(DHT), massive and rather selective calcification of the pancreas is induced by subsequent intraperitoneal administration of egg white. Intravenous injection of egg yolk elicits a calcinosis primarily affecting the reticuloendothelial cells of the liver and the spleen. Yet, other types of calcinosis are induced by intravenous injection of egg white or the intraperitoneal administration of egg yolk. It is assumed that albumen and yolk act as "vital mordants," preparing the tissues of suitably sensitized animals for the subsequent uptake of calcium, distribution of the lesions being dependent upon the special distribution patterns of the mordants themselves.

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Metabolism of Human Amnion Cell Cultures Infected with Poliovirus. II. Utilization of Glucose, Pyruvate and Ribose in Virus Infected Cells. (26701)

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Human amnion cell cultures(1) proved to be a suitable system for investigation of effects induced by poliovirus on the metabolism of the host. It was shown(2) that infected cells utilized glucose by the glycolytic pathway. Levy and Baron(3) showed poliovirus to stimulate glycolysis of monkey kidney cells, and Matzelt(4) found increased activity of the glycolytic enzymes in a similar cell-virus system. This paper is a report of an investigation of the effect of glucose, ribose and pyruvate on virus multiplication. In addition, observations on the synthesis of lactic acid and ribose in infected and uninfected cells are also reported.

Materials and methods. Preparation of cell cultures. Primary human amnion-cell cultures were prepared as previously described(2). Monolayers were formed within 5-7 days.

Virus. A stock of the virus was prepared by inoculating amnion monolayers in milk bottles with the MEF_1 strain of poliovirus type II. After incubation for 72 hours at 37°C the medium was centrifuged, and the supernate containing the virus preserved at −20°C (infectivity titer $10^{7.0}$/ml). Virus titers were determined by inoculating tubes of amnion cells with 10-fold dilutions of the virus suspensions, using 4 tubes per dilution, and following the cytopathic changes microscopically for 7 days. The $TCID_{50}$ of the virus preparations was determined by the method of Reed and Muench(5).

Infection of cell-cultures. The cell cultures were washed with PBS, inoculated with the virus to give a multiplicity of infection of 5-10, and incubated for 1 hour at 37°C; the unadsorbed virus was removed by washing the cell with PBS. Eagle's medium, prepared with glucose, ribose or pyruvate or without any carbohydrate, was added to the cultures, which were reincubated at 37°C.

Chemical determinations. Aliquots of infected and uninfected cells or of cell hydrolysates were used for quantitative determinations of glucose, pyruvate, lactic acid and ribose. Glucose was determined by the Somogyi-Nelson photometric method(6), lactic acid by the method of Barker and Summerson(7), ribose (found in the medium and in the cells) by the method of Drury(8) and pyruvate by reaction with dinitrophenyl hydrazin reagent(9). For determinations of cell-synthesized-ribose, the cells were washed with 3 changes of PBS, released from the

TABLE I. Lactic Acid Production in Uninfected and Poliovirus Infected Human Amnion Cell Cultures.

Hr after re-incubation*	Infected cell cultures — Eagle's medium† containing: Glucose	Ribose	No carbohydrate	Uninfected cell cultures — Glucose	Ribose
	Lactic acid formed (μg/10^6 cells)				
0	85.5‡	35.0	56.5	60.0	20.0
5	169.0	35.0	70.0	102.0	50.0
18	505.0	35.0	70.0	300.0	50.0

* Cell cultures were incubated with poliovirus for 1 hr at 37°C, then washed with PBS to remove unadsorbed virus, Eagle's medium added, and cells reincubated.

† Eagle's medium was modified to contain glucose, ribose (500 μg/ml) or no carbohydrate at all.

‡ Results of cumulative lactic acid formation are expressed in μg/10^6 cells originally present in culture.

glass by combined versene and trypsin treatment(10), counted in hemocytometer, and hydrolyzed with alkali (by heating in a boiling water bath for 1 hour with 0.4 N NaOH).

Results. Relation between glucose uptake and lactic acid production. We have previously reported(2) the increased uptake of glucose by poliovirus infected amnion cell cultures. The present report deals with the lactic acid formed by uninfected and infected cultures.

Both uninfected and infected amnion cell cultures yielded lactic acid when glucose was used as substrate. However, when glucose was replaced by ribose, or when no carbohydrate was added, no lactic acid was formed (Table I). The increase of lactic acid formation in infected cells grown on glucose coincided with the period of virus synthesis (between 7-12 hours after addition of the virus (Fig. 1). During this period glucose was taken up very actively by these cells.

