorbed fowl erythrocytes.) Cells were incubated at 37°C for 20 h when virus was harvested and frozen to -70°C before a plaque titration on chick embryo fibroblasts. Results are expressed as:

$$\log_{10} \frac{\text{PFU released at a given AMD level}}{\text{PFU released in a control with no AMD}}.$$

Effect of AMD on Total RNA Synthesis

Measurements of [^{3}H]uridine incorporation were made on cells seeded in scintillation vials and containing ^{14}C-labeled macromolecules as an internal marker for cell number. Thus 5×10^5 cells were seeded in a 9 cm Petri dish in 10 ml of DC_{10} containing 0.075 μCi/ml of [^{14}C]protein hydrolyzate and grown for 3 days at 37°C. Cells were then trypsinized and plated at 5×10^4 in heat-sterilized scintillation vials in 1 ml of DC_{10} and left overnight at 37°C. This gave a sparse monolayer of cells with each vial containing ~ 2,000 ^{14}C cpm. To determine the effect of AMD on total RNA synthesis 1 ml of DC_{10}

containing AMD was added and cultures were incubated for 90 min at 37°C, then 50 μl of [^{3}H] uridine at 50 μCi/ml was added and incubation was continued for a further 90 min at 37°C.

After removing the medium, cells were washed once with 4 ml of PBS at 4°C, three times with 4 ml of 5% TCA at 4°C, and once with 4 ml of ethanol at 4°C. Vials were than dried at 60°C and the cell monolayer was solubilized in 0.2 ml of hydroxide of hyamine for 1 h at room temperature. Vials were counted in a Packard Tri-Carb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) at settings giving ~ 25% ^{14}C to ^{3}H "spillover", this percentage being determined in each experiment by both the channel ratio method and by inclusion of non-[^{3}H]uridine-incubated vials. Labeling levels were such that for vials with no AMD, the ^{3}H/^{14}C ratio was ~ 100. Results are expressed as percent ^{3}H/^{14}C ratio at a given level of AMD relative to the control without AMD. Eight determinations of the control value and four determinations at each AMD level were made and standard deviations were usually <5%.

Effect of AMD on the Synthesis of Different RNA Classes

BHK and BHK-PV were seeded at 2×10^6/9 cm Petri dish in 10 ml DC_{10} and cultured overnight at 37°C to give a subconfluent monolayer. Medium was replaced with 10 ml of DC_{10} containing 0, 0.05, or 0.25 μg/ml of AMD and the cultures were incubated at 37°C for 90 min. They were then pulsed with either [^{3}H]uridine, 50 μl at 1 mCi/ml (3 mCi/mmol), or [^{14}C]uridine, 50 μl at 17 μCi/ml (22 mCi/mmol). BHK receiving one isotope and BHK-PV the other. Labeling was reversed several times during the course of the experiments, with no alteration in the results obtained.

Incubation was continued for a further 90 min when cells were washed three times in 10 ml of PBS at 4°C. They were then scraped into 10 ml of PBS, and BHK and BHK-PV cells were mixed and pelleted. This pellet was then resuspended in 5 ml of JSK buffer containing pronase at 0.5 mg/ml which had been preincubated for 1 h at 37°C to remove RNase. Incubation was continued for a further 2 h at 37°C, and then 5 ml of JSK-saturated redistilled phenol at 37°C was added. The tubes were vortexed, quenched in ice, and spun at 2,000 g for 15 min to separate the phases. The aqueous phase was then removed and precipitated overnight with 2 vol of ethanol at -20°C. The precipitate was washed once in 10 ml of ethanol, once in 10 ml of ether, and dried under vacuum.

The RNA precipitates were dissolved at 0.5 mg/ml in electrophoresis buffer containing 1% SDS and

run on 2% gels cross-linked with bis-acrylamide (15). Electrophoresis was performed in 90 × 5 mm gels at 5 mA per gel (5 V/cm) for 150 min at room temperature. Gels were then frozen, cut into 2 mm slices, and incubated in 0.2 ml of hyamine for 1 h at room temperature. 5 ml of Bray's scintillation fluid were added and fractions left overnight at 4°C to allow the digested RNA to diffuse from the gel slices. This procedure gave ∼75% recovery of both ^{3}H and ^{14}C. Counting was performed as before at similar settings. For purposes of analysis, corrected ^{3}H counts were obtained, then counts in different regions of the gel were added and expressed as percent of the counts in the same region of a gel containing RNA prepared from cells incubated in the absence of AMD.

