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INFLUENCE OF ADENOVIRUS SOLUBLE ANTIGENS ON HOST CELLULAR METABOLISM

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Summary

At some stages in the replication cycle, adenovirus synthetic and related activities appear to coincide with the stimulation of host cellular metabolism while at other times, significant depression of host cellular activities seem to be caused by some or all of the same processes.

The depression of host cell respiration during early (0-6 h) and late (30-36 h) stages of the virus replication cycle is most noteworthy. It has been shown that these inhibitions are connected with the effect of virus soluble antigens. Isolated antigen, relatively free of virion, affects adversely some respiratory metabolic parameters of the infected cells. Our observations are related to some early studies on virus replication processes and other observed host cellular changes.

Résumé

A certains stades du cycle de multiplication, les activités synthétique et appearentées de l'adenovirus paraissent coincider avec la stimulation du métabolisme cellulaire de l'hôte, tandis qú à d'autres moments, il semble que la baisse significative des activités cellulaires de l'hôte soit causée par quelques-uns des mêmes processus on tous as derniers.

La baisse de la respiration cellulaire de l'hôte pendant les stades initiaux (0 à 6 h) et les stades finals (30 à 36 h) du cycle de multiplication du virus est tout à fait notable. On a montré que ces inhibitions sont liées à l'effet des antigène solubles du virus. Un antigéne isolé, relativement exempt d' infection affecte de fazon défavorable des paramètres respiratoires métaboliques des cellules contaminées.

Nos observations sont liées à celles faites sur d'autres changements cellulaires de l'hôte et à des études préalables menées sur less processus de multiplication du virus.

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Introduction

The adenoviruses have a fairly long replication cycle (Green & Daesch, 1961) and they often evolve relatively slow cytopathic effect (CPE). The replication of the viruses result in rounding up of the cells which are arranged like bunches of grapes. Previous reports have shown stimulation of host glycolysis, increased utilization of acids, (Rozee, Ottley & Van Rooyen, 1957; Fisher & Fisher, 1961; Williams, 1969; Williams & Hannan, 1978) and early inhibition of host cellular functions (Bello & Ginsberg, 1967); Williams (1969), Williams & Hannan (1978), during overall study of host cellular functions.

Apart from the studies of Williams (1969), infection and replication of these viruses have not been observed over the entire length of the virus synthetic cycle. Marked and significant increase in glycolytic and respiration rate including early and late partial inhibition of cell division and host cellular metabolism was reported by Williams (1969). This paper examines the conditions affecting early and late inhibition of host cellular metabolism in the light of the observations of other workers. Pereira and co-workers (Pereira & Kelly, 1957; Pereira, 1958, 1960) demonstrated that toxic components of some of the adenoviruses exert some inhibitory and early cytopathic effects on the ability of cells to support

growth of other viruses.

Lawrence and Ginsberg (1967) noted the uncoating of type 5 adenoviruses, and its relationships to overall host cellular reactions. Bello and Ginsberg (1967) enunciated the inhibition of protein synthesis in type 5 adenoviruses infected cells. This paper presents the effect of adenoviral soluble antigens on some parameters of the host cell metabolism.

Materials and methods

Cells and media. A sub-line of KB cells was propagated in suspension culture. In experiments with growing cultures, unless otherwise stated, Eagle's minimal essential medium containing 5% heat-inactivated horse serum was used.

Stock virus and infection of cultures. The origin and life history of the type 3 adenovirus used have been described (Williams & Hannan, 1978). The virus used in these experiments was passaged several times in KB cells and its identity was confirmed by plaque assay technics (Yong, 1968).

Partial purification of virus. Type 3 adenovirus was partially purified by adsorbing to and eluting from grivet monkey red blood cells as previously described by Williams (1969).

Inactivation of virus by ultra violet (u.v.) light. Some of the materials so purified was u.v.-inactivated by the methods described by Williams and Hannan (1978).

Infection of cells. Live or 'u.v.-killed' virus purified as above was used to infect KB cells at an input multiplicity of $IOTCID_{50}$ per cell. Experimental procedures are the same as described by Williams (1969). Briefly stated, washed cells were obtained from control, live-virus-infected and 'u.v.-killed'-virus-infected cell culture and incubated with 6.0 mmol glucose in Warburg flasks. Oxygen uptake, CO_2 production glucose consumption and lactate production were determined by methods previously described (Williams, 1976).

Analytical methods

Manometric methods. Oxygen consumption and carbon dioxide evolution studies were done by modified conventional manometric methods described by Umbreit (1964).

