Adrenal Corticosteroids Enhance Production of Type-C Virus Induced by 5-Iodo-2'-Deoxyuridine from Cultured Mouse Fibroblasts

(poly(A)/RNA tumor virus/cordycepin)

M. PARAN, R. C. GALLO, L. S. RICHARDSON, AND A. M. WU

Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

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ABSTRACT Induction of RNA "tumor" viruses by 5-iodo-2'-deoxyuridine in mouse fibroblasts is stimulated 5- to 25-fold by glucogenic adrenal corticosteroids. Enhancement of virus production by the hormones is inhibited by low concentration of cordycepin, an inhibitor of poly(A) synthesis.

Similar to mRNA (1), the RNA of RNA tumor viruses (oncornaviruses) both contains a sequence of poly(A) (2-4) and can direct the synthesis of specific proteins in a cell-free system (5, 6). The poly(A) sequence of mRNA is probably added post-transcriptionally during processing of heterogeneous RNA (7, 8). This conclusion is derived from studies with cordycepin (3'-deoxyadenosine), an inhibitor of poly(A) synthesis (7-10), which preferentially affects maturation of mRNA (appearance of mRNA in cytoplasm) while producing little inhibition of the synthesis of heterogeneous nuclear RNA (7, 10). We previously proposed that in the replication cycle of RNA tumor viruses, expression of the provirus genome probably involves mechanisms similar or identical to mechanisms used in the transcriptional process of host DNA to mRNA (11). If this is the case, production of viral RNA will probably be influenced by the same factors that affect synthesis and translation of cellular mRNA. One such group of agents that have apparent significant effects on synthesis and translation of host-cell mRNAs are the adrenal corticosteroid hormones. For instance, these hormones enhance production of tyrosine aminotransferase both in normal rat-liver cells (12) and in cultured rat-hepatoma cells (13). This enhancement of production of tyrosine aminotransferase is probably due to an increase either of mRNA or of the rate of specific polypeptide-chain initiation (14). It was, therefore, of interest to determine if these hormones would affect replication of RNA tumor viruses.

In this report, we describe the effects of glucogenic adrenal corticosteroids and other hormones on induction of oncornaviruses from nonproducing mouse fibroblasts by 5-iodo-2'-deoxyuridine (IdU), the system originally developed by Lowy *et al.* (15). More details on this induction system have recently been reported (16, 17). These cells included fibroblasts both previously infected with Kirsten murine sarcoma viruses (K-MSV) (but not producing virus) and from uninfected 3T3 cells. We find that the adrenal corticosteroids stimulate viral production 5- to 25-fold, and this stimulation is dependent on IdU induction since the hormones alone do not induce virus production. We find that this stimulation is inhibited by cordycepin, an inhibitor of poly(A) formation and, hence, of production of mature functional mRNA.

MATERIALS AND METHODS

Cells and Virus Induction. The cultured cells used for this study were BALB/C-3T3 fibroblasts derived from clone no. 31 of S. A. Aaronson and the transformed nonproducer line of BALB/3T3 infected with Kirsten murine sarcoma virus (K-MSV). This nonproducer was designated as BALB/K-3T3 (16). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal-calf serum and incubated at 37° with 10% CO₂. For most of the experiments, 10⁶ cells were plated in a 100-mm petri dish (Falcon Co.). A more detailed description of these cells has been presented (11).

Procedures of induction of virus production from mouse fibroblasts were similar to those described (11), except that measurement of virus production included an assay of focus-forming units (FFU) as well as of RNA-directed DNA polymerase activity. IdU (P. L. Biochemicals) was added to the cultures at 40 µg/ml for 24 hr. The corticosteroid hormones (all purchased from Sigma Co.), when present, were given either 24 hr before induction or at the time of addition with IdU. Cordycepin (Merck and Co., Incorp., courtesy of Drug Development Branch, National Cancer Institute), when added, was given at the time of induction with IdU at 20 µg/ml. After 24 hr, the cells were washed to remove the inducer and cordycepin (11). In all experiments, the media were harvested and changed daily. The virus particles released into the media were assayed by measurement of RNA-directed DNA polymerase (11) and focus-forming units (19).

