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Inhibition of Host Protein Synthesis in Type 5 Adenovirus-infected Cells

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The effect of type 5 adenovirus infection on the synthesis of host-cell proteins by suspension cultures of KB cells was investigated. Although total protein synthesis continued at a constant rate for approximately 36 hr, net synthesis of five host enzymes (lactic dehydrogenase, acid phosphatase, deoxyribonuclease, fumarase, and phosphoglucose isomerase) was found to stop 16 to 20 hr after infection. The synthesis of alkaline phosphatase stopped 9 to 12 hr after infection. The inhibition of host protein synthesis occurred shortly after the synthesis of viral antigens had begun, accounting for the continued synthesis of total protein. An investigation of the relationship between synthesis of viral antigens and inhibition of host protein synthesis yielded results which suggest that the two processes are in some way coupled.

Adenoviruses comprise a group of deoxyribonucleic acid (DNA)-containing animal viruses which share a common group-specific antigen. Each particular type of adenovirus possesses, in addition, a type-specific antigen by which it can be distinguished from other adenoviruses (18). In recent years, an extensive study has been carried out in this laboratory on type 5 adenovirus. It has been demonstrated that this virus induces the synthesis of viral DNA about 10 hr after infection (9). Approximately 2 hr after the initiation of viral DNA replication, the synthesis of three new antigens, now known to be viral structural proteins, begins (11, 23, 24, 26). Synthesis of these antigens not only follows replication of viral DNA but also is dependent upon it; i.e., the addition of 5-fluoro-2-deoxyuridine (FUdR), a well-characterized inhibitor of DNA synthesis, to cultures of infected cells blocks the production of viral antigens (7). The synthesis of viral antigens, in this respect, is analogous to "late protein" synthesis in phage systems which also follows and may require replication of viral DNA (15, 21).

Although a 12-hr delay exists between the time of infection and the onset of antigen synthesis, when exponentially growing cells are infected this lag is not reflected in the kinetics of total protein synthesis. Thus, the rate of protein synthesis in adenovirus-infected cells continues at the preinfection rate before, as well as after, the initiation of antigen production. The study described in this communication was initiated to determine whether the protein elaborated before the onset of antigen production represents the contined synthesis of host proteins and, if so, whether the production of host proteins continues during the period of antigen synthesis. To follow the synthesis of host proteins selectively, synthesis of six host enzymes was studied in infected cells.

The results obtained indicate that host protein synthesis continues in infected cells until viral antigen production begins. Shortly after synthesis of viral antigens commences, host protein synthesis is inhibited. The data suggest that the block in host protein synthesis is linked to production of viral antigens and is not an independent event occurring simultaneously with the synthesis of viral antigens.

MATERIALS AND METHODS

Tissue culture. All experiments were carried out with suspension cultures of KB cells (5). Cells were routinely propagated in Eagle's minimal essential medium supplemented with 10% calf serum (6). The cell count was adjusted each day to 150×10^3 cells per ml by the addition of fresh media. When cells were to be infected, a 20-hr culture (2×10^5 to 3×10^5 cells/ml) was diluted with an equal volume of Eagle's minimal essential medium so that the final concentration of calf serum was 5%. The concentration of calf serum was readjusted to 10% 2 hr after infection.

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Virus. Type 5 adenovirus was used in all experiments. The strain was identical with that used in previous studies (24). Stock viral preparations were prepared as described previously and stored at −20 C (24).

Biological determinations. Viral infectivity and complement-fixation titrations were carried out as previously described (8). Cell counts were done in triplicate by use of a hemocytometer.

Preparation of antiserum. To prepare anti-viral antiserum, rabbits were given two 4.0-ml intramuscular injections of a concentrated homogenate (protein = 1 mg/ml) of type 5 adenovirus-infected cells mixed with an equal volume of Freund's complete adjuvant and one intraperitoneal infection without adjuvant at 10-day intervals. Animals were bled 10 days after the last injection. Sera were separated from blood clots by centrifugation, inactivated at 56 C for 30 min, and stored at −20 C. Antiserum to KB cells was prepared similarly with extracts of uninfected cells.

