Influence of Potassium on Coxsackie B_2 Virus Growth in Tissue Cultures. (27261)

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Levine *et al.*(1,2) have shown that potassium is required for growth of influenza virus in chicken chorio-allantoic membrane (CAM). Two similar reports(3,4) have shown that this ion is also essential for growth of Newcastle Disease virus (NDV) in CAM. The present study was designed to demonstrate the importance of potassium on growth of Coxsackie B viruses in tissue cultures of monkey kidney (MKC) and monkey testicular cells (MTC).

Materials and methods. Tissue cultures. All experiments were carried out in roller tubes containing epithelial cells derived from monkey kidneys and testes. The MKC were prepared by Youngner's method(5) and grown in medium 199 with 2% calf serum at 36°C. The MTC were prepared from rhesus monkey testicles by the continuous trypsinization method of Rappaport(6) as modified by Tytell *et al.*(7). These cells were planted in a yeast-extract, lactalysate medium containing 5% calf serum. Tissue culture tubes showing confluent cell sheets 6 to 7 days later were washed one day prior to using and incubated with 199 devoid of serum.

Earle's salt solution (ESS), without phenol red, was prepared from a 10-fold concentrated stock solution. Tris (hydroxymethyl aminomethane) buffer was added to this solution to obtain a final concentration of .01 M. The pH was adjusted to 7.4 to 7.6 with 1 N HCl or 1 N NaOH.

ESS, with varying concentrations of KCl, was used throughout these studies as virus growth medium. Concentrations of KCl used were 0.0, 0.4, 0.8 and 1.6 g per liter of this solution. From 10 to 20 tissue culture tubes were used for each concentration of KCl.

Coxsackie B_2 virus was used in most of this work. Most of the cultures were inoculated with $10^{4.5}$ $TCID_{50}$ of virus. Differences in virus adsorption were minimized by inoculating each culture with virus diluted in medium 199 and incubating the cultures for at least 45 minutes at 37°C. Each inoculated culture was then washed 3 times with 3 to 5 ml of the solution containing a specified concentration of KCl. A final 2 ml of the solution with the same concentration of KCl was added to each tube and virus production was followed at 37°C. Samples were taken at certain times, pooled and stored at −20°C. Another set of non-infected cultures were prepared in the same manner and glucose uptake by these cells was measured.

Infectivity titers of Coxsackie virus fluids were determined by microscopic observation for typical cytopathic changes produced by serial 10-fold dilutions of the virus. Titrations were made on confluent sheets of MTC since they were shown to be more sensitive than MKC for detecting Coxsackie viruses by Burnstein *et al.*(8). Occasionally, ½ log dilutions were used. The tubes were incubated at 37°C for 6 days at which time infectivity was determined.

Glucose determinations were made by the anthrone method of Morris(9).

Results. Data in Fig. 1 clearly indicate that potassium enhanced production of infectious Coxsackie B_2 virus from MKC in ESS. This is more evident in first 48 hours of virus growth. After 72 hours of incubation, the amount of virus produced reached a plateau in cells in this solution containing 0.4 to 0.8 g of KCl per liter. Only a small amount of virus was produced at any time in deficient medium. Approximately 500 times more virus was produced in the medium which contained 0.4 g/liter of KCl than was produced in the K deficient medium.

No differences in degree of cellular necrosis produced by virus were observed in all different concentrations of KCl. However, non-specific cellular necrosis was observed in cells in a K deficient medium after 72-hour