RESULTS

Dexamethasone Enhanced IdU-Induced Virus Production from Mouse Fibroblasts. The effect of dexamethasone, a synthetic glucocorticoid, on virus production induced by IdU from BALB/K-3T3 is shown in Fig. 1. In the absence of the hormone, the relative virus production induced by IdU reached a peak activity at the third day after induction, which was similar to reported findings (11). In the presence of the hormone, the peak activity was stimulated at least 10-fold. Kinetics of virus production in the presence of hormone was basically the same as that in the absence of hormone. Similar results were obtained with uninfected BALB/3T3 cells. The results in Fig. 1 also show that this enhancement of virus production is completely dependent on induction of virus by IdU, since no virus production was detected when cells were continuously exposed to dexamethasone alone. This control experiment shows then that dexamethasone enhances virus production induced by IdU and does not, in itself, act as a primary inducer.

Abbreviation: FFU, focus-forming units.

FIG. 1. Kinetics of induction of virus production by IdU and its stimulation by dexamethasone in BALB/K-3T3 cells. 10^6 cells were plated in a 100-mm petri dish. Dexamethasone, when present, was added immediately after the cells were plated, and thereafter, daily throughout induction. IdU was added 1 day after the cells were plated for 24 hr. Days after induction started with addition of IdU. The harvested medium was concentrated 30-fold by ultracentrifugation at $82,000 \times g$ for 60 min at 4° . The pelleted particles were suspended in buffer containing 10 mM Tris·HCl (pH 7.9)–20 mM KCl–1 mM EDTA–1 mM dithiothreitol–50% glycerol. Polymerase activity was assayed in a 50- μ l reaction mixture containing 40 mM Tris·HCl (pH 7.9), 60 mM KCl, 1 mM dithiothreitol, 1 mM Mn acetate 0.2, mM EDTA, 20 mM NaF, 0.1 % Triton X-100, 10 μ g/ml of $(dT)_{12-18} \cdot (rA)_n$ (Collaborative Research), 5.4 μ M $[^3H]$ TTP (Schwarz-Mann Biochemical, specific activity 13,000 cpm/pmol), and 20 μ l of virus solution. $(dT)_n$ was synthesized at 30° for 45 min, and the precipitable radioactivity was then determined. Since the yield of viral enzyme after centrifugation was estimated to be about 20%, the enzyme activity was corrected accordingly. O, Control; Δ , IdU; $\bullet$, IdU + dexamethasone; $\times$, dexamethasone alone.

The optimal concentration of dexamethasone for stimulating virus production was about 10^{-6} M (Table 1). Prednisolone, a natural glucocorticoid, also stimulates IdU-induced virus production with an optimal concentration for stimulation at 10^{-5} – 10^{-6} M (Table 1). This concentration coincides with the range of the physiological concentrations of these hormones (20).

To ensure that the increased polymerase activity, in fact, reflects an increase of virus production, it was necessary first to demonstrate that the increased enzyme activity was of viral origin and correlated with focus-forming units in the culture medium. One convenient, although not definitive, approach to the demonstration of viral RNA-directed DNA polymerase is to measure the preference of the enzyme for $(dT)_{12-18} \cdot (rA)_n$ over $(dT)_{12-18} \cdot (dA)_n$ as template-primer (21–25). Fig. 2 shows the kinetics of $(dT)_n$ synthesis with both $(dT)_{12-18} \cdot (rA)_n$ and $(dT)_{12-18} \cdot (dA)_n$ as template-primer. $(dT)_n$ synthesis was linear for about 60 min when $(dT)_{12-18} \cdot (rA)_n$ was used as template-primer. At the plateau region, the hormone-treated culture showed a 10-fold increase in enzyme activity as compared to the untreated culture. More importantly, the enzymes from both sources showed a strong preference in using $(dT)_{12-18} \cdot (rA)_n$ as template-primer over $(dT)_{12-18} \cdot (dA)_n$. This observation suggests that the enzyme

activity measured was viral. Moreover, as shown in Table 2, viral biological activity (FFU) was demonstrated.

To rule out the possibility that this enhancement of enzyme activity is due to an increase of enzyme activity in each particle rather than to an increase of virus particles, we also measured the FFU of each induced culture, treated or not treated with dexamethasone. Table 2 shows that both the polymerase activity and the number of FFU increased about 13- to 16-fold when the induced culture was treated with dexamethasone. This correlation between enzyme activity and number of FFU provides direct evidence that dexamethasone did stimulate production of virus particles rather than of enzyme alone. This finding is in agreement with that reported by Stephenson *et al.* (26). The amount of enzyme activity per FFU was about 3×10^{-3} pmol/FFU (Table 2) under the assay conditions described.

