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ON THE ROLE OF RIBONUCLEIC ACID IN ANIMAL VIRUS SYNTHESIS

I. Studies with 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole*, ‡

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Use of metabolic inhibitors provides an effective approach to the study of metabolic requirements of virus reproduction (1). In the area of nucleic acid metabolism it has been shown that inhibition of host deoxyribonucleic acid (DNA) synthesis does not inhibit the multiplication of Newcastle disease (2–4) or poliomyelitis virus (2). Thus, host DNA synthesis is apparently not required for the production of several ribonucleic acid (RNA)-containing viruses; whether preformed DNA is necessary is not known.

The present study is concerned with the question whether host RNA plays a role in the reproduction of DNA-containing viruses. This question was explored with the aid of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)[1] and with ribonuclease (RNase).

In this communication the inhibitory effects of DRB on adenosine-8-C^{14} uptake into cell RNA, on C^{14}-L-alanine uptake into cell proteins, and on cellular oxygen consumption are described. Blocking of the inhibitory effect of DRB on influenza virus multiplication by adenosine will be reported. In addition, the inhibitory activity of DRB on adenovirus multiplication will be compared with its effect on influenza, poliomyelitis, and vaccinia virus multiplication (5–7).

In a second communication (8) results of studies of the effects of RNase on influenza B and vaccinia virus multiplication in the chorioallantoic membrane, and on pock formation by vaccinia and herpes simplex viruses will be described

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[1] DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, obtained through the kindness of Dr. Karl Folkers and Dr. Clifford H. Shunk of the Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey.

and the role of RNA in the multiplication of viruses which contain DNA will be discussed.

Materials and Methods

Culture Media.—The medium employed for chorioallantoic membrane cultures contained NaCl, $CaCl_2$, $MgCl_2 \cdot 6H_2O$, Na_2HPO_4, KH_2PO_4, and glucose and it was designated buffered glucosol (9, 10). The pH was 7.28.

Kidney cells obtained from *rhesus* monkeys were grown in Hanks' medium (11) containing 0.5 per cent lactalbumin hydrolysate and 2 per cent calf serum. In experiments with monkey kidney cells either in monolayer or in suspension Eagle's medium (12) without serum was used.

Buffered Saline.—Buffered saline consisted of 0.85 per cent NaCl buffered at pH 7.2 with 0.01 M phosphate.

Viruses.—Seed material of a chick embryo line of influenza B virus, Lee strain, was prepared and stored in a manner described previously (13). It contained 5.4×10^8 EID_{50}[2] per ml. of allantoic fluid.

A tissue culture line of influenza B virus, strain 1760, which had been passed twice in human embryo lung, twice in human embryo kidney, and twice in monkey kidney cells was obtained from Dr. W. J. Mogabgab of Tulane University School of Medicine, New Orleans. To prepare a seed, 42 $TCID_{50}$[3] was inoculated per monkey kidney tube culture and the cultures incubated for 48 hours. The supernatant was collected and clarified by centrifugation. The seed contained 4.3×10^6 $TCID_{50}$ per ml. of tissue culture supernatant as determined in monkey kidney tube cultures at 36°C. The material was divided into small portions and stored at −55°C. Each tube was used but once.

The strain of type 4 adenovirus used was obtained from Parke, Davis and Company. The material received contained 10^3 $TCID_{50}$ of virus per ml. when titered in monkey kidney cells. The strain had been passed repeatedly in such cells. The material was diluted 1:5 and inoculated into monkey kidney tube cultures. After a 48 hour period of incubation the cells were scraped off the glass with a rubber policeman. The cell suspension was frozen and thawed and clarified by centrifugation. It contained 6.2×10^2 $TCID_{50}$ per ml. when titered in monkey kidney cells. The material was divided into convenient portions and stored at −25°C. Each tube was used but once.

Suspension Cultures of Chorioallantoic Membrane.—Intact pieces of chorioallantoic membrane were obtained from 10- or 11-day embryonated chicken eggs and suspended in buffered glucosol according to the procedure described earlier (13). The culture tubes were closed with rubber stoppers and incubated at 35°C. with horizontal shaking.