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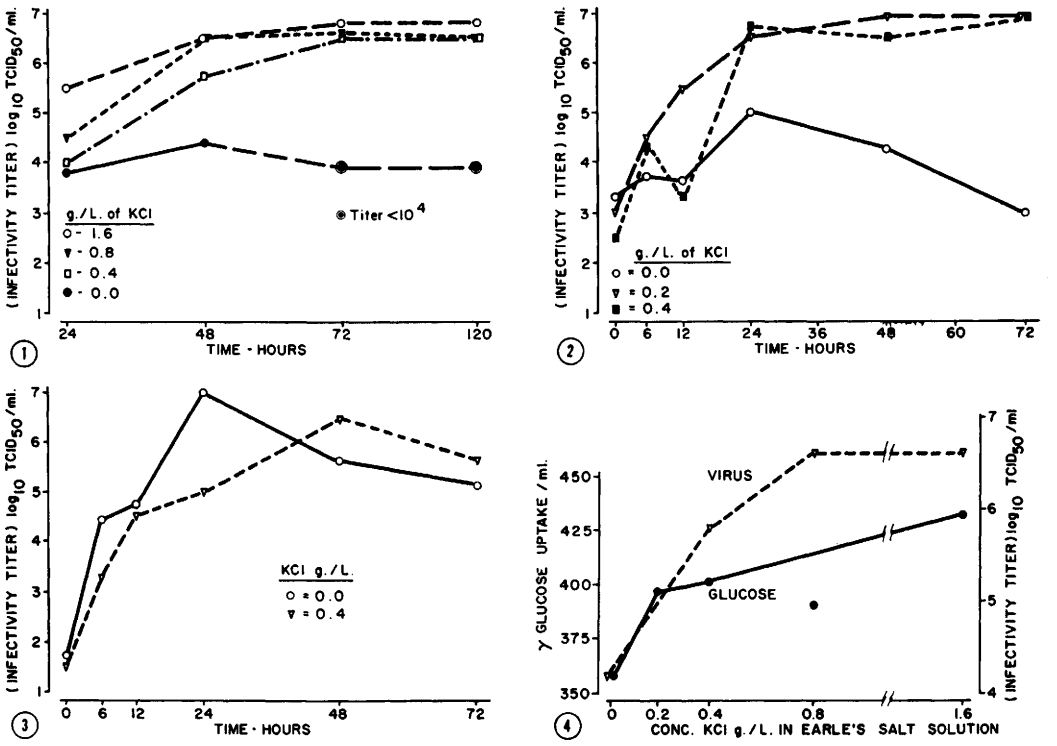

FIG. 1. Effect of KCl in Earle's balanced salt solution on production of infectious Coxsackie B_2 virus in monkey kidney cells.

FIG. 2. Effect of KCl in Earle's balanced salt solution on production of infectious Coxsackie B_2 virus in monkey testicular cells.

FIG. 3. Effect of KCl in Earle's balanced salt solution on production of infectious Coxsackie B_3 virus in monkey testicular cells.

FIG. 4. Effect of KCl in Earle's salt solution on uptake of glucose and on production of infectious Coxsackie B_2 virus in monkey kidney cells after incubation at 37°C for 48 hr.

incubation. Also, early experiments indicated that levels of KCl above 1.6 g/liter were toxic to tissue culture cells. Thus, amount of KCl used and time of a given experiment were limited.

Since it was shown that K influenced growth of Coxsackie B_2 virus in MKC, the next logical step was to investigate whether this result was a reflection of the tissue culture system used. Fig. 2 shows that potassium had about the same effect on virus grown in MTC as was found with MKC; *i.e.*, virus growth was suppressed in a potassium-deficient medium. Potassium seemed to be more critical for growth of Coxackie B_2 virus in MTC since the depletion of potassium in MTC reduced the virus growth more than 2,000 times.

Fig. 3 shows that growth of Coxsackie B_3 virus in MTC was not as adversely affected by depleting potassium from the medium as Coxsackie B_2 virus. Hence, the effect of potassium on virus growth cannot be generalized on all Coxsackie strains, but may also depend upon the specific virus used.

As was stated in the methods, differences in virus adsorption were minimized by allowing virus adsorption to take place in the same medium. Nevertheless, an experiment investigating the possibility that potassium influences virus adsorption to MKC or MTC was designed as follows: A suspension of Coxsackie B_2 virus in medium 199 was dialyzed against 16 volumes of distilled water for 24 hours at 5°C to decrease the concentration of potassium present. This virus suspension was then diluted 1:2 with distilled water. One ml of suspension was added to a roller tube tissue culture which contained 2 ml of Earle's solutions with a specified concentra-

TABLE I. Influence of KCl on Adsorption of Coxsackie B_2 Virus onto MKC and MTC.

KCl conc., g/l	(Infectivity titer) Log_{10} $TCID_{50}$/ml of supernates after 1½ hr Adsorption at 37°C MTC	MKC
.0	5.2	6.2
.4	5.5	5.7
.8	5.5	—
1.6	5.5	5.7

tion of KCl. Four tubes per concentration of KCl were used. Infected cultures were incubated 1½ hours at 37°C and fluids removed, pooled and titrated for residual virus. It can be seen from Table I that adsorption of Coxsackie B_2 virus onto MKC and MTC was not appreciably different in various levels of potassium.

In preliminary experiments pH of medium 199 became progressively more acid with increasing levels of KCl, thus indicating that potassium may influence the rate of glycolysis by MKC and MTC. An attempt was made to correlate glucose uptake by normal (non-infected) MKC with Coxsackie B_2 infected MKC. These results are illustrated in Fig. 4. It is clear from the data that potassium stimulates the rate of glycolysis by MKC and also that a rough correlation between glycolysis and virus production exists.