Virus Induction and Cell Growth. One explanation of the mechanism of the stimulation of virus production by dexamethasone is that dexamethasone enhanced the growth of induced cells, especially since we previously found that IdU-treated cells have a slower growth rate and a lower saturation cell density (11). We examined this possibility by counting the cell number daily during induction. The results of these ex-

FIG. 2. Kinetics of $(dT)_n$ synthesis by endogenous activity of the induced viruses with $(dT)_{12-18} \cdot (rA)_n$ and $(dT)_{12-18} \cdot (dA)_n$ as template-primer. Conditions for enzyme assays were the same as those described in the legend of Fig. 1. Both the $(dT)_{12-18} \cdot (rA)_n$ and the $(dT)_{12-18} \cdot (dA)_n$ concentrations were 10 μ g/ml. $\bullet$, IdU-induced enzyme activity with $(dT)_{12-18} \cdot (rA)_n$; $\times$, IdU-induced enzyme activity with $(dT)_{12-18} \cdot (dA)_n$; $\square$, IdU-induced enzyme activity from dexamethasone-containing culture with $(dT)_{12-18} \cdot (rA)_n$; $\circ$, IdU-induced enzyme activity from dexamethasone-containing culture with $(dT)_{12-18} \cdot (dA)_n$.

periments are shown in Fig. 3. The upper panel shows the profile of the accumulated cell number during induction, and the lower panel shows the kinetics of induction of viral enzyme activity. There is no detectable effect on the cell number by exposing them to dexamethasone either with IdU-treated or untreated cells. This result suggests that the cell number is not a determining factor for the hormone effect. This hypothesis was substantiated by other experiments in which we found no significant increase in the number of infectious centers (unpublished data).

Effect of Dexamethasone is Not Due to Enhancement of IdU Incorporation. The mechanism of induction of virus production by IdU is not known. However, incorporation of IdU into DNA is a prerequisite for successful induction (15, 17). Since the adrenal hormones were present during the entire induction, especially during the first 24 hr in which IdU was present, one can argue that in our experiments the steroids simply act to enhance IdU incorporation into DNA. We investigated this possibility by adding dexamethasone at various time intervals in an experiment in which virus production was induced from BALB/K3T3 cells with IdU. IdU was removed by washing the cells 24 hr after induction. The results are shown in Table 3. Maximum stimulation of virus production occurs when dexamethasone is present between 24 and 48 hr after IdU. Since IdU was present only in the first 24 hr, stimulation by dexamethasone occurs in the absence of IdU. The results in Table 3 also show that prior treatment of the cells with dexamethasone and the presence of dexamethasone at other time periods other than between 24 and 48 hr are not very effective. The magnitude of stimulation of virus production with hormone present between 24 and 48 hr is about the same as that found with the hormone present during the entire experiment (data not shown). It is clear that stimulation of IdU-induced virus production by dexamethasone is not simply due to an enhancement of IdU incorporation into DNA and, furthermore, that dexamethasone acts most efficiently between 24 and 48 hr after induction.

Effect of Other Steroid Hormones and Insulin on Induction of Virus by IdU. Stimulation of virus production is not unique

TABLE 1. Stimulation of IdU-induced virus production from BALB/K-3T3 cells by various concentrations of dexamethasone and prednisolone

Hormone concentration (M)	Relative virus production* pmol/ml	
	Dexamethasone	Prednisolone
0	3.2	3.2
10^{-8}	7.7	N.T.
10^{-7}	22.7	7.6
10^{-6}	56.0	40.4
10^{-5}	29.0	47.8
10^{-4}	N.T.	32.3

* Virus production was measured by RNA-directed DNA polymerase activity present in virus particles. The data were obtained from the peak activity of each culture as shown in Fig. 1 (the second and the third day after induction for prednisolone and dexamethasone, respectively). The data are expressed in pmol/ml of unconcentrated medium. The value of the uninduced control (0.05 pmol/ml) was subtracted from all values. 40 μ g/ml of IdU was used in each experiment. N.T. = not tested.