Uptake of [^{3}H]AMD

Subconfluent cultures in 9 cm Petri dishes similar to those described above were exposed to [^{3}H]AMD at 0.05 μg/ml (0.35 μCi/ml) in 8 ml of DC$_{10}$ for up to 5 h. Cultures were then washed three times with PBS at 4°C, scraped into 10 ml PBS, and pipetted vigorously to produce a homogeneous suspension. This 10 ml was then split into two 5 ml portions and processed separately to give the following.

WHOLE CELL UPTAKE: The 5 ml of cell suspension was centrifuged at 1,000 g for 5 min and the supernatant discarded. The pellet was solubilized by heating to 70°C in 1.2 ml of 0.5 N perchloric acid for 15 min. To 1 ml of this extract was added 2 ml of diphenylamine reagent, and after 16 h incubation at 37°C the OD 600 was determined. By reference to DNA standards, this gave the amount of DNA in the pellet (25). To the other 0.2 ml of extract, 5 ml of Bray's scintillation fluid was added and the radioactivity was determined in a Packard Tri-Carb scintillation counter. The absolute disintegrations per minute in the pellet were calculated and the results are expressed as disintegrations per minute per microgram DNA.

NUCLEAR UPTAKE: The 5 ml of cell suspension was centrifuged as above and the pellet was resuspended in 2 ml of RSB diluted 1 in 3 in distilled H$_2$O (20) and left on ice for 10 min. It was then homogenized with 40 strokes of a Dounce homogenizer and spun at 2,000 g for 1 min. This nuclear pellet was resuspended in 2 ml of RSB, and 0.3 ml of a mixture of 10% sodium deoxycholate (1 part) and Tween-40 (2 parts) was added. The tubes were vortexed for 5 s, and a final pelleting of the nuclei was performed as before. The disintegrations per minute per microgram of DNA in this pellet were determined as described above, and this was termed the "nuclear uptake."

RESULTS

Effect of AMD on FPV Replication in Normal and Transformed Cells

FPV is sensitive to relatively low doses of AMD administered in the first 2–3 h after infection (2). FPV is a myxovirus and as such contains a single-stranded RNA genome which is replicated via an RNA-dependent RNA polymerase (12) and therefore it would not be expected to be AMD sensitive. It has been generally supposed that the AMD sensitivity of myxovirus is due to a requirement for the synthesis of some host cell function used early in the replication cycle (2).

A difference between several normal and transformed cell systems in their ability to replicate FPV in the presence of AMD has been previously reported (27, 17, 19). These studies showed that FPV replication in chicken, mouse, and hamster cells transformed by both DNA and RNA tumor viruses was much less sensitive to AMD than in normal cells. These finding were confirmed in this study for BHK cells and for their DNA and RNA tumor virus-transformed derivatives (BHK-PV and BHK-RSV, respectively). Both tumor virus-transformed cells were consistently less AMD sensitive in their ability to support FPV replication (Fig. 1), BHK-PV being the least AMD sensitive.

These results were obtained using transformed cells which had been cultured for many generations after transformation comparing them with relatively low passage BHK. In order to show that their resistance to AMD was associated with their transformed physiology and not to a difference in their history compared with the BHK control, clones of freshly transformed cells were studied along with carefully matched controls cells; 10 clones of polyoma virus-transformed BHK (BHK-PV-1 to 10) were less sensitive to the inhibitory action of AMD, with respect to FPV growth, than 5 clones of BHK. The range of sensitivities of BHK-PV-1 to 10 lay between that of BHK-RSV and that of the high passage BHK-PV.