Preparation of cell extracts. At various times after infection, 50-ml samples of the cell suspension were withdrawn from the culture vessel and centrifuged for 10 min at 160 × *g*. The cell pellet was washed once with 10.0 ml of cold 0.15 M NaCl buffered with 0.01 M sodium phosphate, *p*H 7.2 (PBS), and stored at −20 C until used. Extracts were prepared by dispersing the cell pellets in 5.0 ml of 0.01 M glycyl-glycine buffer (*p*H 7.4) and treating the suspension for 2 min in an M.S.E. ultrasonic disintegrator (60-w model). Each sample was then divided into five 1.0-ml fractions, and those not immediately used were kept at −20 C.

Enzyme assays. Acid phosphatase was always assayed on the day that extracts were prepared since small decreases in activity were observed after storage at −20 C. The enzyme was assayed by a modification of the procedure of Lowry (14). The reaction mixture contained 300 μmoles of sodium acetate (*p*H 4.7), 3.0 μmoles of *p*-nitrophenyl phosphate, and 30 to 90 units of enzyme, in a final volume of 3.0 ml. After incubation of the mixture at 37 C for 15 min, the reaction was terminated by the addition of 2.0 ml of 0.2 N NaOH. Colorimetric determinations were then carried out by use of a Klett-Summerson photometer with a 420-mμ filter. One unit of enzyme is defined as the amount giving a reading of one Klett unit.

Alkaline phosphatase was assayed by the same procedure used for acid phosphatase. Glycine buffer (*p*H 9.5) was substituted for sodium acetate.

Lactic dehydrogenase was assayed in a Zeiss spectrophotometer by following the oxidation of reduced nicotinamide adenine dinucleotide ($NADH_2$). The reaction mixture contained 300 μmoles of potassium phosphate buffer (*p*H 6.8), 1.0 μmole of sodium pyruvate, 0.3 mg of $NADH_2$, and 10 to 30 units of enzyme, in a final volume of 3.0 ml. After addition of substrate, the reaction mixture was held at 23 C, and the changes in absorption at 340 mμ were recorded at 1-min intervals. One unit of enzyme is defined as the amount causing a change of 0.001 in optical density per minute at 23 C.

Fumarase was assayed in a Zeiss spectrophotometer by a modification of the procedure of Racker (19). The reaction mixture contained 300 μmoles of tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 8.2), 150 μmoles of sodium malate, and 10 to 30 units of enzyme, in a final volume of 3.0 ml. After addition of the substrate, the changes in absorption at 240 mμ were recorded at 1-min intervals. One unit of enzyme is defined as the amount causing a change of 0.001 in optical density per minute at 23 C.

Phosphoglucose isomerase was assayed by a modification of the procedure of Slein (22), in which the amount of fructose-6-phosphate formed was determined by the method of Roe (20). The reaction mixture contained 50 μmoles of Tris buffer (*p*H 8.2), 5.0 μmoles of glucose-6-phosphate, and 30 to 90 units of enzyme in a final volume of 1.0 ml. After incubation of the mixture at 37 C for 15 min, the reaction was terminated by the addition of 3.5 ml of 2 N HCl. To this solution, 0.5 ml of a developing reagent containing 0.1 g of resorcinol and 0.25 g of thiourea in 100 ml of glacial acetic was added. The solution was heated for 10 min at 80 C, cooled for 5 min at room temperature, and read in a Klett-Summerson photometer with a 540-mμ filter. One unit of enzyme is defined as the amount giving a reading of one Klett unit.