In uninfected amnion cells, the molar ration between lactic acid formed and glucose consumed was 1.56, showing that 80% of the glucose was utilized through the glycolytic pathway (Table II). In the infected cells the lactic acid:glucose ratio was 0.9, indicating that only 46% of the glucose was transformed to lactic acid. This lower ratio was obtained despite the fact that in poliovirus infected cells, glucose uptake was twice as high as in uninfected cells (2.93 μM and 1.43 μM per 10^6 cells, respectively).

Effect of poliovirus on pyruvate uptake. Primary amnion monolayers incubated in Eagle's medium with pyruvate or ribose during an observation period of 36 hours showed the same morphological characteristics as cultures in presence of glucose. Pyruvate consumption, as measured by its disappearance from the medium, amounted to 2-4 μmoles per 24 hours. Cells infected with poliovirus showed diminished uptake of pyruvate, beginning 5-7 hours after infection. However, the cells continued to incorporate pyruvate during the next 20 hours. Virus release from cells incubated with pyruvate was only 1% of that formed in presence of glucose (Table III).

Effect of poliovirus on ribose uptake. Poliovirus infected cells incubated in Eagle's

TABLE II. Glucose Consumption and Lactic Acid Formation in Infected and Uninfected Human Amnion Cell Cultures.

Exp. No.	Infected cells* Glucose consumed, μM	Lactic acid formed, μM	Lactic acid / Glucose	Uninfected cells Glucose consumed, μM	Lactic acid formed, μM	Lactic acid / Glucose
1	2.0	1.8	.90	1.6	2.5	1.56
2	3.1	2.8	.90	1.6	2.4	1.50
3	3.7	3.6	.97	1.1	1.8	1.63

* Cell cultures incubated with poliovirus for 1 hr at 37°C, washed with PBS to remove unadsorbed virus, Eagle's medium added and cells reincubated.

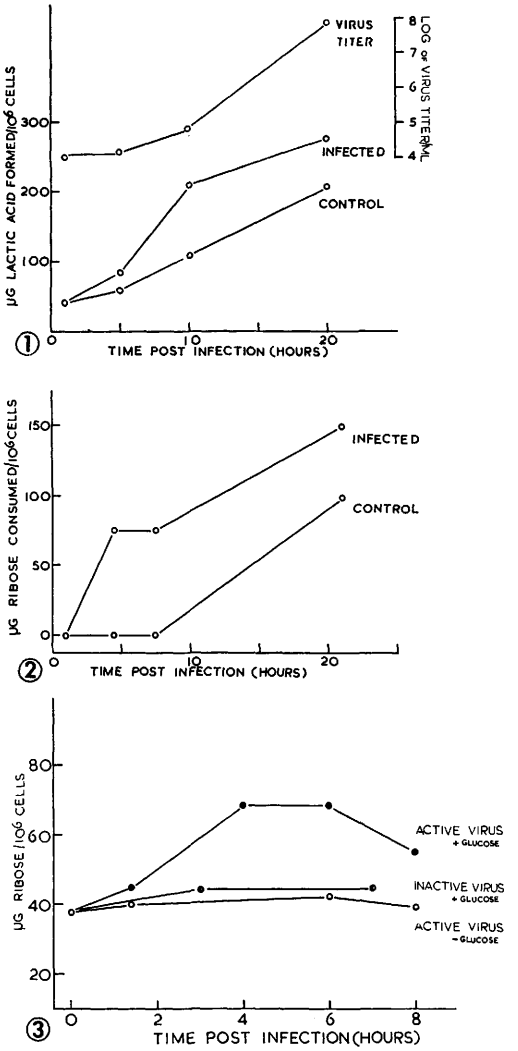

FIG. 1. Correlation between lactic acid formation and poliovirus production in human amnion cell cultures. Cell cultures incubated with poliovirus for 1 hr at 37°C, then washed with PBS to remove unadsorbed virus, Eagle's medium added and cells reincubated. Amount of virus was determined by inoculating tubes of amnion cells with 10-fold dilutions of the virus. See *Methods*.

FIG. 2. Ribose consumption by poliovirus infected amnion cells. Cell cultures were incubated with poliovirus for 1 hr at 37°C, then washed with PBS to remove unadsorbed virus. Eagle's medium containing ribose was added, and cells were reincubated.

FIG. 3. Increase of intracellular ribose content during poliovirus infection. Amnion cell cultures were incubated with poliovirus in Eagle's medium with or without glucose. At various intervals, cells were washed with PBS, released from the glass by combined versene and trypsin treatment, counted in a hemocytometer, hydrolysed with alkali, and ribose content determined.

TABLE III. Effect of Ribose and Pyruvate on Poliovirus Production by Amnion Cell Cultures.