Suspension Cultures of Monkey Kidney Cells.—Primary cultures of *rhesus* monkey kidney cells were grown in 32 ounce prescription bottles in Hanks' medium supplemented with lactalbumin hydrolysate and calf serum. Complete cell sheets formed in 7 to 8 days at which time the cells were removed with 1:5000 versene and suspended in Eagle's medium without serum. The total cell count was determined in a hemocytometer after staining with crystal violet. The concentration of the cell suspension was adjusted by addition of Eagle's medium and aliquots were transferred into culture tubes. These were stoppered and incubated at 35°C. with horizontal shaking.

Tube Cultures of Monkey Kidney Cells.—Monkey kidney cell tube cultures were secured from Microbiological Associates, Inc. When used the cultures were 9 to 10 days old.

Hemagglutination Titrations.—Yield of influenza virus was measured by a modified fractional dilution procedure which was described in an earlier report (13), and it was ex-

[2] EID_{50} signifies 50 per cent egg infective doses.

[3] $TCID_{50}$ signifies 50 per cent tissue culture infective doses.

pressed in hemagglutinating units per milliliter. The amount of chorioallantoic membrane per ml. of culture medium was 3.8 cm.^{2}.

Infectivity Titrations.—Yield of adenovirus was measured by a 50 per cent infective endpoint titration procedure in monkey kidney cell cultures.

Determination of Adenosine-8-C^{14} Uptake into RNA.—Suspensions of chorioallantoic membrane or monkey kidney cells were incubated at 35°C. for 3 hours with continuous horizontal shaking in the presence of adenosine-8-C^{14}. The membranes or cells were then washed with buffered glucosol, treated with 2 per cent perchloric acid for 1½ hours at 4°C. and washed again. The membranes were homogenized and the homogenate was placed on planchettes and dried at 70°C. overnight. Suspensions of monkey kidney cells were directly placed on planchettes and dried. Radioactivity of samples was determined in a nuclear ultrascaler with gasflow and a micromil window, and it was expressed in counts per minute per amount of membrane or number of cells used.

To establish that the radioactivity was due to incorporation of adenosine-8-C^{14} into RNA, the acid-extracted membranes were treated with ribonuclease in appropriate control experiments. Such treatment reduced the count by 75 to 85 per cent.

Determination of C^{14}-L-Alanine Uptake into Protein.—Suspensions of chorioallantoic membrane or monkey kidney cells were incubated at 35°C. for 3 hours with continuous horizontal shaking in the presence of C^{14}-L-alanine. In the preparation of protein for counting the procedure of Allfrey *et al.* (14) was used. The membrane or cell suspensions were mixed with equal volumes of 20 per cent trichloroacetic acid (TCA) and held for 30 minutes at room temperature (RT). The membranes or cells were then washed twice with 10 per cent TCA and heated in 10 per cent TCA at 90°C. for 20 minutes to remove nucleic acids. The residue was washed once in 10 per cent TCA at RT and then extracted with hot 95 per cent ethanol for 3 minutes and with a 2:1:1 mixture of ethanol, ether, and chloroform at 50°C. to remove lipids. The residue was washed once in ethanol-ether-chloroform, suspended in ether, and dried in a hot bath. The protein fraction was then homogenized in acetone and collected on filter paper disks. Radioactivity was measured in an autoscaler, and expressed as counts per minute per milligram of protein.

Oxygen Uptake Measurements.—Krebs' modification (15) of Pardee's method (16) was used. The method permits direct determination of oxygen uptake when respiring cells are suspended in a medium buffered with bicarbonate. In both the original and modified procedure constant CO_2 concentration in the atmosphere is maintained by the use of a solution of diethanolamine in the center well. The solution of diethanolamine is equilibrated beforehand with the atmosphere to be employed. The solution used consisted of 4 M diethanolamine and 0.1 per cent thiourea and the atmosphere was 2 per cent CO_2 in air. One-third of the required amount of solution was gassed through a sintered glass filter with 100 per cent CO_2 for 30 minutes and then mixed with the ungassed portion. The mixture was gassed with 2 per cent CO_2 at 35°C. for 2 hours and stored. Before use the solution was gassed with 2 per cent CO_2 for an additional 2 hour period.