Deoxyribonuclease was assayed by measuring the conversion of DNA to acid-soluble material. The reaction mixture contained 300 μmoles of sodium acetate buffer (*p*H 4.7), 180 μg of salmon sperm DNA, and 10 to 35 units of enzyme, in a final volume of 1.8 ml. The mixture was incubated for 2 hr at 37 C, and the reaction was terminated by the addition of 0.20 ml of 35% perchloric acid. After standing for 15 min at 0 C, the mixture was centrifuged for 15 min at 3,000 × *g*. The supernatant solution was removed with a pipette and read at 260 mμ in a Zeiss spectrophotometer. A blank obtained by adding acid to an identical reaction mixture at zero-time was subtacted from this value. Since linearity with enzyme concentration was observed only between optical density readings of 0.100 and 0.450, a standard curve relating optical density to enzyme concentration was prepared. A reading of 0.100 was defined as 10 units of enzyme activity. Between optical density readings of 0.100 and 0.450, increases of 0.136 equaled 10 additional units.

DNA and protein determinations. DNA and protein determinations were performed on 1.0-ml fractions of the cell extracts. Two ml of cold 10% trichloroacetic acid was added, and after 30 min at 0 C the precipitate which formed was sedimented by centrifugation for 15 min at 3,000 × *g*, washed once with 2.0 ml of cold 5% trichloroacetic acid, and finally resuspended in 1.0 ml of 5% trichloroacetic acid. The suspension was heated for 45 min at 90 C, chilled, and centrifuged. The supernatant solution was used for DNA determinations and the precipitate, after being dissolved in 2.0 ml of 0.2 M Na_2CO_3–0.1 M NaOH, was used for protein determination. DNA was determined by the Burton modification of the diphenylamine reaction (2), and protein, by the method described by Lowry (13).

RESULTS

Effect of adenovirus infection on protein synthesis. Previous studies carried out with resting

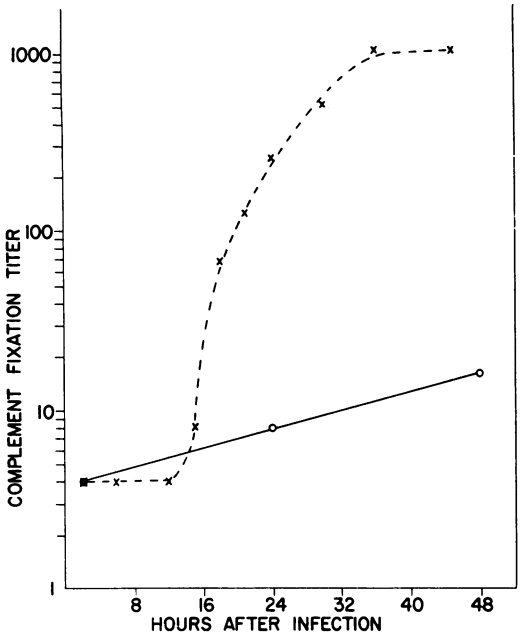

FIG. 1. *Production of complement-fixing antigens in infected cells. Two 900-ml cultures of KB cells containing 100 × 10^3 cells per ml were incubated at 36 C. One of the cultures was infected with an input multiplicity of 200 PFU per cell while the other culture was untreated. At 2 hr after infection, calf serum was added to the medium to give a concentration of 10%. At the indicated times, samples were withdrawn and complement-fixation assays were done. Infected, ×; control, ○.*

cells demonstrated that new complement-fixing antigens are produced in cells infected with type 5 adenovirus (11, 24). The kinetics of antigen synthesis in exponentially growing cells are shown in Fig. 1. Under the conditions employed in these experiments, the antigens were first detected 12 to 15 hr after infection. Their rate of synthesis increased until 20 to 24 hr after infection, after which synthesis proceeded at a linear rate. Net protein synthesis continued in infected cultures at a linear rate for about 36 hr, despite the fact that cell division was inhibited about 12 hr after infection. The total amount of protein synthesized by 36 hr was essentially equal to the amount made in a control culture of uninfected cells. Similar results have been reported in cells infected with type 2 adenovirus (10). These data obtained with exponentially growing cells are in contrast to those obtained when resting cells are infected. Under conditions of arrested cell division and depressed cellular biosynthesis, an actual increase in the rate of protein synthesis is observed when antigen synthesis begins (25).