FIG. 3. Effect of dexamethasone on cell number and virus production induced by IdU from K-3T3 cells. (Upper panel) Accumulated cell number during induction of viruses by IdU in cells. (Lower panel) Rate of induction of virus production assayed by RNA-directed DNA polymerase activity. Induction was initiated at day 2 after the cells were plated. Enzyme assays were performed as described in the legend to Fig. 1. O, Control; Δ , IdU; $\bullet$, IdU + dexamethasone; $\times$, dexamethasone alone.

to dexamethasone. All the natural and synthetic glucogenic hormones tested enhanced induction (Table 4). The stimulation with nonproducing cells ranges from 2-fold (cortisone) to 25-fold (dexamethasone). With BALB/3T3 cells, the range of stimulation was 1.8-fold (cortisone) to 7.5-fold (dexamethasone). Apparently, this stimulatory activity is specific for glucogenic adrenal steroids, because it does not occur when estrogenic or androgenic hormones were used (Table 4). Perhaps this is simply due to the fact that these two classes of hormones have different modes of action. Alternatively, it could be due to the fact that fibroblasts are not target cells for estrogenic and androgenic hormones. It will be interesting to see whether the differential potential for stimulation of virus production by various adrenocorticoids has any correlation with the potential of induction of tyrosine aminotransferase synthesis by these hormones.

TABLE 2. Relationship between RNA-directed DNA polymerase activity and viral transforming activity*

Dexamethasone	RNA directed DNA polymerase activity (pmol/ml)	Viral transforming activity† (FFU/ml)	Specific viral activity‡ (pmol/FFU) × 10 ⁻³
None	15.1	5.1 × 10 ⁴ /ml	3.0
10 μM	236.7	6.5 × 10 ⁴ /ml	3.6
Fold stimulation by hormone§	15.6	12.7	

* Cultured cells (BALB/K-3T3 cells) in the absence and in the presence of hormone were induced with IdU, and the media were collected daily and assayed for RNA-directed DNA polymerase and focus-forming ability in normal rat-kidney cells. Values presented were obtained from results 3 days after addition of IdU, the time of maximum virus induction. Polymerase assays were the same as those described in the legend to Fig. 1.

† Focus-forming assay was as follows: 0.2 ml of virus-containing medium was added to normal rat-kidney cells treated with DEAE-Dextran (25 μg/ml) in 60-mm plates. After 1 hr absorption, all plates were washed with 2 ml of phosphate-buffered saline (pH 7.2) and fed with 3 ml of Dulbecco's Modified Eagle's Medium supplemented with 5% fetal-calf serum. Media were changed on days 2 and 4, and the foci were stained with Giemsa and counted on day 6.

‡ Specific viral activity is defined as the amount of RNA-directed DNA polymerase activity (pmol/45 min per ml) per FFU.

§ Fold stimulation is defined as follows: (Activity in the presence of hormone)/(Activity in the absence of hormone).

Insulin enhanced a post-transcriptional synthesis of tyrosine aminotransferase in rat hepatoma cells (27). To test whether insulin could also control virus production stimulated by dexamethasone, we measured the effect of insulin alone and together with dexamethasone on IdU-induced virus production. The results are also shown in Table 4. Continuous exposure of the culture to insulin at 5–10 μg/ml does not enhance IdU-induced virus production or stimulation by dexamethasone. On the contrary, a small degree of inhibition was observed in both cases.

Cordycepin Depresses the Stimulation of IdU-Induced Virus Production by Dexamethasone. We previously showed that

TABLE 3. Dexamethasone enhances type-C virus production after removal of IdU

Presence of dexamethasone* (hr)	Relative virus production† (pmol/ml)
None	119.2
–24–0	161.5
0–24	446.2
24–48	692.3
48–72	126.9

* The time of addition of IdU is taken as 0 time.

† The procedure for virus induction from BALB/K-3T3 cells is the same as that described in the legend of Fig. 1 except that dexamethasone was added at the time indicated. IdU was present only in the first 24 hr of induction.

TABLE 4. Effect of various steroid hormones and insulin on induction of type-C virus by IdU from BALB/K-3T3 and BALB/3T3 cells

Hormones*	Relative virus production† (pmol/ml)	
	BALB/K-3T3	BALB/3T3
None	3.7	4.4
Dexamethasone	92.5	33.5
Prednisolone	81.0	18.5
Fludrocortisone	76.5	17.5
Hydrocortisone	61.0	14.8
Corticosterone	41.2	10.4
Cortisone	7.2	7.6
Androstenedione	5.9	2.5
Testosterone	4.4	2.5
Progesterone	3.4	4.7
β-Estradiol	2.4	1.2
Insulin	1.8	—
Dexamethasone + insulin	50.0	—

* The concentration of all steroid hormones was 1 μM. The concentration of insulin was 10 μg/ml.