Eagle's medium* containing:	Net increase of virus titer,† log/ml	P‡
Glucose	4.0 ± .0 §	
Ribose	2.5 ± .15	<.01
Pyruvate	1.88 ± .23	<.01
No carbohydrate	2.0 ± .24	<.01

* Cell cultures incubated with poliovirus for 1 hr at 37°C, washed with PBS to remove unadsorbed virus, Eagle's medium added, and cells reincubated. Results obtained after incubation for 20 hr.

† Log virus titer obtained by subtracting the initial virus titer in the culture fluid from the titer of virus reached 24 hr post-infection.

‡ Probability of obtaining a larger value of t by chance. Level of significance is $P < 0.05$.

§ Mean ± stand. error (S.E.) of mean. S.E. = $\sqrt{\frac{\Sigma d}{n(n-1)}}$ (Ref. 13).

medium containing ribose (instead of glucose), took up the sugar immediately and to a greater extent than uninfected controls (Fig. 2). However, despite the increased uptake of ribose by infected cells, the amount of virus was no greater than that produced by cells incubated in Eagle's medium without added carbohydrate (Table III). The virus yield was only 1-5% of that obtained from cells incubated in presence of glucose.

Effect of poliovirus on intracellular ribose synthesis. Infection with poliovirus stimulated intracellular ribose synthesis in cell cultures containing glucose. Formation of ribose began 2-4 hours after infection and continued for 6 hours, *i.e.*, up to the appearance of cytopathic changes, then the production of intracellular ribose decreased. Uninfected cells, or those incubated with heat inactivated poliovirus showed no change in intracellular ribose content. Ribose synthesis was not observed in infected cells incubated in Eagle's medium lacking glucose (Fig. 3).

Discussion. The metabolism of primary human amnion cell cultures is mainly glycolytic, as indicated by the conversion of 80% of the glucose consumed into lactic

●—●, infected cells in a medium containing glucose; ○—○, infected cells in a medium without added carbohydrate; ◒—◒, cells in a glucose containing medium incubated with heat inactivated virus.

acid. No lactic acid was formed by cells in the absence of glucose or in presence of ribose (instead of glucose). Infection of cell cultures with poliovirus stimulated lactic acid formation. Similar findings were made by Levy and Baron in poliovirus infected monkey kidney cell cultures(3). However, determination of the lactic acid formed is not sufficient to evaluate the metabolic pathways of the infected cells. The infected cells not only produced more lactic acid, but consumed much more glucose than uninfected controls. Thus, whereas in uninfected cells the ratio of lactic acid formed to glucose consumed is about 1.6, in infected cells it is only 0.9.

It was previously shown(2) that inhibitors of the oxidative pathways, *i.e.*, cyanide and azide, had no effect on poliovirus formation. We have shown in the present paper that pyruvate utilized aerobically by infected cells did not support virus synthesis. This affords an additional proof that oxidative pathways are not involved in virus formation.

The metabolic shift in the infected cells as shown by the low lactic acid:glucose ratio does not necessarily indicate an increase in oxidative metabolism. Rather, it is assumed that during infection glucose possibly takes part in metabolic processes which do not lead to lactic acid formation.

When ribose was substituted for glucose in the medium it was found to be incorporated by cells immediately after infection. Despite the stimulated ribose uptake, the virus yield was only 1-5% of that formed in presence of glucose, as much as in cells incubated in absence of any added carbohydrate (Table III). The findings that ribose cannot substitute for glucose in poliovirus production may perhaps be explained by not participating in energy yielding processes which lead to lactic acid formation (Table I). Darnell and Eagle(11) also showed that ribose could not support polio-virus replication in HeLa cell cultures. However, no data were represented by them to show whether ribose was utilized by these cells.

Determinations of intracellular ribose in infected cells showed an increase in ribose content during 6 hours and a decrease following the appearance of cytopathic changes in the cells. Ribose was formed only when glucose was present in the medium, whereas none was formed in its absence. The finding of increased intracellular ribose synthesis may be connected with the increased RNA synthesis in poliovirus infected HeLa cells as described by Ackerman(12).

Summary. 1. Infected amnion cells take up more glucose and produce more lactic acid than uninfected controls. However, the lactic acid:glucose ratio is 0.9 in infected and 1.6 in control cells. This finding indicates that poliovirus infected amnion cells use glucose through different metabolic channels than uninfected cells. 2. Both infected and uninfected cultures take up ribose from the medium, the infected cells being more active. The ribose consumed cannot supply the proper carbohydrate source for virus formation. Infected, but not uninfected cells, synthesize ribose, if glucose is present in the medium. 3. Both infected and uninfected cells take up pyruvate but its uptake is diminished following infection. Pyruvate cannot replace glucose for virus synthesis.

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