Selection of enzymes and efficiency of enzyme release. The data described above led to two questions. (i) Is host protein synthesized during the period prior to viral antigen synthesis? (ii) Does host protein synthesis continue during the period of viral antigen synthesis? To answer these questions, the synthesis of specific host enzymes was studied in cells infected with type 5 adenovirus.

The measurement of cellular enzymes, although uncomplicated, contains several pitfalls. The first is the danger that varying inactivation of the enzymes may occur prior to assay. This problem has been obviated by selecting for study only stable enzymes. The availability of a rapid, accurate, and convenient assay was the only other criterion used to select the enzymes to be measured. The enzymes selected were acid and alkaline phosphatase, phosphoglucose isomerase, lactic dehydrogenase, fumerase, and deoxyribonuclease. All except acid phosphatase could be stored for at least 1 week at −20 C with no detectable loss in activity.

Another possible source of error when enzyme levels are compared is inefficient and, therefore, variable release of enzymes from the cells under study. Mechanical breakage of cells, for example, has been shown to release only a fraction of the alkaline phosphatase activity released by deoxycholate treatment (4). The method for preparing cell extracts in the experiment to be described, i.e., sonic treatment of cells in a hypotonic medium, reproducibly released maximal levels of the enzymes studied, provided the cell debris was not removed. Comparable values were obtained for alkaline phosphatase when cell extracts were prepared either by sonic treatment or by treatment of cell suspensions with 0.5% deoxycholate. Since sonic treatment for 1 to 4 min gave similar results, there was little danger that anomalous values would be caused by minor differences in the efficiency of sonic disruption.

Synthesis of host enzymes in infected cells. Synthesis of the six host enzymes listed was studied in infected and uninfected cell cultures. The results of a representative experiment are summarized in Fig. 2. In the infected cells, all the enzymes, with the exception of alkaline phosphatase, were synthesized at the same rate as in the control for 16 to 20 hr. By 20 hr after infection, however, increases in enzyme activities had ceased. Alkaline phosphatase differed only in that synthesis stopped about 12 hr after infection. The results of these experiments are consistent with the bulk of the protein synthesized prior to antigen synthesis being host material. The results also suggest that host protein syn-

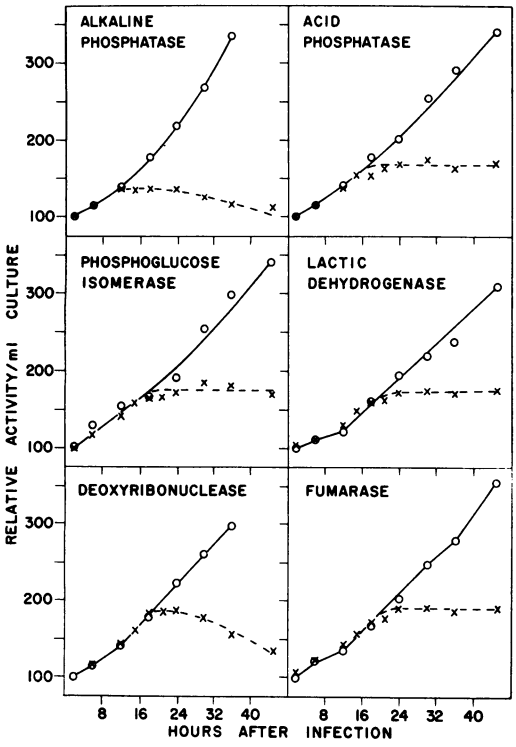

FIG. 2. *Effect of type 5 adenovirus infection on synthesis of host enzymes. Conditions were as described in Fig. 1. At the indicated times, samples were withdrawn and used for enzyme assays as described in Materials and Methods. The values are expressed as relative activity per milliliter of culture with the 2-hr sample from the control normalized to a value of 100 for each enzyme. Infected,* ×; control, ○.

thesis was inhibited during the period of viral antigen synthesis. The latter conclusion, however, was not unequivocal, and the following alternatives were considered. (i) The release of enzymes from infected cells may be slower than from control cells. Thus, the low values observed in extracts of infected cells may reflect the fact that only a fraction of the enzyme content of these cells is actually being assayed. (ii) The enzymes in infected cells may be more sensitive to sonic inactivation than those in control cells. If this were true, the lower values could be attributed to a higher fraction of inactivated enzymes. (iii) Infection may result in the production of substances which are enzyme inhibitors. (iv) Infection may result in the depletion of essential enzyme activators. (v) Infection may result in an increase in the general rate of enzyme breakdown or inactivation. If this were true, the observed inhibition of enzyme accumulation would not be equivalent to inhibition of enzyme synthesis.