† Relative virus production is expressed as pmol/ml of RNA-directed DNA polymerase activity. For details of these data, see footnote to Table 1.

cordycepin inhibits induction of RNA tumor virus by IdU if cells are exposed to cordycepin during an early critical period (12–18 hr after addition of IdU) (11). Since the mechanism of action of cordycepin is probably due to inhibition of poly(A) synthesis (7, 9), which then affects mRNA formation, it is our tentative conclusion then that the block of virus production with cordycepin is due to inhibition of the synthesis of the poly(A) component of the RNA of type-C viruses. The effect of cordycepin on the dexamethasone enhancement of IdU-induced production of virus is shown in Table 5. In the absence of hormone, virus production was completely inhibited by cordycepin (20 μg/ml). When hormone was included, cordycepin still inhibited virus production by about 97%. Since cordycepin was removed 24 hr after induction while the hormone was continuously present, the fact that the hormone stimulatory activity was inhibited suggests that the action of the hormone depends on events that occur in the first 24 hr of induction, which was shown to be inhibited by cordycepin.

TABLE 5. Effect of cordycepin on the enhancement of type-C virus production by dexamethasone

IdU (40 μg/ml)	Dexamethasone (1 μM)	Cordycepin (20 μg/ml)	Relative virus production* (pmol/ml)	% Inhibition by cordycepin
–	–	–	1.1	N.A.†
+	–	–	119.2	N.A.
+	–	+	4.7	96.1
+	+	–	692.2	N.A.
+	+	+	24.7	96.5

* These data were obtained from the peak activity (the third day) of the kinetic curve of IdU induction as shown in Fig. 1.

† N.A. = not applicable.

DISCUSSION

Mechanism of action of the adrenal corticosteroids

Utilization of host transcriptional and translational machineries for replication of RNA tumor viruses is a simple model for explaining the mechanism involved in the later stages of virus formation. Adrenal corticosteroids (dexamethasone and other glucogenic corticoids) induced an increased synthesis of tyrosine aminotransferase (12), and this induction was inhibited by cordycepin (28, 29). Our findings that the same hormones stimulate production of oncornavirus induced by IdU and that this stimulating activity was depressed by cordycepin tend to support the model that RNA tumor viruses utilize host transcriptional and translational machineries for their replication. Recently the same hormones were also found to stimulate production of polyoma virus in mouse-embryo fibroblasts (30). It is, of course, possible that these hormones stimulate virus production by a mechanism different from that of induction of tyrosine aminotransferase synthesis.

Stimulation of virus production by adrenal corticoid hormones depends on the induction process by IdU. This absolute requirement of induction itself strongly argues that the hormone effect is secondary, rather than acting as an "inducer." This stimulatory activity seems to be a post-induction event because maximal stimulation was obtained even when hormones were given after removal of IdU (24–48 hr after induction). Another result that supports a post-induction effect is the observation that the infectious centers (the cells that were actually induced) did not increase to a significant degree (1- to 2-fold, unpublished data), although virus production was stimulated about 10-fold. Thus, the hormones do not appear to promote inducibility of the cell population, but rather to enhance virus production from those cells that were already induced.

Glucogenic steroids bind to cytoplasmic receptor sites ("steroid-binding protein") and then these hormone-receptor complexes appear in the nucleus (31, 32). Other studies have shown that steroid-induced rat hepatoma cells treated with pactamycin still contained an increased concentration of nascent tyrosine aminotransferase molecules. Since pactamycin can prevent initiation but not completion of polypeptide chains (33), these results were interpreted to indicate that the adrenal corticoid hormones increase specific messenger for the enzyme or increase the specific initiation of the messenger (14). Our findings of stimulation of viral production by adrenal corticosteroids might provide a convenient system to distinguish between these two possibilities.

The enhancement of virus production from induced cells by adrenal corticosteroids obviously increases detectability of virus production. Thus, these findings may also be useful in attempting to induce viruses from human tumor cells. Finally, we have recently found that the same hormones enhance virus production 3- to 5-fold from BALB/K-3T3 cells infected with Rauscher leukemia virus and also increase virus production from some murine cell lines that produce virus at low titer (manuscript in preparation). Therefore, these findings may also be of practical use in achieving greater production of type-C virus from mammalian virus-producing cell lines.