Effect of AMD Pretreatment on FPV Replication in Normal and Transformed Cells

Since FPV replication is only sensitive to AMD within the first 2–3 h after infection the difference between normal and transformed cells may be
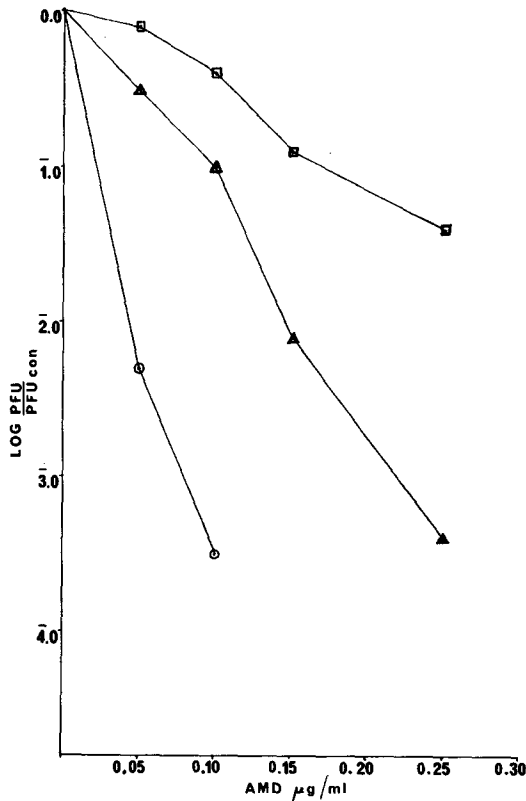
FIGURE 1 Effect of AMD on FPV replication in normal and transformed cells. AMD was added to cultures immediately after infection with FPV, and yield was determined at 24 h as described in Methods. BHK, —O—; BHK-PV, —□—, BHK-RSV, —△—.

due to AMD acting less quickly in transformed cells. An experiment was designed to test this possibility.

AMD at 0.05 µg/ml was added to a series of replicate cultures at various times from 5 h before to 3 h after infection with FPV. Yield of FPV was determined at 24 h and results were expressed relative to a control not treated with AMD (Fig. 2). These experiments showed quite clearly that the difference between normal and transformed BHK was maintained even when cells received up to 5 h pretreatment with AMD; thus it is not due to a difference in the rate of attainment of maximal inhibition by AMD.

Effect of AMD on RNA Synthesis in Normal and Transformed Cells

TOTAL RNA SYNTHESIS: The primary effect of AMD on cells is the strong inhibitory action it

exerts on RNA synthesis (7), so relative sensitivities of RNA synthesis to AMD in normal and transformed BHK cells were determined. Most observations utilized a 90 min incubation with AMD at up to 0.25 µg/ml followed by a 90 min incubation with [³H]uridine. These times and AMD levels were based on the observation that drug applied at these levels for up to 4 h was not cytotoxic as determined by washing out drug and determining efficiency of colony formation. Thus, secondary effects are probably minimized. Exactly similar results were obtained with a 30 min AMD incubation followed by a 30 min [³H] uridine pulse.

A consistent and significant difference in sensitivity to AMD was found, RNA synthesis of the two transformed cell lines being two to three times more resistant to AMD. A typical result for BHK, BHK-PV, and BHK-RSV cells is shown in Fig. 3. Note again that BHK-RSV is inter-
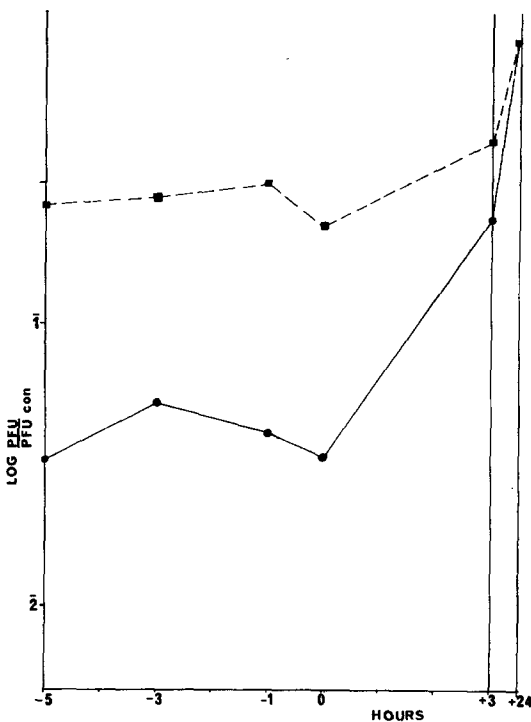