TABLE 1. *Effect of time of sonic treatment on enzyme activities in extracts of infected cells*

Time of sonic treatment	Relative activity (per cent of maximum) of[a]					
	Alkaline phosphatase	Acid phosphatase	Lactic dehydrogenase	Fumerase	Deoxyribonuclease	Phosphoglucose isomerase
min						
0	100	100	92	74	95	93
1	92	94	94	98	100	98
2	92	88	100	98	98	100
4	94	84	94	100	96	100

[a] Enzyme assays were carried out as described in Materials and Methods, and the highest value obtained for each enzyme was standardized at 100.

Release of enzyme activity from infected cells. The first alternative investigated was that enzymes are released from infected cells at a slower rate than from uninfected cells, resulting in spuriously low values. The results of experiments to explore this possibility, summarized in Table 1, indicate that enzymes are readily released from infected cells; in most cases, maximal levels were obtained even in the absence of sonic treatment. These data also indicate that the reduced levels in infected cells are not the result of increased sensitivity to sonic inactivation.

Absence of inhibitors and activators. To determine whether the reduced enzyme levels in infected cells resulted from the formation of enzyme inhibitors or the absence of enzyme activators after viral infection, samples of infected and control cultures were assayed both separately and after mixing. The results (Table 2) indicate that enzyme activities measured in the mixtures were equal to the sums of the individual assays. This evidence implies that neither enzyme activators nor inhibitors were responsible for the phenomenon described.

Evidence for inhibition of enzyme synthesis. The results of these experiments indicate that net accumulation of the enzymes studied ceased by 20 hr after infection. They do not prove, however, that enzyme synthesis was affected, since an increase in the rate of enzyme inactivation to the point where inactivation equaled synthesis would also prevent a net increase in enzyme activity. The view that the actual synthesis of enzymes was inhibited implies that the protein made after 20 hr is entirely viral. This predication was tested by determining at various times after infection, what fraction of the ^{14}C-valine incorporated during a 10-hr pulse could be precipitated by antiserum to viral antigens.

Four 100-ml cultures of KB cells, containing

TABLE 2. *Additive effect of enzyme activities in mixed extracts of control and infected cells*[a]

Extract	Alkaline phosphatase[b]	Acid phosphatase[b]	Lactic dehydrogenase[c]	Fumerase[c]	Phosphoglucose isomerase[c]	Deoxyribonuclease[c]
			(Units)			
Infected	20	20	8.2	8.4	12.9	23
Control	60	58	15.8	17.0	33.8	42
Infected + control	78 (80)	90 (93)	23.2 (24.0)	24.2 (25.4)	44.0 (46.7)	61 (65)

[a] Conditions for preparation of extracts were identical to those described in Fig. 2. For each assay, an equal volume of extract from the control or infected culture was used. The figures in parentheses are the calculated values, assuming no interaction of the extracts.

[b] Based on a 36-hr sample from infected or control culture.

[c] Based on a 45-hr sample from infected or control culture.

TABLE 3. *Precipitation of proteins synthesized after type 5 adenovirus infection by homologous adenovirus antiserum*[a]

Time ^{14}C-valine was present (hr)	Total ^{14}C-valine incorporated into soluble proteins (counts per min per ml of culture)	^{14}C-valine precipitated by type 5 antiserum (%)
3–13	1,258	5
13–23	1,413	51
23–33	1,319	83
33–43	1,048	88

[a] Four cultures of KB cells were infected with a viral input multiplicity of 200. Individual cultures were pulsed with ^{14}C-valine for 10-hr periods at the times indicated. After the pulse, extracts were prepared and incubated with type 5 adenovirus antiserum as described in text.