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- Adesnik, M., Salditt, M., Thomas, W. & Darnell, J. E. (1972) *J. Mol. Biol.* **71**, 21–30.
- Lai, M. M. & Duesberg, P. H. (1972) *Nature* **235**, 383–386.
- Gillespie, D., Marshall, S. & Gallo, R. C. (1972) *Nature New Biol.* **236**, 227–231.
- Green, M. & Cartas, M. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 791–794.
- Lucas-Lenard, J. & Lipmann, F. (1971) *Annu. Rev. Biochem.* **40**, 409–448.
- Siebert, W., Konings, R. N. H., Bauer, H. & Hofschneider, P. H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 888–891.
- Darnell, J. E., Philipson, L., Wall, R. & Adesnik, M. (1971) *Science* **176**, 507–510.
- Philipson, L., Wall, R., Glickman, G. & Darnell, J. E. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2806–2809.
- Abelson, H. T. & Penman, S. (1972) *Biochim. Biophys. Acta* **277**, 129–133.
- Penman, S., Rosbash, M. & Penman, M. (1971) *Proc. Nat. Acad. Sci., USA* **67**, 1878–1855.
- Wu, A. M., Ting, R. C., Paran, M. & Gallo, R. C. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3820–3824.
- Lin, E. C. C. & Knox, W. E. (1957) *Biochim. Biophys. Acta* **26**, 85–88.
- Thompson, E. B., Tomkins, G. M. & Curran, J. F. (1966) *Proc. Nat. Acad. Sci. USA* **56**, 296–303.
- Scott, W. A., Shields, R. & Tomkins, G. M. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2937–2941.
- Lowy, D. R., Rowe, W. P., Teich, N. & Hartley, J. W. (1971) *Science* **174**, 155–156.
- Aaronson, S. A., Todaro, G. J. & Scolnik, E. M. (1971) *Science* **174**, 157–159.
- Teich, N., Lowy, D. R., Hartley, J. W. & Rowe, W. P. (1973) *Virology* **51**, 163–173.
- Aaronson, S. A. & Weaver, C. (1971) *J. Gen. Virol.* **13**, 245–252.
- Ting, R. C., Yang, S. S. & Gallo, R. C. (1972) *Nature New Biol.* **263**, 163–166.
- Forsham, P. H. & Melmon, K. L. (1968) in *Textbook of Endocrinology*, ed. Williams, R. H. (W. B. Saunders Co., Philadelphia, London, and Toronto), chap. 5, p. 309.
- Baltimore, D. & Smoler, D. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1507–1511.
- Gallo, R. C., Abrell, J. W., Robert, M. S., Yang, S. S. & Smith, R. G. (1972) *J. Nat. Cancer Inst.* **48**, 1185–1189.
- Goodman, N. D. & Spiegelman, S. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2203–2206.
- Robert, M. S., Smith, R. G., Gallo, R. C., Sarin, P. S. & Abrell, J. W. (1972) *Science* **176**, 798–800.
- Gallo, R. C., Sarin, P. S., Smith, R. G., Bobrow, S. M., Sarngadharan, M. G., Reitz, M. S. & Abrell, J. W. (1973) in *DNA Synthesis In Vitro* (proceedings of the 2nd Annual Steenbock Symposium), in press.
- Stephenson, J. R., Reynolds, R. K. & Aaronson, S. A. (1972) *Virology* **48**, 749–756.
- Gelehrter, T. D. & Tomkins, G. M. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 390–397.
- Butcher, F. R., Bushnell, D. E., Becker, J. E. & Potter, V. R. (1972) *Exp. Cell Res.* **74**, 115–123.
- Dethlefsen, L. A., Levison, B. & Tomkins, G. M. (1971) in *Abstracts* (11th Annual Meeting of the American Soc. Cell Biol.), p. 75.
- Morhenn, V., Rabinowitz, Z. & Tomkins, G. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1088–1089.
- Rousseau, G. G., Baxter, J. D. & Tomkins, G. M. (1972) *J. Mol. Biol.* **67**, 99–115.
- Baxter, J. D., Rousseau, G. G., Benson, M. C., Garcea, R. L., Ko, J. & Tomkins, G. M. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1892–1896.
- Lodish, H. F., Housman, D. & Jacobson, M. (1971) *Biochemistry* **10**, 2348–2356.