Into the center well of each Warburg flask were placed two 22 x 40 mm. pieces of filter paper, one rolled into a cylinder and the other folded in accordion fashion. The diethanolamine solution was placed in the center well (0.5 ml.) and into two side arms (0.5 ml. in each) with a syringe and needle. Monkey kidney cells were suspended in Eagle's medium, which contained 0.4 gm. of $NaHCO_3$ per liter.

EXPERIMENTAL

Blocking of the Inhibitory Effect of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on Influenza Virus Multiplication.—Earlier attempts to block

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with various metabolites the inhibitory effect of DRB on influenza virus multiplication were unsuccessful (5). A new series of experiments was carried out in which DRB was used at a somewhat lower concentration.

From each of 12 embryonated eggs eight 1.9 cm.² pieces of chorioallantoic membrane were obtained with a round punch. They were washed twice in buffered glucosol and distributed into 8 tubes measuring 40 x 200 mm. Each tube contained 3.6 ml. of medium with or without adenosine or guanosine and received one piece of chorioallantoic membrane from each of 12 eggs. The tubes were incubated for 1 hour at 35°C. with horizontal shaking after which 1.8 ml. of buffered glucosol with or without DRB was introduced per tube. Immediately there-

TABLE I

Blocking of Influenza Virus Inhibitory Effect of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) with Adenosine in the Chorioallantoic Membrane (CAM) in Vitro

	Yield of virus	
	Hemagglutinating units per 3.8 cm.² of CAM	Per cent of control
Control	36*	100
DRB‡	11§	31
Adenosine‖	38	106
Adenosine‖ + DRB‡	38	106
Guanosine‖	47	131
Guanosine‖ + DRB‡	10	28

* Represents mean of two determinations, which gave 32 and 40 respectively.
‡ 40 μM
§ Represents mean of two determinations, which gave 13 and 10 respectively.
‖ 400 μM

after all tubes received $10^{6.1}$ EID_{50} of Lee virus contained in 0.6 ml. of medium. The cultures were incubated for 41 hours and the yield of virus was determined in the medium by the hemagglutination technique.

As can be seen in Table I, 400 μM adenosine completely blocked the virus inhibitory effect of 40 μM DRB whereas 400 μM guanosine failed to do so. In other experiments adenosine blocked partially or failed to block the relatively greater virus inhibitory effect of DRB used at higher concentrations.

Inhibition by DRB of Adenosine and Alanine Uptake in the Chorioallantoic Membrane.—The effects of DRB on uptake of adenosine-8-C^{14} into RNA and of C^{14}-L-alanine into proteins of the chorioallantoic membrane were determined.

For the preparation of cultures groups of 6 eggs were used. From each of 6 eggs three 3.8 cm.² pieces of chorioallantoic membrane were obtained. The pieces were washed twice in buffered glucosol and distributed into 3 tubes measuring 40 x 200 mm. In experiments on adenosine-8-C^{14} uptake chorioallantoic membranes were suspended in 4.2 ml. of buffered

glucosol with or without DRB. Each tube received one piece from each of 6 eggs, and then 1.8 ml. of a solution of adenosine-8-C^{14} in buffered glucosol was added. The amount of isotope per tube was 0.14 μc. Final volume of medium was 6 ml. In experiments on incorporation of C^{14}-L-alanine into the protein fraction a similar procedure was used. The amount of C^{14}-L-alanine per tube was 0.5 μc.

The procedure for incubation of membrane cultures and the methods for determination of uptake of labeled precursors into RNA and proteins were described above.

The results of a representative experiment are summarized in Table II. At a concentration of 55 μM DRB caused 56 per cent inhibition of uptake of adenosine-8-C^{14} into RNA but only 16 per cent inhibition of uptake of C^{14}-L-alanine into proteins of the chorioallantoic membrane. With 28 μM DRB less

TABLE II

Inhibitory Effect of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on Uptake of Adenosine-8-C^{14} into RNA and of C^{14}-L-Alanine into Proteins of Chorioallantoic Membrane (CAM) in Vitro

	Uptake per 3 hrs.			
	Adenosine-8-C^{14}*		C^{14}-L-alanine‡	
	Net c.p.m. per 23 cm.² of CAM	Inhibition	Specific activity of protein	Inhibition
		per cent	*c.p.m./mg.*	*per cent*
Control	362	0	290	0
DRB, 28 μM	207	43	274	6
DRB, 55 μM	161	56	244	16

* 0.024 μc./ml.
‡ 0.084 μc./ml.

inhibition was obtained but the relationship between inhibition of the two processes was similar.