150×10^3 cells per ml, were infected with a viral input multiplicity of 200. Individual cultures were pulsed with 15 μc of ^{14}C-valine for 10-hr periods. After the pulse, a 50-ml sample was withdrawn from each culture and centrifuged for 10 min at $160 \times g$; the cell pellet was washed with 10.0 ml of cold PBS. To prepare extracts, the cells were suspended in 5.0 ml of cold PBS and sonically treated for 2 min. The extracts were dialyzed for 16 hr at 4 C against 100 volumes of PBS and were centrifuged for 30 min at $3,000 \times g$. The supernatant solution which contained 90% of the incorporated ^{14}C-valine was used to determine total ^{14}C-valine incorporated into soluble proteins. To measure the fraction of radioactivity incorporated into nonviral proteins, 0.24 ml of the supernatant solution was incubated with 0.24 ml of type 5 adenovirus antiserum. After 2 hr at 37 C, 0.24 ml of PBS was added and the samples were centrifuged for 20 min at $1,000 \times g$. A 0.50-ml portion of the supernatant solution was placed in a planchet, dried, and counted. The radioactivity remaining in the supernatant solution was compared with samples treated similarly with serum from unimmunized rabbits to determine the fraction specifically precipitated. A small correction (5%) was necessary to correct for differences in self-absorption by the two sera.

These counts not precipitated by viral antibody were considered a maximal estimate of host protein synthesized during the 10-hr period. The more direct method of testing the quantity of protein precipitated by antiserum to host protein was precluded by our inability to precipitate significant quantities of host protein with specific antibody. The data summarized in Table 3 indicate that over 80% of the ^{14}C-valine incorporated into protein later than 23 hr after infection was precipitated by virus-specific antibody. Thus, even if all of the radioactivity that was not precipitated represented continued synthesis of host proteins, the rate of synthesis was less than 20% of that in uninfected cells.

Coordination of antigen synthesis with inhibition of host protein synthesis. The fact that host protein synthesis was inhibited after synthesis of viral antigens began suggested the possibility that the two processes might be related. To investigate this possibility, the synthesis of host proteins was studied in cells infected under conditions in which the synthesis of viral antigens was blocked. This was accomplished by the addition of FUdR to an infected culture. FUdR inhibits the replication of viral DNA and in so doing blocks the synthesis of viral antigens (7). The addition of thymidine prevents the block in antigen synthesis, indicating that FUdR acts by virtue of its inhibitory effect on thymidylate synthesis rather than by some less specific mechanism.

Although antigen synthesis is not initiated in the presence of FUdR, net protein synthesis continues. The data presented in Fig. 3 indicate that the addition of a 2×10^{-6} M FUdR at the time of infection caused only a slight depression in the rate of protein synthesis when compared with the control which received FUdR and thymidine. Apparently, the toxic effects of unbalanced growth (3) were somehow minimized in cells infected with type 5 adenovirus, since uninfected cells treated with FUdR