FIGURE 2 Effect of AMD pretreatment on FPV yield in normal and transformed cells. AMD (0.05 µg/ml) was added to cultures at various times before and after infection with FPV. The time of infection was termed 0 and along the abscissa is time relative to infection. Yield was determined at 24 h as described in Methods. BHK, —●—; BHK-PV, —■—.
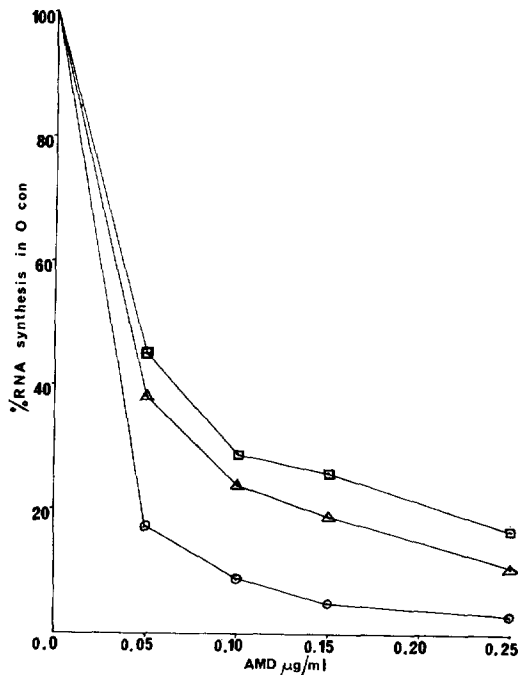
FIGURE 3 Effect of AMD on RNA synthesis in normal and transformed cells. Cultures were incubated with AMD for 90 min and pulsed with [³H]uridine for a further 90 min. They were then treated as described in Methods. BHK, —O—; BHK-PV, —□—; BHK-RSV, —△—.

mediate in its sensitivity to AMD between BHK and BHK-PV. It was also found that a correlation in the behavior of the freshly transformed clones in this system and in the FPV system existed, e.g. BHK-PV-1, a relatively resistant clone in its FPV replicative capacity, was also resistant in RNA synthesis. This effect extended to all five clones tested.

This difference in RNA sensitivity has not to our knowledge been reported previously; in fact, results reported by Malluci and Skehel (19), in which quite similar experiments were performed, suggested that no difference exists. Malluci and Skehel, though, were using a different cell system, i.e., secondary mouse embryo fibroblasts and cells derived from a polyoma-induced mouse tumor. These cells showed the difference in FPV sensitivity to AMD reported here, but in contrast showed equal RNA synthesis sensitivity. Since detailed results for the RNA synthesis experiments were not presented, it is not possible to further discuss this discrepancy.

Effect of AMD on the Synthesis of Different RNA Classes

Over a [³H]uridine pulse of 90 min, label would be expected to be incorporated into all major species of RNA. Thus, it seemed possible that the observed two- to threefold difference in sensitivity of RNA synthesis was due either to a generalized two- to threefold difference in sensitivity of all classes of RNA or to a selectively decreased sensitivity in only one class.

Accordingly, RNA synthesized in the absence of AMD and in the presence of 0.05 and 0.25 μg/ml AMD was analyzed on polyacrylamide gels. All major RNA classes were labeled in the 90 min pulse, with the majority of label in ribosomal RNA species (45S, 32S, 28S, and 18S), a significant amount in heterogeneous nuclear RNA (HnRNA) of size from 45S to 18S (1, 21), and lesser amounts in 4S and 5S RNA which this percent gel was incapable of resolving (Fig. 4).

In the presence of 0.05 μg/ml AMD, synthesis of the ribosomal species was virtually eliminated, while HnRNA and 5S RNA syntheses were left relatively unaffected. At 0.25 μg/ml, both HnRNA and 4S and 5S RNA syntheses were significantly reduced. This agrees with results obtained by others (22).

For the quantatitive analysis of these results the plots of the counts were divided into four regions as shown in Fig. 4.

(a) 45S heterogeneous nuclear RNA;
(b) 45–18S RNA, in the control largely ribosomal species but in the presence of AMD mainly HnRNA;
(c) 18S–4 and 5S RNA, a region with virtually no counts which was not quantitated;
(d) 4 and 5S RNA, transfer RNA and the ribosome-associated 5S RNA.

These results were quantitated as described in Methods and are tabulated in Table I. They show quite clearly that the difference in sensitivity of two- to threefold observed for total incorporation into RNA applies equally to all the classes analyzed.