A question arose whether or not potassium deficiency produced an irreversible change in MTC, so that they were incapable of producing virus. To test this hypothesis, 2 sets of cultures were infected and prepared as previously described. One set was incubated with a potassium-deficient medium while the other set had medium containing 0.8 g/liter of KCl. After 12-hour incubation at 37°C, the fluids in half of the cultures of each set were discarded and the cells were washed. Cells which originally contained potassium-deficient medium received medium containing 0.8 g/liter of KCl while other cells originally in potassium containing medium received potassium-deficient medium. From Table II it can be seen that potassium deficiency for 12 hours did not produce an irreversible change in MTC, making them incapable of producing virus. However, there seemed to be a lag phase of at least 24 hours after replenishing potassium to the potassium-deficient cells before they were capable of producing virus equal to the cells in a non-deficient-potassium medium.

TABLE II. Reversal of KCl Deficiency Inhibition of Infectious Coxsackie B_2 Virus Production in MTC.

KCl conc., g/l		(Infectivity titer) Log_{10} $TCID_{50}$ at 37°C			
Initial	After 12 hr	6 hr	12 hr	24 hr	48 hr
.0		3.7	3.6	5.0	4.3
	.8	—	—	4.0	7.0
.8		3.5	3.0	6.5	7.0
	.0	—	—	5.8	4.7

Discussion. The importance of cations in growth of animal viruses is becoming more apparent. Levine *et al.*(1,2) have shown that potassium deficiency did not support growth of the PR8 strain of influenza virus in minced chorio-allantoic membranes (CAM) of chicken embryos. These workers further demonstrated that deficiency reduced respiration of normal and virus-infected tissues by about 10%, and that these effects could be reversed by addition of potassium. Similar results obtained by Zuschek *et al.*(3) showed that potassium ions stimulated growth of NDV in CAM and that high levels of calcium interfered with growth of NDV in the same tissues. Gohd and Huang(10) have shown a requirement of Ca for growth of PR8 virus in CAM. High levels of $CaCl_2$ in mixture 199 will interfere with production of polio virus MEF-type II in MKC.

Although potassium ions were found to be necessary for production of infectious Coxsackie B_2 virus from MKC and MTC, the exact function of potassium for growth of this virus remains unknown. Since potassium was not required for virus attachment but did stimulate glycolysis by cells, it is postulated that potassium may be involved in energy mechanisms (glycolysis or oxidative phosphorylation) utilized for synthesis of Coxsackie B_2 virus. This observation cannot be generalized since Coxsackie B_3 was not adversely influenced by potassium deficiency under a similar condition. This was quite surprising since these viruses are serologi-

cally related, presumably both (B_2 and B_3) viruses need the same energy sources. Also, potassium may be necessary for penetration of virus into cells. This possibility was not investigated in the present study, but Levine *et al.* have evidence that potassium may be necessary for effective penetration of influenza (PR8) virus into chick cells and for intracellular synthesis of new virus. This evidence is lacking for the role of potassium in growth of Coxsackie B_2 virus.

Summary. Potassium ion augments the amount of infectious Coxsackie B_2 virus produced from MKC and MTC. Production of infectious Coxsackie B_2 virus was reduced 500 to 2,000 times in MKC and MTC by depletion of potassium from their medium. Similar results were not obtained with Coxsackie B_3 virus. Potassium was not required for virus attachment, but did stimulate glycolysis of tissue culture cells. Potassium could be replaced in a deficient medium after 12 hours of infection and still obtain maximal yields of infectious virus in MTC.

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Catecholamines in Homologous Heart Grafts.* (27262)

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Goodall and Kirschner(1) reported that cervicothoracic ganglionectomy in dogs results in a considerable decrease of the cardiac content of norepinephrine and epinephrine. However, since these catecholamines were not entirely depleted by this procedure, the possibility arose that a fraction of cardiac catecholamines could be stored in other than sympathetic structures, thus remaining unaffected by surgical denervation. Studies(2,3) on cardiac sensitivity to epinephrine after similar denervation yielded contradictory results, probably because complete sympathetic denervation is difficult to accomplish. More recently Cooper, Gilbert, Bloodwell and Crout (4) described a technic for ablation of all known autonomic structures in the mediastinum in dogs. They found a depletion of 50% of the catecholamine content in the denervated hearts within 1 to 2 days. Almost complete loss of ventricular catecholamines was noted on the third day after operation.

The present report concerns a study of the rate of catecholamine depletion in heart in which denervation was complete. This was accomplished by transplanting the heart into the neck of adult mongrel dogs and determining its catecholamine content at certain intervals after this operation. Wegmann and Kako(5) have reported that 74% of the catecholamines in the heart homogenates of puppies were found in the sediment obtained by subsequent low and high speed centrifugation. The present paper includes results on the possible influence of transplantation on distribution of catecholamines in the cell fractions of heart homogenates.

Technic. Twenty-seven puppy hearts were transplanted according to the technic previ-

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