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Received April 2, 1954. P.S.E.B.M., 1954, v86.

Intracellular Pattern of Nucleic Acid in Virus Infection.* (21085)

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(Introduced by Thomas Francis, Jr.)

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Upon infection with herpes virus, the heart and liver of chick embryos reveal an enlargement which results from an increased rate of cellular proliferation. This is paralleled by a markedly increased rate of nucleic acid synthesis such that the total nucleic acid of the liver may be 70% greater than normal(1). While these first observations were concerned with differences in the whole organ which were a result of the increased mass of the liver, the present paper deals with observations at the cellular level.

The amount of nucleic acid per cell has been estimated in normal and viral infected tissues. Nuclei isolated from herpetic liver have been analyzed for the nucleic acids, and data concerning the intracellular distribution of deoxyribonucleic acid and ribosenucleic acid have been obtained. Parallel observations have been made of the nucleic acid pattern of the Ehrlich ascites tumor infected with influenza virus.

Methods and materials. Virus. The herpes virus used was the Armstrong 1166 strain. It had undergone 54 passages in mouse brain and was adapted to the embryonate egg by 40 passages on the chorionic membrane(2). The WS strain of Type A influenza virus was used in the studies with the Ehrlich mouse carcinoma. This virus was maintained for several years by continuous passage in tissue culture and since has undergone 102 passages in mouse brain. It will multiply both in the brain and lungs of mice. After adaptation to the Ehrlich ascites tumor, it has been passed serially in that tissue 40 times(3). *Tissue.* The Ehrlich mouse carcinoma was adapted to ascitic form by Dr. G. A. LePage and in this laboratory has undergone 60 passages in the ascitic form in Swiss white mice (Webster strain). *Fractionation.* A 20% homogenate of liver was prepared in 0.25 M sucrose containing .0018 M $CaCl_2$. The disruption of the tissue was achieved with the Potter-Elvehjem glass homogenizer. The resulting preparation was diluted to 4% and fractionated essentially by the procedure of Hogeboom and Schneider(4). A nuclear and a cytoplasmic fraction were obtained. The latter contained the soluble proteins as well as the mitochondria and microsomes. All operations were performed at temperatures

* These studies were aided by a grant from the National Foundation for Infantile Paralysis.

TABLE I. Nucleic Acid Content per Cell of Normal and Virus Infected Chick Liver.

	No. nuclei* $\times 10^{-7}$	NA-P $\times 10^{7}$/cell‡	DNA-P $\times 10^{7}$/cell‡	RNA-P $\times 10^{7}$/cell‡	$\frac{\text{DNA-P}}{\text{RNA-P}}$
Normal†	33.4 ± 1.3	16.8 ± .8	4.4 ± .5	12.4 ± .8	.35
Infected†	34.2 ± 1.5	16.7 ± 1.2	4.3 ± .6	12.4 ± .7	.35

* No. of nuclei in 400 mg moist liver.
† Each value (± stand. dev.) is the average of 7 determinations.
‡ Calculated on assumption that there is 1 cell per nucleus and expressed as μg of nucleic acid phosphorus per cell.

between 4° and 0°C. *Nuclei counts.* To determine the number of nuclei per unit weight of liver, suitable dilutions of a 20% homogenate of tissue were made in 1% citric acid. The nuclei hardened by citric acid were then counted in a hemocytometer. *Nucleic acids.* Deoxyribonucleic acid (DNA) and ribosenucleic acid (RNA) were determined by the procedure of Schneider(5). The RNA was calculated from the difference between the total nucleic acid and the DNA. Phosphate was estimated by the modified Fiske and Subbarow method(6).