Inhibition by DRB of Adenosine and Alanine Uptake in Monkey Kidney Cells.—The effects of DRB on uptake of adenosine-8-C^{14} into RNA and of C^{14}-L-alanine into proteins of monkey kidney cells were determined.

In experiments on uptake of adenosine-8-C^{14} groups of 25 x 150 mm. tubes were set up and each received 4×10^6 cells suspended in 0.5 ml. of Eagle's medium. To the suspension was added 1.0 ml. of Eagle's medium with or without DRB. Each tube then received 0.5 ml. of adenosine-8-C^{14} in Eagle's medium. The amount of isotope per tube was 0.2 μc. Final volume of medium was 2 ml.

In experiments on uptake of C^{14}-L-alanine groups of 40 x 200 mm. tubes were set up and each received 20×10^6 cells suspended in 2.5 ml. of Eagle's medium. To the suspension was added 5.0 ml. of Eagle's medium with or without DRB. Each tube then received 2.5 ml. of C^{14}-L-alanine in Eagle's medium. The amount of isotope per tube was 1 μc. Final volume of medium was 10 ml.

The cells were incubated and processed as described above.

Results of 4 experiments are summarized in Table III. As can be seen DRB at a concentration of 95 μM caused 58 per cent inhibition of uptake of adenosine-8-C^{14} into RNA but only 20 per cent inhibition of uptake of C^{14}-L-alanine into proteins of monkey kidney cells.

Inhibition by DRB of Oxygen Uptake in Monkey Kidney Cells.—It was reported previously that 38 μM DRB had no effect on oxygen consumption by the chorioallantoic membrane (1, 5, 17). At this concentration DRB caused

TABLE III

Inhibitory Effect of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on Uptake of Adenosine-8-C^{14} into RNA and of C^{14}-L-Alanine into Proteins of Monkey Kidney Cells in Vitro

Experiment	Uptake per 3 hrs.					
	Adenosine-8-C^{14}*			C^{14}-L-alanine‡		
	Net c.p.m. per 4 × 10^6 cells		Inhibition	Specific activity of protein§		Inhibition
	Control	DRB, 95 μM		Control	DRB, 95 μM	
			per cent	*c.p.m./mg*	*c.p.m./mg.*	*per cent*
A	85	36	58	47	28	40
B	84	46	45	84	70	17
C	79	23	71	40	33	17
D	54	24	56	48	45	6
Mean.....			58			20

* 0.1 μc./ml.
‡ 0.1 μc./ml.
§ Obtained from 20 × 10^6 cells.

75 per cent inhibition in yield of influenza virus. At a concentration 5 times greater a slight reduction (5 to 10 per cent) in oxygen uptake was observed. The effect of DRB on oxygen uptake by monkey kidney cells was determined because multiplication of polio and adenoviruses was studied in such cells.

Oxygen uptake was measured by the direct method in the Warburg apparatus at 35°C. The center well and the side arms contained a solution of 4 M diethanolamine and 0.1 per cent thiourea (15) which had been equilibrated with 2 per cent CO_2 in air. One ml. of a suspension of monkey kidney cells in Eagle's medium was introduced per flask followed by 1 ml. of Eagle's medium with or without DRB. The flasks were connected to manometers and 2 per cent CO_2 gas was passed through the flasks for 1 hour with shaking. The system was then closed and readings begun. Two flasks were employed per variable in each experiment.

The results summarized in Table IV show that DRB at a concentration of 95 μM caused 29 per cent inhibition of O_2 uptake by monkey kidney cells.

Inhibition by DRB of Adenovirus Multiplication in Monkey Kidney Cells.— Previously it was shown that the inhibitory activity of DRB on influenza B (5) and vaccinia (7) virus multiplication in the chorioallantoic membrane *in vitro* was closely similar. The 75 per cent virus inhibitory concentration of DRB was 38 μM with influenza B and 32 μM with vaccinia virus. In monkey kidney cells the 75 per cent virus inhibitory concentration of DRB with type 2 poliovirus was 57 μM (6). The inhibitory activity of DRB on the multiplication of influenza B and type 4 adenovirus multiplication was determined in monkey kidney cells.