Separation of antigen by cesium chloride technique. Crude virus was prepared by growing virus as either monolayer or suspension cultures. Two-millilitre portions of the product were mixed with cesium chloride in the ratio of cesium chloride: virus preparations, 1:10. Centrifugation was carried out at 70000*g* for several hours. The upper band represented the antigenic component (Norrby, 1968; Everett & Ginsberg, 1957). It contained some residual viral activity. The lower band contained viral activity of above 10^8 $TCID_{50/0\cdot2\ ml}$, it represents the purified, reasonably soluble antigen-free product. The upper portion was collected by means of sterile syringe and needle. To collect the virus, a hole was punctured at the bottom of the centrifuge tube and the band drained into a container.

Titrations. All preparations were checked for toxic activities by adding the preparation to a monolayer of cells growing on converslips in Leighton tubes. The ability of the preparations to cause cytotoxic effect evidenced and judged by complete or partial detachment of cells from the surface of the glass as seen under a phase contrast microscope was carried out (Pereira, 1958).

Virus assay. Virus yield was assayed via Haemagglutination and infectivity titration methods as routinely done.

Toxin assay. A toxin unit is described as that amount of material that led to toxic effect on the cells after 24 h of contact. By this time whole cell sheets are detached from glass surfaces and microscopical examination show that damages have been done to cells as evidenced by morphological distortions of these cells.

Cytotoxic units. This is defined as the amount of antigenic preparation that caused almost complete detachment of cells from glass surface coupled with gross morphological changes observed as above within 24–48 h after treatment. The morphology and integrity of the cells were examined and determined under phase contrast microscopes.

Results

Respiration in cells infected with partially purified type 3 adenovirus

Table 1 shows pattern of oxygen uptake and carbon dioxide evolution in KB cells infected with type 3 adenovirus. The metabolism of the host cells during the entire replicating cycle of the virus are indicated. Note early and late inhibition of respiration.

Preparation and determination of toxin units

A comparison of the various viral preparation and the total amount of soluble antigens expressed in units is shown in Table 2. The crude virus has the bulk of the toxic factor.

Respiration in cells infected with type 3 adenovirus relatively free from soluble antigen

Figure 1 represents a typical pattern of oxygen uptake and carbon dioxide evolution. It is noteworthy that the early inhibition of the parameters (compare Table 1 above) are no longer in evidence.

TABLE 1. Respiration-carbon dioxide evolution and oxygen uptake in type 3 adenovirus-infected* cells expressed in micromoles per 10 million viable cells per hour

After infection (h)	Cells†	Carbon dioxide (mmol)	Oxygen uptake (mmol)	Carbon dioxide $\left(\%\frac{V}{C}\right)^{\dagger}$	Oxygen uptake $\left(\%\frac{V}{C}\right)^{\dagger}$
6	C	0·59	1·05		
	V	0·40	0·67	67·80	63·81
12	C	0·47	0·45	134·42	135·55
	V	0·61	0·61		
18	C	0·33	0·74	136·6	121·62
	V	0·45	0·90		
24	C	0·35	0·83		
	V	0·45	0·61	128·57	73·74
30	C	0·48	0·92	70·83	52·91
	V	0·34	0·53		
36	C	0·65	1·59		
	V	0·50	0·83	76·92	52·2

†C = control uninfected cell; V = virus-infected cell
*Type 3 adenovirus used in the above was only partially purified

TABLE 2. A comparison of the toxin levels in different viral material preparations

Material	Infectivity (TCID 50/0·2 ml)	Haemagglutination (HAU/0·4 ml)	Cells (Toxin unit/ml/10^7)
Crude virus	5	65 136	8 533
Heat-inactivated virus	<1·0	nd	nd
u.v.-inactivated virus	2·0	512	2 133
Partially purified (adsorption to and elution from red blood cells)	8·0	16 284	2 133
CsCl upper band (soluble antigen)	<2·0	32 568	1 600
CsCl lower band (antigen-free virus)	nd	4 096	533

nd = Not determined.