Effect of AMD Pretreatment on [³H]Uridine Incorporation in Normal and Transformed Cells

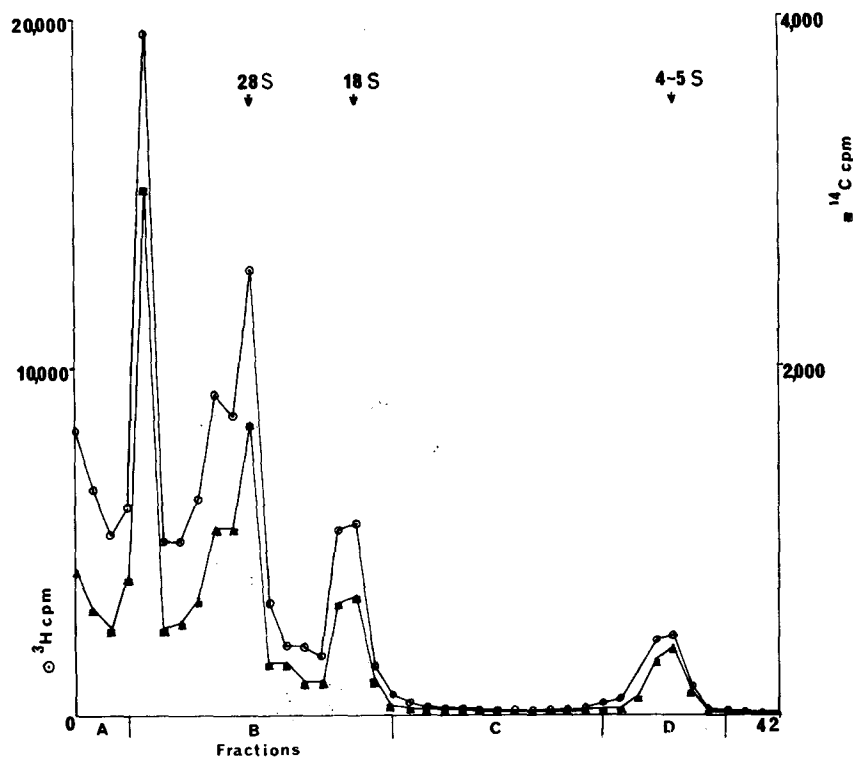As was stated in the section describing the effect of AMD pretreatment on FPV replication

FIGURE 4 Size distribution of RNA extracted from normal and transformed cells. Cultures were labeled for 90 min with [³H]uridine, and whole cell RNA was extracted and electrophoresed as described in Methods. The arrows indicate position of migration of RNA from 24 h labeled cultures run in parallel. BHK, —O—; BHK-PV, —□—.

TABLE I

	BHK percent control incorporation				BHK-PV percent control incorporation				Ratio	BHK-PV percent control / BHK percent control		
AMD	Total	A	B	D	Total	A	B	D	Total	A	B	D
$\mu g/ml$	%	%	%	%	%	%	%	%				
0	100	100	100	100	100	100	100	100	—	—	—	—
0.05	22	29	15	35	43	64	27	71	2.0	1.9	2.0	2.0
0.25	6	9	4	13	18	24	11	25	3.2	3.2	2.8	2.3

Effect of AMD on different RNA classes in normal and transformed cells. After exposure to AMD for 90 min, cultures were labeled with [³H]uridine for a further 90 min. Whole cell RNA was extracted and electrophoresed as in Methods, and separations similar to that shown in Fig. 4 were obtained. After correcting for ^{14}C-^{3}H "spillover," counts in regions A (>45S), B(45-18S), and D(4-5S) were normalized to the counts in the same region of the gel of RNA from the non-AMD-treated control.

in normal and transformed cells a possible reason for a difference in AMD effect is that in transformed cells AMD acts at a slower rate. Thus, an experiment was performed which was analogous to the FPV pretreatment experiment except that

30 min [³H]uridine pulses were used rather than a determination of FPV replicative capacity.

In the system described for the determination of total cell RNA, AMD (0.25 μg/ml) pretreatments of between 30 and 240 min were followed

by 30 min pulses of [³H]uridine, with harvesting as before. On analysis of the results, curves of the type shown in Fig. 5 *a* were obtained. This agrees with the observation of others (22), in that virtually maximal inhibition is reached within 2 h

and no further significant reduction in RNA synthesis occurs up to 5 h. The difference of two- to threefold in sensitivity of normal and transformed cells is maintained at all times up to and including 4 h; i.e., pretreatments longer than 90 min do not reduce the observed difference. The difference therefore appears not to be in the rate of attainment of maximal inhibition but rather in the magnitude of the maximal or "equilibrium" inhibition contained. This is graphically represented in Fib. 5 *b*, in which the rate of inhibition of RNA synthesis is plotted taking the value at 4 h as 100% inhibition. Similar curves are obtained for both cell types.