TABLE IV

Inhibitory Effect of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on Oxygen Uptake by Monkey Kidney Cells in Vitro

Experiment	No. of cells, × 10^6	Cumulative O_2 uptake, *μl. per 3 hrs.*		Inhibition
		Control	DRB, 95 μM	
				per cent
A	13.2	70	47	33
B	11.3	66	50	24
Mean.....				29

In a series of experiments with influenza B virus the inoculum was varied between 50 and 500 $TCID_{50}$ per ml. Eagle's medium was used with or without DRB. The compound was employed at varying concentrations. Final volume was 1 ml., and the tubes were incubated at 36°C. for 48 hours. The amount of virus produced was determined in the supernatant by the hemagglutination technique. Yield in the presence of DRB was expressed as a percentage of control yield and plotted against concentration of DRB. From the graph the concentration of DRB required to restrict the yield of virus to 25 per cent of control value was interpolated.

In experiments with type 4 adenovirus the inoculum was 62 $TCID_{50}$ per ml. After a 48 hour period of incubation cells were scraped off the glass with a rubber policeman and transferred to nitrocellulose tubes. The cell suspension was frozen and thawed five times and clarified by centrifugation. The amount of virus present was determined by infectivity titrations in tube cultures of monkey kidney cells. The concentration of DRB required to restrict the yield of virus to 25 per cent of control value was derived in a manner described above.

It was found that DRB caused 75 per cent inhibition of influenza B virus at 23 μM and of that of adenovirus at 30 μM. Thus the inhibitory activity of DRB on the multiplication of the DNA-containing adenovirus was similar to its activity on the multiplication of the RNA-containing influenza virus. However, with the RNA-containing poliovirus DRB was only one-half as active (6).

Inhibitory Activity of DRB.—Summaries of results obtained with DRB in two host cell systems are shown in Tables V and VI. In Table V the inhibitory activity of DRB on the multiplication of influenza, vaccinia, polio,

TABLE V

Virus Inhibitory Activity of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)

Culture	Virus	Concentration of DRB causing 75 per cent inhibition in virus yield
		μM
Chorioallantoic membrane	Influenza*	38 (5)
" "	Vaccinia‡	32 (7)
Monkey kidney cells	Polio*	57 (6)
" " "	Influenza*	23
" " "	Adeno‡	30

* RNA viruses.
‡ DNA viruses.

TABLE VI

Inhibitory Effects of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) in the Chorioallantoic Membrane and in Monkey Kidney Cells in Vitro

Culture	DRB	Process	Time of observation	Inhibition
	μM		hrs.	per cent
Chorioallantoic membrane	55	Influenza virus multiplication	36	>95 (5, 18)
		Vaccinia virus multiplication	48	>95 (7)
		Adenosine-8-C^{14} uptake into RNA	3	56
		C^{14}-L-alanine uptake into proteins	3	16
		Oxygen uptake	3	0 (5, 17)
Monkey kidney cells	95	Poliovirus multiplication	48	95 (6)
		Influenza virus multiplication	48	>95
		Adenovirus multiplication	48	>95
		Adenosine-8-C^{14} uptake into RNA	3	58
		C^{14}-L-alanine uptake into proteins	3	20
		Oxygen uptake	3	29

and adenoviruses is recorded. In Table VI inhibition of adenosine-8-C^{14} uptake into cell RNA, of C^{14}-L-alanine uptake into cell proteins, and of cellular oxygen uptake is described.

DISCUSSION

The structure of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (5) and the time of its action early in the latent period of the reproductive cycle of influenza virus (18) suggested that DRB inhibits influenza virus multiplica-

tion through inhibition of RNA synthesis (19). The results of the present studies on the biochemical mechanism of action of DRB indicate that this compound is, indeed, an inhibitor of RNA synthesis.

The fact that DRB is highly active as an inhibitor of multiplication of vaccinia and adenoviruses suggests that RNA is of decisive importance in the reproduction of these DNA-containing viruses.

