in these experiments, since the observed results with arginase were unexpected. Apparently the change in body weight, which may be related to nitrogen balance, affects both arginase and lactic acid dehydrogenase similarly, but shows less of the effects on arginine synthetase activity. This may be of importance to the overall protein economy of the animal as both arginase and lactic acid dehydrogenase do not appear to be limiting or pace setting enzymes in either the urea cycle or the glycolytic scheme. In contrast, arginine synthetase, which apparently is the limiting enzyme in the urea cycle and therefore probably the "pacemaker enzyme", increases during excessive protein breakdown regardless of the nutritional state of the animal, even when the other 2 enzymes show significant decreases in activity as during fasting. The synthetase also shows a greater percentage increase than arginase in all cases of increased protein catabolism, except after administration of glucocorticosteroids. Thus, arginine synthetase may be a better indicator of nitrogen catabolism than arginase. Further studies on the importance of nitrogen balance and hormonal dietary interrelationships are being conducted using more than one treatment per animal and using surgically altered animals.

Summary. All treatments increasing protein catabolism produced an increase in arginine synthetase activity. Arginase activity was increased only in cases where the increased protein breakdown was accompanied by an increase or maintenance of body weight. The changes in lactic acid dehydrogenase, used as a control enzyme, more nearly parallel those in arginase and show very little relationship to changes in arginine synthetase. It has been suggested that arginine synthetase would be a better indicator enzyme of nitrogen catabolism than arginase.

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Hemagglutinin Loss by Vaccinia Virus and Its Relationship to Virus Adaptation in Tumor and L Cells. (27216)

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An earlier report described how vaccinia virus strain IHD-E lost its hemagglutinin-producing ability on serial passage in the Ehrlich ascites tumor(1). This loss occurred along with a marked increase in ability of the virus to multiply in the tumor, and to cause lysis of the tumor cells. At the time, there was no way of determining how closely integrated the hemagglutinin loss was with the other concomitant changes in the virus. It became possible to examine this relationship when a hemagglutinin-negative virus was derived in an L cell culture system containing a hemagglutinin inhibitor from the Ehrlich tumor(2). The present report describes this examination.

Materials and methods. Viruses TF-1, TF-32, and TF-33 are respectively the first, thirty-second, and thirty-third passages of

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TABLE I. Virus Multiplication and Tumor Cell Destruction Following Inoculation of 5-Day-Old Tumors with 200 p.p.u. of TF-32 Virus.

Days after inoc.	Tumor age, days	Log of p.p.u. of virus/.05 ml of tumor	Total tumor cells $\times 10^8$ Control*	After infection with virus TF-32
3	8	1.8	9.7	9.6
4	9	2.2	9.9	10.2
5	10	2.9	14.8	14.7
6	11	3.6	15.2	16.4
7	12	4.2	15.7	14.5
8	13	4.1	14.3	14.7

* Control mice did not receive any virus.

vaccinia virus IHD-E through L cell cultures containing hemagglutinin inhibitor (ascitic tumor fluid)(2). In this series the virus became hemagglutinin-negative at the seventeenth passage. Viruses HS-1 and HS-33 represent the first and thirty-third passages of vaccinia virus IHD-E through L cells in a horse serum medium, which was the control medium in the study mentioned earlier(2). Both cell culture lines of virus originated from the same specimen of IHD-E virus.

In the analysis of the effect of virus TF-32 on the hyperdiploid Ehrlich ascites tumor, 200 pock-producing units (p.p.u.) of virus were inoculated into each of the 5-day-old tumors employed. Subsequently, 5 tumor-bearing mice were sacrificed daily for 8 days. Equal amounts of tumor from each daily sampling were pooled and used for virus assay and tumor cell counts.

Studies with L cells were initiated by inoculating 2000 p.p.u. of each type of virus per bottle of cells. The TF viruses were cultivated in cells grown in a medium with a tumorous ascitic fluid base, and the HS viruses were cultivated in a medium with a horse serum base(2). For assay purposes, equal volumes of fluid from 3 bottles of each kind of virus-infected culture were harvested and pooled daily. The sampled bottles were then discarded from the study.

Additional details concerning materials and methods have been published(1,2).

Results. The multiplication curve of virus TF-32 in the tumor was found to be identical with that reported earlier for the IHD-E virus(1). Total tumor cell counts over the 8-day period were also identical with those encountered following inoculation of the tumor with the IHD-E virus, and revealed no marked oncolytic action on the part of the TF-32 virus. The pertinent data are presented in Table I.