Uptake of [³H]AMD in Normal and Transformed Cells

The simplest explanation for the phenomena described above is that less AMD is bound per unit of DNA in transformed cells than in normal cells, during incubation of any duration. This proviso is necessary since both systems described differ in the equilibrium inhibition achieved rather than the rate at which they attain this equilibrium.

Experiments were performed in an attempt to detect any such differences in binding. The whole cell uptake results showed quite conclusively that a difference existed and that the magnitude of this difference was sufficient to account for the differential effect on RNA synthesis observed. The normal cell binds between two and three times as much [³H]AMD per unit of DNA as the transformed cell.

When these measurements were made for BHK-RSV cells and for polyoma-transformed clones a correlation was found with the other phenomena; e.g., BHK-PV-1 took up less AMD than BHK-PV-8. This is displayed graphically in
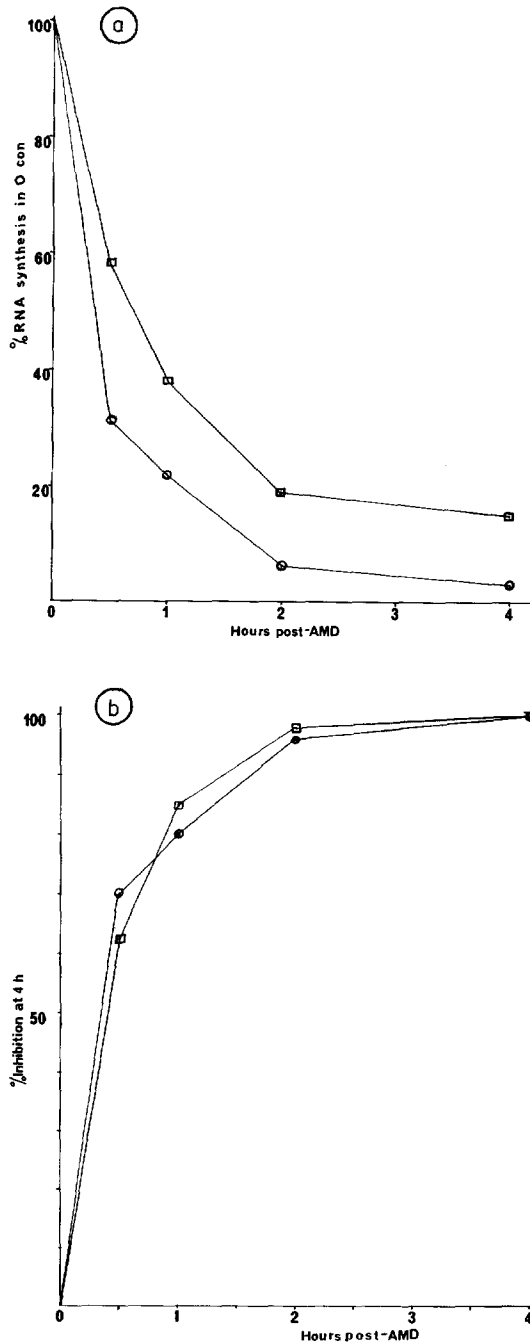
Figure 5 Effect of AMD pretreatment on RNA synthesis in normal and transformed cells. AMD (0.25 µg/ml) was added to replicate cultures at 0 h, and [³H]uridine was added at times shown. Cultures were incubated for 30 min after addition of [³H]uridine, and then harvested as described in Methods. BHK, —O—; BHK-PV, —□—. (*a*) The ordinate is a plot of percent RNA synthesis compared with a culture that was labeled for 30 min in the absence of AMD. (*b*) The ordinate is a plot derived from the same data as 5 *a* but expressing percent inhibition, taking the inhibition at 4 h as 100%.

Fig. 6, for the correlation between RNA synthesis inhibition and [³H]AMD uptake (at 0.05 μg/ml for 90 min for both parameters).

