the 111 million counts/minute injected. This result compares with a less sensitive earlier estimate of maximum catabolism of AIB in the rat of 0.02% (1).

Discussion. Although the test amino acid actually was present at very low concentrations, we believe that we should take the effective extracellular level entering the concentration process for most cells to be roughly 2 millimolar, representing the sum of the various neutral amino acids of plasma, since the concentrative process distinguishes only weakly among the neutral amino acids.

The foregoing effect of age was shown for a number of tissues in the rat, and undoubtedly is not limited to muscle in man. Upon stunting rats by calorie-restriction extent of tissue concentration associated itself more closely with body weight than with age(2). According to our interpretation, tissue "amino-acid hunger" is quantitatively associated with potentiality for growth or protein synthesis.

Summary. 1. Injected $C_1{}^{11}\alpha$-aminoisobutyric acid was much more strongly concentrated by skeletal muscle in children than in men. The greater general uptake by tissues could be recognized by the resultant consistently lower plasma level in the young. 2. The absence of catabolism of this amino acid was shown by observation of less than 0.002% of the injected C^{14} in expired air during 6 hours, counting respiratory CO_2 in the form of Li_2CO_3. After 24 hours average concentration in the body water was about 4 times that in the plasma water. A renal clearance of 56 ml of plasma per min was observed.

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Changes in Protein and Nucleic Acid Content on HeLa Cells Infected with Equine Abortion Virus.*† (24500)

DOROTHY J. MOORE‡ AND CHARLES C. RANDALL
Dept. of Microbiology, University of Mississippi School of Medicine, Jackson

Study of the effects of equine abortion virus (EAV) on hamster liver has been reported (1,2). In this host, a rapidly lethal hepatitis usually results in death of the animal within 18 hours and is characterized by very numerous conspicuous intranuclear inclusions. Sequential studies of the infection have shown increased RNA and protein in infected nuclei isolated in citric acid, DNA remaining constant. A search for a better defined, easily attainable and readily susceptible system with which to investigate further the nature of chemical changes induced in hamster liver cells infected with EAV led to the use of HeLa cultures and this adaptation has been reported recently(3). Investigators have utilized tissue culture to demonstrate certain changes in host cell due to virus infection. Kovacs(4) demonstrated marked changes in activities of 9 enzyme systems of poliomyelitis-infected Rhesus monkey kidney epithelium. Maassab,

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Loh, and Ackermann(5) reported a sustained high uptake of P^{32} by the nuclear fraction of HeLa cells infected with poliovirus, in which the quantities of nuclear RNA and DNA remained fixed. However, the net synthesis of cytoplasmic RNA was 2.5 x normal. Levy, Rowe, Snellbaker, and Hartley(6) noted a rapid and prolonged stimulation of cellular uptake of radioactivity of inorganic P^{32} and glycine-2-C^{14}, with no change in concentration of P, RNA, or DNA of adenovirus type 2-infected HeLa cells. In contrast Boyer, Leuchtenberger, and Ginsberg(7), by means of Feulgen microspectrophotometry, demonstrated an increase in DNA content of HeLa cells infected with adenovirus, types 1 to 4. The data of Newton and Stoker(8) show that HeLa cells infected with herpes after 3-4 days contain double the DNA content of normal cells and little or no RNA, yet number of cells obtained from infected cultures increased rather than diminished. The results reported here are part of a general study of the effect of equine abortion virus on susceptible cells. Certain changes in protein and nucleic acid content of the infected HeLa cell are here reported.

Methods. The virus used has been adapted to HeLa cells as noted above(3). *Tissue culture.* The strain of HeLa cells used has been adapted to horse serum in this laboratory for approximately 4 years. Stock cultures were grown in a medium consisting of 20% horse serum and 80% equal parts of mixture #199 of Morgan, Morton and Parker(9) and Earle's balanced salt solution (BSS). Subcultures were established in 250 ml serum bottles and fed every other day. Replicate cultures, 7 days of age, containing approximately 1 x 10^7 cells, were washed 3 times with BSS to eliminate horse serum. Two or more cultures were inoculated with 1 ml of virus suspension containing approximately 10^3 to 10^4 50% infectious doses (ID_{50}) and remained at room temperature for 30 minutes. Controls and infected cultures were incubated at 37° C with 10 ml of a 50-50 mixture of human ascitic fluid and BSS. After 48 hours the infected cultures apparently had undergone complete cytopathic change. Infected cells and maintenance mixture from one bottle of each passage were harvested, frozen and ground. The resulting viral suspension was stored at −50°C in sealed vials. Fresh cultures were inoculated from material of the previous passage. *Enumeration of nuclei.* Aliquots (1/10) of each sample were removed for nuclei counts utilizing the citric acid method of Sanford *et al.*(10). At least 2,000 nuclei were counted in every instance. *Chemical determination.* The remainder of sample containing a minimum of 10^7 cells was washed 4 times with physiological saline frozen and lyophilized. DNA, RNA and protein were determined in Beckman Model B spectrophotometer using matched silica cells. Methodology for the various chemical determinations has been cited(2). The various procedures were performed on extracts of whole cells rather than on nuclei isolated in citric acid, because of the possible loss of protein and RNA during citric acid isolation as reported by Kay *et al.*(11). Nucleic acids were extracted by the method of Schneider(12) with minor modifications as reported by Bracken and Randall(2). The important advantage of the Schneider procedure as discussed by Webb and Levy(13) is that no separation of DNA from RNA is required since determinations are based on characteristic color reaction for pentoses and desoxypentoses. Relative merits of a number of methods are cited in this review. Diphenylamine reaction of Dische(15) was used to estimate DNA content/cell. The results were compared with similarly tested controls prepared from purified DNA (Mann Laboratories). RNA was determined by the orcinol method of Mejbaum(14). The results were compared with similarly tested standards of commercial yeast nucleic acid (Mann Laboratories). All data were corrected for DNA interference according to Schneider(12) utilizing similarly tested DNA standards. Protein values were determined by a modification of the method of Robinson and Hogden(2). The protein content of whole cell preparations was compared with Armour's protein standard and crystallized edestin (Worthington Biochemical Lab.).