Results. Nucleic acid of normal and herpes infected livers. Inoculation of an adapted strain of herpes simplex virus on the chorionic membrane of an egg is followed by viral infection not only of the membrane but also of the liver, heart, and brain of the embryo. Because of the size of the organ, the extensive lesions obtained, and the ease of cellular fractionation, the liver was chosen as a suitable material for these studies. Eighteen 12-day-old eggs were inoculated via the chorionic membrane with 0.2 ml of herpes virus of titer $10^{-5.5}$ ID_{50} for eggs. After incubation for 3 days at 38°C, the eggs were opened and the livers of the embryos were removed. In all cases the infection had spread to the embryo and there was evidence of extensive hepatic lesions. A control group of eggs which had not been infected was treated in like fashion. From the harvested tissues, 20% homogenates were prepared in 0.25 M sucrose which contained .0018 M $CaCl_2$. Suitable dilutions of the 20% homogenates were made in the solution of calcium and sucrose used originally to prepare the homogenates. For each procedure 2 aliquots of the diluted homogenate were taken in order to obtain duplicate values. The number of nuclei per ml of homogenate was determined. Each gram of moist embryonic liver contains approximately 80 x 10^7 nuclei. The count per unit weight of moist tissue is slightly but consistently greater in the infected tissue. The results are in agreement with previous determinations(1). With this value for the number of nuclei and the analysis of other aliquots for nucleic acid, the amounts of DNA and RNA per cell were calculated.

The data from 7 similar experiments were combined and recorded in Table I. On the average, these cells contained 16.8 x 10^{-7} μg of nucleic acid phosphorus which is distributed between RNA and DNA in 3:1 ratio. It is clear from the data of Table I that on the basis of the nucleic acid analyses the infected tissue is undistinguishable from normal liver. Within the limitations of the method, the total nucleic acid and the ratio of DNA to RNA are found to be unchanged by the infection which has produced extensive and visible alterations in the liver.

Intracellular distribution of nucleic acids. When infected tissue is subjected to controlled cellular disruption the major part of the herpes virus is found in the cytoplasmic fractions of the cell(2,7). The detection of viral nucleic acid in the cytoplasmic fractions by the methods employed here would not be expected. However, alteration in the intracellular distribution of nucleic acids might be anticipated from other considerations. If herpes virus is formed at some extranuclear site as postulated, the synthesis of viral nucleic acid in the cytoplasm or the transfer of nucleic acid from the nucleus to cytoplasm might be in excess of that found ultimately in the viral body. Since DNA is confined to the nucleus of the normal cell, and the other cellular fractions are essentially free of DNA,

TABLE II. Intracellular Distribution of Nucleic Acids in Normal and Virus Infected Chick Liver.

Preparation*	Normal DNA-P mg†	Normal RNA-P mg†	Infected DNA-P mg†	Infected RNA-P mg†
Whole homogenate	.15	.40	.14	.37
Nuclei	.14	.26	.13	.24
Supernate‡	.01	.15	.01	.14

* Each preparation is equivalent to 400 mg moist liver.

† Nucleic acid phosphorus in mg/preparation.

‡ Fraction contains soluble cytoplasmic elements, mitochondria and microsomes.

the nuclear and cytoplasmic fractions of normal and infected tissues were separated and analyzed for both nucleic acids. The resulting data are recorded in Table II. Approximately one-third of the total RNA is found in the nucleus of the embryonic chick liver. Despite the nuclear aberrations seen in infected cells, the amount of nuclear DNA and RNA is unaltered, nor does one find abnormal amounts of contaminating DNA in the extranuclear fractions. The evidence of these experiments suggests that the development of herpes virus proceeds without any gross alterations in the amount of nucleic acid per cell, in its distribution between RNA and DNA, or in the distribution of nucleic acids within the major cellular fractions.

Nucleic acid of control and infected tumor cells. Since the cells of herpetic liver contain on the average 36% less mitochondria than normal liver cells, a minimum value of 36% can be set for the fraction of cells involved in an infected tissue. If, as one might suppose, the destruction of mitochondria in an infected cell is less than 100%, then the number of infected cells in a tissue may be considerably greater than 36% (3). However, comparisons of the type made here are always between a mixture of normal and infected cells on the one hand and normal cells on the other. Because it seemed that any metabolic alteration would be more readily demonstrated with a system containing a high degree of cellular infection, the host-virus system composed of influenza virus and the Ehrlich mouse ascites tumor was included in the study. Mice inoculated 7 days previously with 2 x 10^6 ascites tumor cells were infected with 0.1 ml of a preparation of adapted influenza virus. This inoculum had a titer in eggs of $10^{-7.5}$ ID_{50}. After 22 to 28 hours when the titer of the peritoneal fluid had reached a maximum, fluids containing cells were removed from infected and control groups of mice. The cells were removed from the fluid, washed and counted. Separate aliquots were analyzed for RNA and DNA. The amounts of these nucleic acids per cell were calculated and the resulting values are found in Table III. The proportions of DNA to RNA are much higher in these cells than in those of the chick liver. However, the comparison of normal and infected tissue is completely consistent with that found in Table I. In other experiments tumor tissue was removed from mice which were infected with influenza virus 42 hours previously and in which the ascites cells had begun to aggregate and form solid tumors. To obtain comparable control material, a few uninfected mice were chosen which had solid tumors in the peritoneal cavity. The amounts of nucleic acids per gram of moist tissue were determined in each case and the resulting values presented in Table III. Again it will be noted that the pattern and distribution of nucleic acid between RNA and DNA are the same in control and infected tissue. These data with influenza virus like those from the herpetic infection indicate no measurable change in the amount of RNA or DNA per cell that is induced by infection with influenza virus.