To confirm and refine these results, binding to the cell nucleus was studied. A difference similar to that found with whole cells (two- to threefold) was observed; furthermore, it was again maintained up to 5 h (Fig. 7). This experiment is open to the criticism that AMD could be leached out of the nucleus by exposure to hypotonic conditions and deoxycholate. Thus, no comment can be made on the intracellular distribution of AMD, nor are the values for AMD bound per DNA claimed to give a true indication of the state in the intact cell nucleus. Since more than 70% of the label was associated with the nuclear fraction and
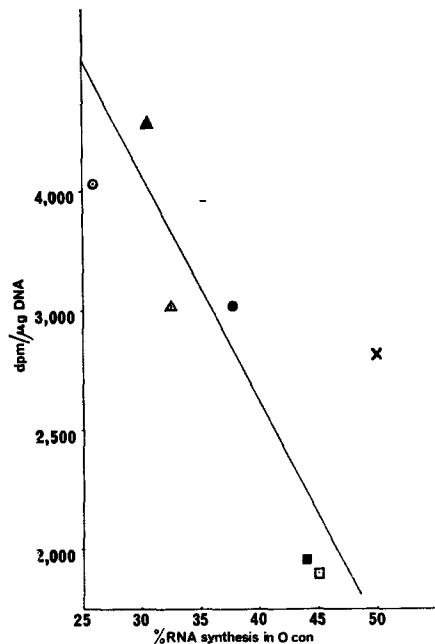

Figure 7 The uptake of [³H]AMD to the nuclei of normal and transformed cells. After the indicated exposure to [³H]AMD (0.05 μg/ml), the cultures were harvested and nuclei prepared as described in Methods. BHK, —O—; BHK-PV, —□—.

since the experiment confirms results obtained with intact cells, it is probably a meaningful comparison of normal and transformed BHK nuclear AMD binding.

DISCUSSION

The initial observations of Zavada (27) that FPV replication is more sensitive to AMD in normal chicken or hamster cells than in their transformed derivatives would seem to be explained, for BHK at least, by a difference in RNA synthesis sensitivity to AMD. Thus, presumably, a higher rate of synthesis of the unknown, cell-coded component can occur in the transformed cell in the presence of AMD because of the higher level of RNA synthesis maintained in the presence of AMD. This two- to threefold increased resistance of RNA synthesis in the transformed BHK is maintained after a long pretreatment with AMD under conditions in which maximal inhibition has been achieved, so that this phenomenon does not appear to be the result of a lower rate of action of the drug due to increased cell size, or to rate of diffusion across the cytoplasm or membranes.

The fact that the two- to threefold difference in sensitivity of RNA synthesis is not confined to

Figure 6 The correlation between [³H]AMD uptake and RNA synthesis inhibition in BHK, BHK-PV, BHK-RSV, and BHK freshly transformed with polyoma virus. Cultures were exposed to [³H]AMD (0.05 μg/ml) for 90 min, and whole cell uptake in disintegrations per minute per microgram DNA was determined as described in Methods. Parallel cultures were exposed to nonlabeled AMD (0.05 μg/ml) for 90 min and to [³H]uridine for a further 90 min, and percent RNA synthesis, compared with a non-AMD-treated culture, was determined as in Methods. BHK, —O—; BHK-PV, —□—; BHK-RSV, —Δ—; BHK-PV-1, —X—; BHK-PV-6, —●—; BHK-PV-7, —■—; BHK-PV-8, —▲—.
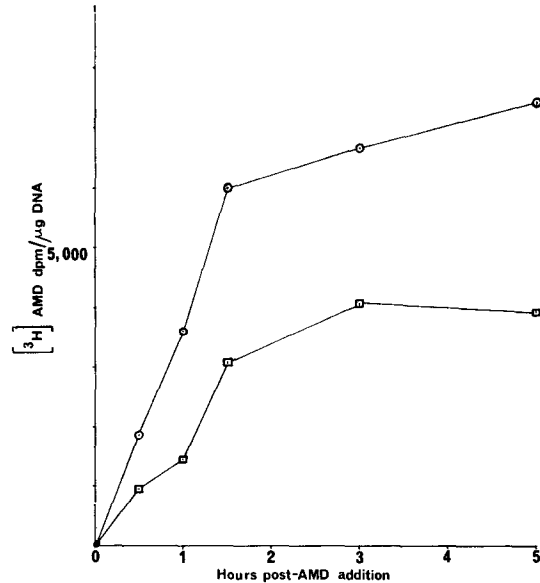
any one type of RNA but extends equally to all classes studied shows that this difference does not occur as a result of some altered stability of one particular type of RNA in transformed cells, as has been suggested by Malluci et al. for mouse cells (18, 19). Both these observations could best be explained by assuming that in transformed BHK, two to three times fewer AMD molecules are bound per unit DNA.