Results. The data are summarized and compared statistically in the accompanying

TABLE I. Comparison of Normal and Infected HeLa Cells.*

	Normal	Infected	Δ	P†
DNA	31.0	40.2	+ 9.2	<.01
RNA	40.3	41.0	+ .7	>.05
Protein	336.0	525.0	+189.0	<.01

* All values are expressed as mg $\times 10^{-8}$/cell.
† Probability of obtaining a larger value of t by chance. Level of significance is $P \leqq 0.05$.

Table. Values represent quantities/whole cell. It is interesting to note that whereas DNA and protein are increased significantly, total RNA is not changed during infection. The DNA and RNA control values for uninfected whole cells are similar to that for another type of tissue culture cell, the L cell, as reported by Kuchler, who employed similar methods(16). RNA content of normal HeLa cells is in agreement with the data of Newton and Stoker(8) but their DNA value is about ½ that reported here. The problem of evaluating data arrived at by different methods remains a pressing one.

It was of interest to determine whether any of the material pertinent to the investigation could be determined in the maintenance medium or wash fluid of normal and infected cells. Neither RNA or DNA could be detected in the maintenance fluid by the method employed nor protein in the final saline wash of the cells.

Discussion. From our results it is apparent that HeLa cells infected with EAV contain increased amounts of DNA and protein. The latter observation, to our knowledge, has not been reported previously.

Analyses on whole cells have the advantage that the constituents under study can be measured without loss or change that may occur in cell fractionation technics. However, by use of the latter method it would be interesting to compare nuclear and cytoplasmic RNA and protein. The mechanism of the protein increase is unexplained at present. The possibility obtains that during the process of infection altered cell permeability may permit increased amounts of protein from the nutrient fluid to enter the cell, or the change may be attributable to increased protein synthesis.

Relative to the nature of chemical changes induced by viruses, it does not seem wise to equate different viruses or different host cells to one another. However, it is of interest to note that biochemical changes induced by EAV differ in HeLa cells and hamsters. From data on hamster liver cell nuclei DNA apparently remains constant during all stages of infection whereas protein and RNA increase. Admittedly, these data rest on cell fractionation studies and it is possible that there is no overall increase of protein and RNA; at least there is an increase in nuclear content of these constituents. The nature of the increase in DNA relative to the infected HeLa cell, which is malignant, is not known.

As far as HeLa cell is concerned, a number of virus infections are associated with inclusions (adenovirus, EAV, herpes) and apparently result in the induction of increased amounts of DNA. On the other hand, Maassab, Loh and Ackermann(5) have demonstrated increase in cytoplasmic RNA in poliomyelitis-infected HeLa cells.

The question invariably arises as to the composition of the virus in question. The data do not permit an analysis of this problem. It is not likely that intracellular virus particles in themselves would be present in such number as to be detectable by the relatively gross chemical methods employed. The concurring remarks of Newton and Stoker(8) are of interest. Bracken(17) has reported recently an electron microscopy study of EAV in the hamster liver. The small aggregates of intranuclear virus particles are not equivalent to the large intranuclear inclusion visualized by the light microscope.

We have reported increase in certain cellular constituents. It is plausible to suggest that the increased materials are due to altered cell metabolism as a manifestation of virus infection. Elucidation of these phenomena await further investigation of the infected cell and its products.

Summary. HeLa cell cultures aged 7 days were infected with equine abortion virus, incubated for 48 hours and compared with control cultures as to content of RNA, DNA and protein. DNA and protein were elevated significantly, whereas RNA was unchanged. The observation that cell protein is increased as a

result of virus infection of a tissue culture system has not been reported previously.

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