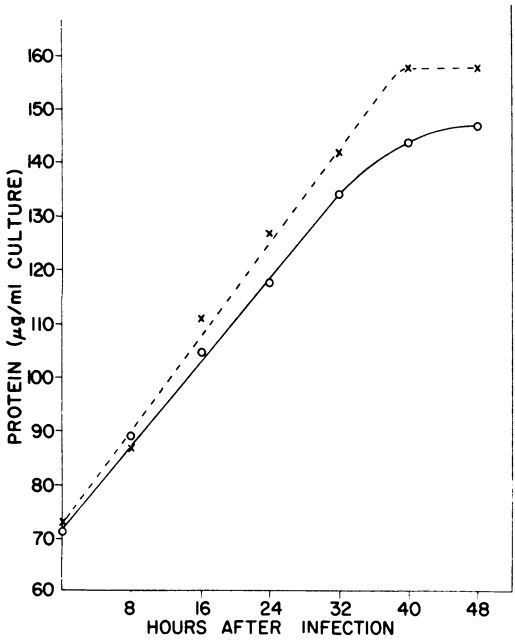

FIG. 3. *Effect of FUdR on protein synthesis in cells infected with type 5 adenovirus. Two 450-ml cultures of KB cells containing 120 × 10^3 cells per ml were incubated at 36 C. Both cultures were infected with a viral multiplicity of 200 PFU per cell. After infection, FUdR was added to both cultures at a final concentration of 2 × 10^{-6} M. One culture also received thymidine at a final concentration of 10^{-5} M. At the indicated times, samples were withdrawn and concentrations of protein were determined. FUdR, ○; FUdR + thymidine, ×.*

synthesized only 70% as much protein in 36 hr as infected cells (*unpublished data*). Since viral antigens, which normally constitute over 80% of the protein made later than 23 hr after infection, were not made in FUdR-treated cells, the continued synthesis of total protein indicates that host protein synthesis continued in the treated, infected cells past the time when inhibition normally occurs. Otherwise, inhibition of host synthesis would be reflected in an inhibition in total protein synthesis.

Experiments to test the above interpretation were carried out. The data summarized in Fig. 4 indicate that acid phosphatase, fumarase, and deoxyribonuclease continued to be synthesized past the time when inhibition occurred in the infected culture which received thymidine in addition to FUdR. Although phosphoglucose isomerase was synthesized at the uninhibited rate for only 16 hr, synthesis continued longer in the FUdR-treated cells than in the cells that received FUdR and thymidine. Synthesis of this enzyme was apparently sensitive to the toxic

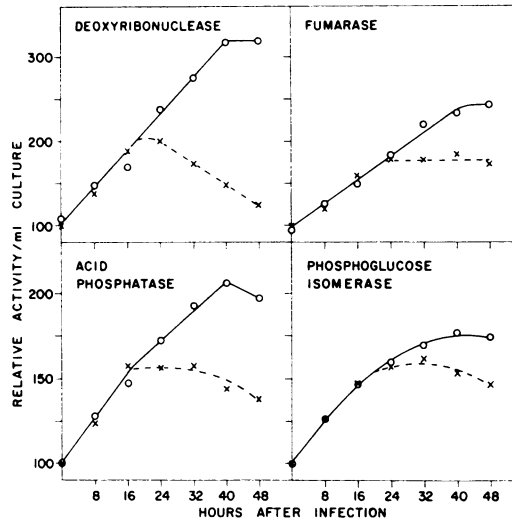

FIG. 4. *Effect of FUdR on enzyme synthesis in type 5 adenovirus-infected cells. Conditions were as described in Fig. 3. At the indicated times, samples were withdrawn and used for enzyme assays as described in Materials and Methods. Values are expressed as relative activity with the zero-hour sample from the FUdR + thymidine culture normalized to a value of 100 for each enzyme. FUdR, ○; FUdR + thymidine, ×.*

effects of unbalanced growth (3). The synthesis of lactic dehydrogenase was even more sensitive to the toxic effects of FUdR and for that reason could not be included in the study. It should be pointed out, however, that infected cells treated with FUdR synthesize more lactic dehydrogenase than uninfected cells treated similarly. Alkaline phosphatase synthesis was in-

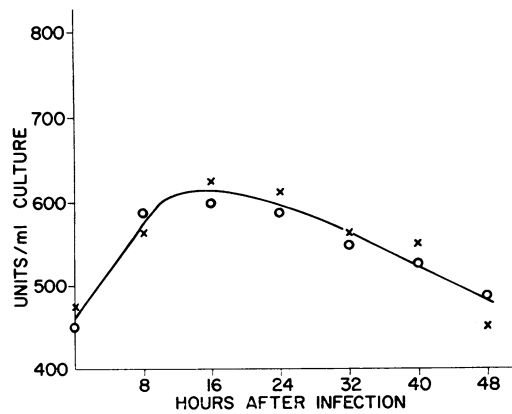

FIG. 5. *Effect of FUdR on alkaline phosphatase synthesis in cells infected with type 5 adenovirus. Conditions were as described in Fig. 3. At the indicated time, samples were withdrawn and assayed for alkaline phosphatase activity as described in Materials and Methods. FUdR, ○; FUdR + thymidine, ×.*