An additional aspect of the matter under discussion developed from a study on the multiplication of the HS and TF viruses in L cell cultures (Table II). The data draw comparisons between the multiplication of viruses HS-1, TF-1, HS-33, and TF-33. At the onset of serial passage there is no significant difference between the proliferation of the IHD-E virus in L cells growing in a horse serum or a tumor fluid medium. At the thirty-third passage in these media the 2 viruses still show comparable growth curves. There was, however, an increased adaptation to the L cell, as seen in the significant increase in the multiplication of both viruses. Comparison with virus from the first passage shows a 2-log increase in infectivity over the major portion of each growth curve.

Discussion. As previously described(1), although the IHD-E virus can be fully adapted to the Ehrlich ascites tumor by serial passage it is initially able to multiply in the tumor only to a low degree, and no oncolytic activity is seen unless very large doses of virus are employed. The original virus is referred to as IHD-E. The fully tumor-adapted virus is designated IHD-T. It is evident that the TF-32 virus acts in the tumor in a manner identical with the IHD-E virus, except that it does not produce hemagglutinin. This answers the question of how closely related hemagglutinin production is

TABLE II. Multiplication of Viruses HS-1, TF-1, HS-33, and TF-33 in L Cell Cultures.

Day	Log of p.p.u. per 0.05 ml of culture medium HS-1	TF-1	HS-33	TF-33
0	1.0	1.0	1.0	1.0
1	.9	1.0	1.7	1.6
2	1.0	1.0	2.2	2.0
3	1.2	1.5	2.8	3.0
4	2.0	2.6	3.8	4.5
5	2.2	2.8	4.5	4.9
6	3.0	3.0	4.9	5.0
7	3.3	3.9	5.0	5.3
8	4.0	4.3	5.0	5.3

with ability for maximum multiplication in the tumor, and oncolytic activity. Hemagglutinin-producing ability can be lost without the virus acquiring these two properties.

The L cell studies provide further evidence that the presence of hemagglutinin inhibitor, and not an increased ability to multiply in a host system, is the most important factor in determining hemagglutinin loss, and that as a result of prolonged passage in L cells both the HS-33 and TF-33 viruses showed a marked increase in ability to multiply in the L cell. The HS-33 virus, which was not exposed to hemagglutinin inhibitor, however, did not show a loss of hemagglutinin.

Summary. Following the observation that vaccinia virus became hemagglutinin-negative when fully adapted to the Ehrlich ascites tumor, the question arose as to whether or not the hemagglutinin loss was an intrinsic step in the adaptation process. In the present study it was possible to show that the loss of hemagglutinin by vaccinia virus and an increased ability to multiply in a host system are independent transitions, but may occur simultaneously.

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Uptake of Glucose, Amino Acids and Vitamins from a Medium by Human Amnion Cell Cultures Infected with Measles or Reovirus.* (27217)

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The only nutrients of the medium essential for synthesis of poliomyelitis virus in cell cultures have been shown to be glucose and glutamine in full-grown monolayer cultures of HeLa cells(1,2), and cystine and glucose in monkey kidney tissue cultures(3), while none are needed in concentrated suspensions of ERK cells(4). On the other hand, Eagle and coworkers(2,5) have shown that the nutritional requirements for poliomyelitis virus synthesis in HeLa cells depend on cell population density. If diluted cell suspensions are used in a medium containing glucose and glutamine as sole nutrients, rapid depletion of the free amino acid pool of the cells occurs and the capacity of the cells to synthesize the virus is greatly reduced(5). It is possible that a similar loss of amino acids may occur even with a complete medium if the nutrients are consumed during lengthy incubation of cell cultures such as is necessary for propagation of some viruses.

The present investigation is one of a series of studies dealing with the nutrients required in human amnion cell cultures for propagation of various viruses with relatively long incubation times. This report describes the changes in concentrations of glucose, amino acids and vitamins of the B-group in Eagle's minimum essential medium(5) in human amnion cell cultures infected with measles and reovirus.

Materials and methods. Cells. Human amnion cells of a continuous line, strain Utrecht, obtained from Dr. R. Doorschodt, Hygienisch Laboratorum der Rijks Universiteit, Utrecht, Holland, were grown in Roux bottles or Carrel flasks in a medium containing 20% calf serum and 0.5% lactalbumin hydrolysate in Hanks' solution. The growth medium was changed twice during the 6-day growth period. At the time of virus inoculation the number of cells was 25 to 30 million per Roux bottle containing 60 ml of medium, and 5 to 7 million per Carrel flask containing 10 ml of medium.

Cell culture medium. In all experiments where glucose, amino acids and vitamins of the B-group were determined, a single lot of Eagle's minimum essential medium contain-

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