Studies with isotopically labeled AMD confirm this hypothesis, and the results with freshly isolated polyoma-transformed BHK which show ranging AMD sensitivities for both FPV replication and RNA synthesis, and ranging AMD uptakes, indicate that the relation between differential uptake and differential sensitivity is probably causal. These observations support results obtained by Subak-Sharpe (26) who found that cytotoxicity of AMD, maintained at very low levels in the media of BHK and polyoma-transformed BHK, was observed at lower levels of AMD in BHK. The results presented here on the differences in the inhibition of RNA synthesis and the amount of AMD taken up provide an explanation for the differences in cytotoxicity observed. Other authors have reported specific differences between various cells transformed by polyoma virus and their normal counterparts in AMD sensitivity. Malluci et al. (18) have shown that cell coat repair after trypsinization is continued for much longer in polyoma mouse tumor cells after the addition of AMD, at 0.5 µg/ml, than in mouse embryo fibroblasts. The results presented here suggest that this difference may be due to a higher rate of continued RNA synthesis in their polyoma-transformed cells.

Differences between various cell lines have also been the subject of several reports. Sawicki and Godman (24) found that HeLa cells are much more sensitive to the cytotoxic effect of AMD than Vero, W138, or L cells. Their results show that on a per cell basis, HeLa cells take up more AMD than the other cells, which is of course in agreement with these findings, although they claim that this difference is not large enough to account for differential cytotoxicity. Benedetto and Djaczenko (3) have shown that 37RC green monkey kidney cells, which show lower amounts of inhibition of RNA synthesis per amount of AMD added than HeLa cells, also show a much higher recovery rate after AMD is removed. This observation parallels a difference shown by Sawicki and God-

man in that Vero cells (also a green monkey kidney cell line) were found to lose AMD at an elevated rate compared with HeLa, W138, and L cells.

Several possibilities exist for the mechanism of the difference observed here, the major ones being as follows.

(a) The transformed cell maintains a lower internal AMD concentration by some mechanism of selective transport or permeability, perhaps similar to those described above for AMD removal by Benedetto and Djaczenko, and Sawicki and Godman. Many cases in which AMD-sensitive and AMD-resistant cells have been compared have implicated differences in permeability to the drug (4, 8, 13, 14, 23). Some evidence also exists for plasma membrane involvement in differential sensitivity; thus, Kessel and Bossman (13) found that when an L 5178Y murine leukemia cell was selected for AMD resistance, an altered cell surface glycoprotein fraction was found. Riehm and Biedler (23) found that treatment of AMD-resistant Chinese hamster cells with Tween-80, a potent surfactant, increased AMD sensitivity and uptake.

In fact, generalized transport differences have been shown to occur for many normal transformed cell systems, e.g. 3T3 and PV-3T3 (6), 3T3 and MSV-3T3 (10, 11) chick embryo fibroblasts, and RSV-transformed derivatives (9). These differences are in the rate of uptake of amino acid analogues, or substituted sugars, and usually transformed cells show higher rates of uptake. This is, of course, the reverse of the AMD situation described here, where uptake rates are greater in the normal cell. The previous generalization is not, however, without its exceptions, and Hatanaka et al. (9) found that the substance 3–0 methyl glucose was taken up at a greater rate in the normal mouse embryo cell that in its MSV-transformed derivative. Of course, none of the above substances is similar in structure to AMD, and the kinetics are further complicated by the fact that AMD has a specific target within the cell to which it binds (7).

(b) The DNA of the transformed cell is less available to AMD. It is difficult to conceive of differences in the binding of AMD to DNA due to some intrinsic structural feature of the DNA, but since DNA in the cell is much folded and in intimate association with several classes of protein, it may well be rendered less available to AMD. If this is different for normal and transformed cells, with the DNA of normal cells being more avail-

able, then such a mechanism could account for the observed difference. Darzynkiewicz et al. (5) found that, for normal and phytohemagglutinin (PHA)-stimulated lymphocytes, both acridine orange binding and [³H]AMD binding in the nucleus increase upon stimulation by PHA; the authors presume this to be due to increased availability of the DNA. Perhaps an analogous mechanism could account for the differenc described here.

In conclusion, it has been shown that a hitherto unreported difference exists in the uptake of the antibiotic AMD. This difference may be a reflection of a more fundamental difference between normal and tumor cells, and experiments are in progress to determine the nature and significance of the phenomenon.

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