hibited in both the FUdR-treated culture and in the control, i.e., FUdR plus thymidine (Fig. 5). This inhibition cannot be attributed to the toxic effect of FUdR, since synthesis of the enzyme in uninfected cells treated with FUdR proceeded for longer periods of time than in infected cells. It appears that the early inhibition of alkaline phosphatase synthesis is effected by a mechanism distinct from that which inhibits the synthesis of the other five enzymes studied. Since several other examples of a specific alteration in the rate of alkaline phosphatase synthesis in cultured cells are known to be caused by such diverse factors as hormones and alterations in the ionic strength of the culture medium, the atypical behaviour of this enzyme in infected cells may not be representative of host protein synthesis in general (4, 17).

DISCUSSION

The results described indicate that infection of KB cells with type 5 adenovirus has little effect on host protein synthesis until after viral antigen synthesis begins. Host protein synthesis is then markedly inhibited. Whereas synthesis of host protein is probably blocked completely by 20 hr after infection, synthesis of total protein in the infected cells is not impaired until about 36 hr after infection. The phenomenon, therefore, is one of selective rather than general inhibition of protein synthesis. The protein made from 20 to 36 hr after infection consists largely, if not entirely, of viral antigens. This has been demonstrated by the specific precipitation of ^{14}C-valine incorporated during this period by antiserum directed against viral antigens. However, the absence of material precipitable by virus-specific antibody early in infection is not, in itself, sufficient evidence that host protein is made during this period. New proteins that are coded for by the virus but which are poor immunogens, or which are not readily precipitated by the antiserum used, would be undetected by this method. The failure to precipitate a significant amount of host protein by antiserum directed against uninfected cells serves to illustrate this point. However, if one accepts the assumption that the producion of the host enzymes studied reflects the synthesis of all host proteins, the demonstration that during the initial 16 hr after infection specific host enzymes were synthesized at the same rate as in uninfected cells indicates that host, rather than viral, proteins make up the bulk of the material made during this period.

The experiments in which FUdR was used indicate that inhibition of host protein synthesis in infected cells requires synthesis of viral DNA. Hence, it is unlikely that an "early protein" synthesized only before production of viral DNA is responsible for the inhibition. Since DNA synthesis is required both for initiating the production of viral antigens and inhibiting the synthesis of host proteins, a "built-in" control is present for eliciting the inhibition at the time that synthesis of viral proteins is taking place. This effect is manifested in the lack of a quantitative alteration in the rate of total protein synthesis despite the extensive qualitative alteration in the kinds of proteins made. It is conceivable that inhibition of host protein synthesis increases the efficiency of production of viral antigens. This seems especially likely in exponentially growing cells in which protein synthesis is probably proceeding at a maximal rate before infection. The precise way that inhibition is coordinated with synthesis suggests that some component of the reactions involved in synthesizing viral antigens, or alternatively, one of the antigens synthesized, may be involved in the inhibition. Indeed, one of the viral antigens can inhibit protein synthesis when added directly to cultured cells (9). It is not yet certain, however, that the latter phenomenon is affected by the same mechanism which brings about inhibition in infected cells. The selective inhibition of protein synthesis in cells infected by an adenovirus appears analogous to the inhibition of host protein synthesis in cells infected with some coliphages (1, 12, 16) and poliovirus (27). In contrast to adenovirus-infected cells, however, these viruses block protein synthesis immediately after infection rather than during the synthesis of viral subunits.

Conceivably, the inhibition of host protein synthesis could occur at any of the following three steps: (i) transcription of host messenger RNA (mRNA), (ii) transport of host mRNA into the cytoplasm, and (iii) translation of host mRNA. Recent evidence (9) indicates that synthesis of host protein is inhibited at some step after transcription of host mRNA, indicating that the block involves interference with either the transport or translation of host mRNA.

ACKNOWLEDGMENTS

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