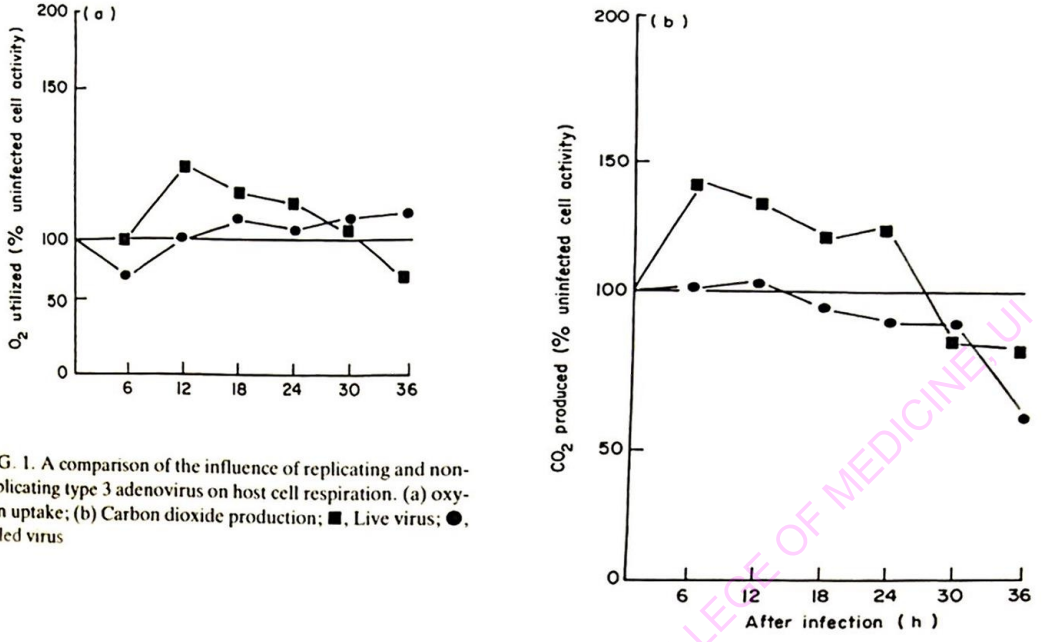

FIG. 1. A comparison of the influence of replicating and non-replicating type 3 adenovirus on host cell respiration. (a) oxygen uptake; (b) Carbon dioxide production; ■, Live virus; ●, killed virus

TABLE 3. A comparison of the effect of toxic components of type 3 adenovirus soluble antigen on respiration of KB cells

Material added to metabolic mixture	Oxygen (mmol/10^7 viable cells/min)			
	60 min	120 min	180 min	240 min
Control crude virus	1·66	3·31	5·22	6·62
Soluble antigen (CsCl upper band)	1·24	2·80	4·35	6·00
Virion (CsCl upper band)	1·32	2·98	4·55	6·21
u.v.-inactivated virion (CsCl lower band)	1·16	2·13	4·06	5·71

TABLE 4. A comparison of the effect of toxic components of the soluble antigen of u.v.-light-inactivated type 3 adenovirus on respiration KB cells*

Material added to metabolic mixture	Oxygen (mmol/10^7 viable cells/min)					Control (%)
	30 min	60 min	90 min	120 min	150 min	
Control	0·88	1·76	2·64	3·43	3·96	100
ble antigen 3 toxin units)	0·79	1·58	1·94	2·77	3·52	88·89
activated (virion 5·3 toxin)	0.70	1·67	1·98	2·82	3·61	91·16

e data presented here attempts to compare the effect of comparable amount of toxin units derived from both antigen and inacti- l partially purified virus. It was not possible to include a similar data for soluble antigen free virus as CTC was very low (see e 1). Larger and unmanageable volume of virus material would be required.

Effect of soluble antigen on cell respiration

The data presented in Tables 3 and 4 show a comparison of the respiration rate of cells incubated with partially purified virus with virus relatively free of soluble antigen free virus. Note relatively inhibitory effect of the presence of soluble antigens.

Discussion

Rowe *et al.* (1955) reported the first important observation that tissue culture medium of adenovirus-infected cells turned acidic faster than the medium or non-infected cultures. Fisher and Ginsberg (1957) confirmed this and indicated that this was due to accumulation of organic acids.

Williams (1969), Williams and Hannan (1978) and Fargey (unpubl. comm.) consistently showed that upon infection, the respiration rate of host cell was significantly depressed very early (6 h) and later (30–36 h) after infection. A typical one-step growth cycle of the virus in KB cells (Williams, 1969) showed little or no difference between the biochemical sequence of events as compared to other adenoviral replication cycles (Green, 1961).

A time-course virus production study on monolayer cell cultures confirmed the observations regarding early cytopathic effect credited to the adenoviruses (Pereira, 1958).