Discussion. Subsequent to infection of the chick liver with herpes virus, several changes are detectable There is a loss of mitochondrial material and a relative change in the abilities of the tissue to oxidize succinic and α-ketoglutaric acids(1). Despite these alterations, the static pattern of the nucleic acids seems to remain fixed as the transformation of the normal cell to a viral producer is accomplished (Table II). The rather large increase (70%) in the rate of nucleic acid synthesis by the liver, as reported previously, must result entirely from the accelerated cellular proliferation(1). The increased production of liver nucleic acid is paralleled by an increased number of cells which on the average contain the normal amount of nucleic acid.

No unnatural amount of nuclear material

TABLE III. Nucleic Acid Content of Control and Influenza Infected Ascites Tumor Cells.

Tissue	Cells/ml $\times 10^{-6}$	NA-P* $\times 10^6$/cell	DNA-P* $\times 10^6$/cell	RNA-P* $\times 10^6$/cell	DNA-P / RNA-P
Control†	19.7 ± 3.5	5.5 ± .2	2.4 ± .0	3.1 ± .2	.79
Infected‡ 22-28 hr	21.8 ± 4.6	5.3 ± .4	2.2 ± .2	3.2 ± .2	.69

Tissue	—	NA-P mg/g§	DNA-P mg/g§	RNA-P mg/g§	DNA-P / RNA-P
Solid tumor control	—	.97	.45	.52	.87
Solid tumor infected 42 hr	—	1.00	.47	.52	.90

* Nucleic acid phosphorus expressed in μg per cell.
† Each value (± stand. dev.) is average of 2 determinations.
‡ " " " " " " 3 " .
§ mg of nucleic acid phosphorus per g of moist tissue.

(DNA) is found with the cytoplasm, as might be expected if fragmentation of the nucleus were a major feature of infection. These data, as well as those of other recent studies(8), support the concept of a stable structural integrity including the external cellular membrane which is maintained throughout the period of viral development and liberation. Apparently the subtle metabolic injuries which must occur are manifest only later in a general dissolution of cellular structure.

It should also be noted that, while there are aberrations in the nucleus (inclusion bodies) which are Feulgen staining in infected cells, this abnormality was not detectable by the chemical analyses for DNA and RNA (Tables I and II). Quite possibly this finding is an inevitable result of the limitations of the experiments which lie in the grossness of the methods, and measurements of the dynamic state of the nucleic acids might be much more rewarding. However, as yet, there is no clear evidence that the nuclear bodies contain significant amounts of infectious virus, and the nature and origin of these structural changes remain unexplained. That the nuclear aberrations appear close upon the initiation of infection is an extremely interesting observation (9), but does not allow one to decide whether they are developmental forms of the virus or secondary cellular injury(2).

Summary. The nucleic acid content per cell of chick livers was found to be unaltered as a consequence of infection with herpes virus. The distribution of the nucleic acid between ribosenucleic acid and deoxyribonucleic acid is the same in normal and infected tissues. The intracellular distribution of nucleic acids was determined and the data suggest a remarkable stability of the nucleus of the infected cell. Within the extent of the investigation, the nucleic acid pattern of the Ehrlich ascites tumor after infection with influenza virus was found to parallel that of the herpetic liver cell.

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Received April 21, 1954. P.S.E.B.M., 1954, v86.