In an earlier report it was shown that conversion of chlorinated benzimidazoles to corresponding β-D-ribofuranosides did not increase their inhibitory activity on vaccinia virus multiplication (7), although their inhibitory activity on the multiplication of RNA-containing influenza virus was greatly enhanced by such a structural modification (5). The view was expressed that this difference may have been due to the type of nucleic acid present in the several viruses.

In the earlier analysis of data, little emphasis was placed on the fact that the chlorinated parent compounds were considerably more active against vaccinia than influenza virus and the fact that the corresponding ribofuranosides were in absolute terms not more active against influenza than vaccinia virus. The view may be taken that conversion of chlorinated benzimidazoles to corresponding ribofuranosides failed to increase further the inhibitory activity against vaccinia virus because the parent compounds themselves were of high activity.

The complexity of factors which determine susceptibility of different viruses to inhibitory compounds is underlined by the fact that in monkey kidney cells multiplication of adeno or influenza virus was more susceptible to inhibition by DRB than that of poliovirus. As has been stated before (6) the factors involved may concern not only quantitative differences in the metabolic requirements of these viruses, but also differences in the location of sites of synthesis of virus materials, differences in the effects of these viruses on the metabolism of host cells, and other factors. The type of nucleic acid present in the virus particle does not appear to be of decisive importance in determining susceptibility to inhibition by DRB.

The precise mechanism whereby DRB interferes with the incorporation of a number of RNA precursors into RNA remains uncertain. In the present study only adenosine was used. However, in studies with isolated calf thymus nuclei it was found (14) that DRB also inhibited orotic acid incorporation into RNA. Two explanations may be considered: (*a*) DRB acts at a level basic to incorporation of all precursors into RNA; or (*b*) Interference with incorporation of one precursor results in failure of incorporation of all precursors.

The demonstration that adenosine can block the inhibitory effect of DRB on influenza virus multiplication provides suggestive evidence that DRB acts as a metabolic antagonist of adenosine or of a precursor of adenosine. It is of considerable interest in this connection that guanosine failed to block the inhibitory effect of DRB. In studies on blocking of the inhibitory effect of

DRB on alanine uptake into proteins of isolated calf thymus nuclei a similar result was obtained in that adenosine but not guanosine was capable of partially blocking the inhibitory effect of DRB (20).

Of great interest are the findings (21) that turnover of nucleolus-associated RNA is much faster than that of nucleoplasmic RNA and that incorporation of precursors into nucleolus-associated RNA is two times more sensitive to inhibition by DRB than incorporation into nucleoplasmic RNA. As was pointed out above a similar difference in susceptibility to inhibition was observed with influenza and polioviruses.

Some indication of chemical specificity of action of DRB is provided by the findings that at concentrations which were markedly inhibitory for incorporation of adenosine into RNA, incorporation of alanine into cell proteins and cellular oxygen uptake were only slightly reduced.

The slight effect observed on incorporation of alanine into proteins does not mean that RNA was of little importance for protein synthesis in the chorioallantoic membrane or in monkey kidney cells. It merely suggests that the process of incorporation of alanine into proteins was not directly or uniformly geared to uptake of adenosine into RNA.

It has also been shown that at concentrations which inhibit uptake of labeled precursors into RNA, DRB has little or no effect on uptake of thymidine into DNA of isolated calf thymus nuclei (20).

Additional evidence in support of the conclusion that RNA is of vital importance in the reproduction of several animal viruses which contain DNA will be given in the communication which follows (8). Possible ways in which RNA of one or another kind may play such a role will be discussed.

SUMMARY

Adenosine, but not guanosine, was capable of blocking the inhibitory effect of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on influenza virus multiplication in the chorioallantoic membrane *in vitro*. At virus inhibitory concentrations DRB caused marked inhibition in uptake of adenosine-8-C^{14} into RNA of uninfected host cells, but it had little effect on uptake of C^{14}-L-alanine into host cell proteins or on cellular oxygen consumption. The activity of DRB in inhibiting multiplication of the DNA-containing adenovirus was similar to its inhibitory activity on multiplication of the RNA-containing influenza virus. These and earlier results are discussed from the point of view of the important role of RNA in the reproduction of DNA-containing viruses.

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