Following speculation that the inhibition of host cellular metabolism may be linked to adenoviral soluble antigens or other viral-related protein material, an attempt was made to check the direct effect of relatively nucleic acid-free antigens of type 3 adenovirus on the rate of KB cell respiration. The experiment was designed to allow for a comparison of the effect of intact virion, virion alone and antigen alone on host cellular metabolism. The experiment further tested the effect of killed virus on the nature of the inhibition under investigation.

Live, infective, complete, partially purified, replicating, type 3 adenovirus stimulates host metabolism (Table 1) as well as cause inhibition of respiration at 6 h and 20–30 h after infection, in a manner comparable to previous observations (Williams, 1969; Williams & Hannan, 1978).

It is noteworthy that relatively antigen-free type 3 adenovirus also shown to be capable of replication did not cause depression of the parameters of respiration at 6 h after infection. These parameters are however depressed late in the viral replication cycle. It is considered interesting that separated soluble antigen all by itself caused appreciable depression in both oxygen uptake and carbon dioxide evolution when incubated with these cells (Tables 3 and 4).

The depression of both of these parameters later in the viral replication cycle as shown in Fig. 1 is perhaps to be expected. These may be explained in one or two ways. Either, that the observation represents the influence of freshly made new viral soluble antigens resulting from new viral replication or that they are expression of cellular damage which may also be creditable to new viral synthetic activities.

Our data probably support the general belief that upon infection, viruses take over the control of host metabolic machinery. The early decline in respiration rate may well represent a manifestation of this phenomenon, while later depression may be a reflection of damage to cells — both successfully and or abortively infected.

Acknowledgment

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References

Bello, L.J. & Ginsberg, H.S. (1967) Inhibition of host protein synthesis in type 5 adenovirus infected cells. *J. Virol.*, **1**, 843.

Everett, S.F. & Ginsberg, H.S. (1958) A toxin-like material separable from type 5 adenovirus particles. *Virol.*, **6**, 770.

Fisher, T.N. & Fisher, E. (1961) Host cell metabolic alterations following herpes simplex infection. *Virol.*, **13**, 308.

Green, M. (1966) Biosynthetic modifications induced by DNA animal viruses. *Ann. Rev. Microbiol.*, **20**, 189.

Green, M. & Deusch, G.E. (1961) Biochemical studies on adenovirus multiplication. II. Kinetics of nucleic acid protein synthesis in suspension cultures. *Virol.*, **13**, 169.

Lawrence, D.H. & Ginsberg, H.S. (1967) Intracellular uncoating of type 5 adenovirus deoxyribonucleic acid. *J. Virol.*, **1**, 851.

Norrby, E. (1968) Biological significance of structural adenovirus components. *Curr. Top. Microb. Immuno.*, **43**, 1.

Pereira, H.G. & Kelly, B. (1957) Dose response curves of toxic and infective actions of adenoviruses in HeLa cell cultures. *J. Gen. Microb.*, **17**, 517.

Pereira, H.G. (1958) A protein factor responsible for the early cytopathic effect of adenoviruses. *Virol.*, **6**, 601.

Pereira, H.G. (1960) A virus inhibitor produced in HeLa cells infected with adenoviruses. *Virol.*, **2**, 590.

Rowe, W.P., Hartley, J.W., Reizman, B. & Levy, H.B. (1958) Characterization of a factor formed in the course of adenovirus infection of tissue cultures causing detachment of cells from glass. *J. Exptl. Med.*, **108**, 713.

Rozee, K.R., Ottey, L.J. & Van Rooyen, C.E. (1957) Some metabolic effects of adenovirus infection in HeLa cells. *J. Gen. Microb.*, **3**, 1015.

Umbreit, W.W. (1964) *Manometric Techniques* (eds W.W. Umbreit, R.H. Burris and J.F. Stauffer) 4th ed. Burgess, Minneapolis.

Williams, B.A.A. (1969) Studies on the replication of type 3 adenovirus in relation to host cellular carbohydrate metabolism. PhD Thesis, University of Manitoba Canada.

Williams, B.A.A. (1976) Carbohydrate metabolism in KB cells. *Biochem. Exp. Biol.* **12**, 357.

Williams, B.A.A. & Hannan, C.K. (1978) Glucose breakdown by the hexose-mono-phosphate pathway in KB cells infected with type 3 adenovirus. *J. West. Afr. Sc. Ass* (in press)

Yong, D. (1968) Plaque assay by adenoviruses. MSc Thesis University of Manitoba, Winnipeg, Canada.

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