are specific for acid-fast materials is recommended for the study of the membranes formed in lipid pneumonia where unsaturated lipids are suspected and for other familiar lipo-protein combinations, as more sensitive than the brightfield microscopy with Ziehl-Neelsen staining.

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Some Biochemical Aspects of Herpes Infection.* (17829)

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Inoculation of an adapted strain of herpes simplex virus on the chorio-allantoic membrane of an egg is followed by viral growth not only in this membrane but also in the liver, heart, spleen, and brain of the embryo (1). The pathology and histology of the herpetic infection of these tissues has been described by Anderson(1) while the pathology of the same infection in tissue culture has been given by Rivers, Haagen and Muckenfuss(2). This work is a preliminary study of some of the biochemical changes which characterize the host tissue during the course of this viral infection. It was from the viewpoint of learning the nature of the alterations which take place in the metabolism of the infected tissues that measurements were made: of the rate of growth of the tissues; of two of the respiratory enzyme systems; of changes in the nucleic acid composition; and of the protein content. The results of these findings and some of their significance are discussed in the following.

Experimental. In all experiments the Armstrong 1166 strain of herpes simplex virus was used. Previously it had undergone 54 passages in mice and 37 passages in eggs. The inoculation was made on the chorio-allantoic membrane of 12-day-old embryonated eggs. The tissues were harvested after 3 days and enzyme determinations were made on pooled samples of this moist tissue. Other pooled samples were frozen and brought to dryness *in vacuo*. These desiccated samples were used for nucleic acid and nitrogen determinations.

Methods. The protein nitrogen was determined on samples of tissue which were precipitated from an aqueous suspension with trichloroacetic acid and washed repeatedly with alcohol and ether. The nitrogen was measured by the Koch-McMeeken modification of Nessler's method(3). To determine the number of nuclei per unit weight of liver, a 10% homogenate of the tissue was prepared in 1.0% citric acid(4). The citric acid hardened nuclei were stained with methyl green and counted in a hemocytometer after appropriate dilution. The phosphate was determined by the modified Fiske and Subbarow procedure(5). Succinoxidase determinations were made using the method of Potter and Schneider(6). The α-ketoglutaric acid oxidase activity was measured by the method described by Ackermann(7). The desoxyribosenucleic acid (DNA) was determined by both the Schmidt-Thannhauser(8) and by the Schneider(9) procedures. The

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TABLE I.
Effect of Herpes Infection on Mass of the Embryo, Embryonic Liver, and Embryonic Heart.

Tissue	No. of embryos	Liver wt, mg Dry	Liver wt, mg Moist	Heart wt, mg Dry	Heart wt, mg Moist	Embryo wt, g Moist
Normal	18-43	34.4	172	12.0	88.3	11.46
Viral	17-36	47.2	245	14.6	118	10.20

TABLE II.
Nitrogen and Moisture Content of Embryonic Tissues Infected with Herpes Virus.

Tissue	No. of embryos	Total* N	Protein* N	Moisture†
Normal Heart	18	11.0	10.2	86.8
Herpetic Heart	17	10.8	10.0	87.4
Normal Liver	22	11.6	10.2	79.5
Herpetic Liver	22	11.8	10.9	80.8

* % of dry weight.
† % of moist weight.

TABLE III.
Succinoxidase and α-Ketoglutaric Acid Oxidase Activities of Embryonic Tissues Infected with Herpes Virus.

Tissue	No. of embryos	Succinoxidase Qo_2*	Succinoxidase Total organ†	α-Ketoglutaric oxidase Qo_2*	α-Ketoglutaric oxidase Total organ†
Normal Heart	22	12.1	53.5	8.0	36.2
Viral Heart	21	8.8	51.3	6.3	36.5
Normal Liver	18	16.9	154	9.6	87.7
Viral Liver	17	17.6	234	6.9	91.5

* Microliters oxygen per 20 mg moist tissue per 10 min.
† Microliters oxygen per total organ per 10 min.

pentosenucleic acid (PNA) was determined by Schneider's method(9).

Results. Effect of virus on growth. Three days after inoculation of herpes virus, the moist weights of liver and heart are 42 and 34% greater, respectively, than the organs of normal embryos (Table I). These results are in agreement with the findings of Anderson(1). However, in addition to these gross observations, analyses were made which revealed no significant differences in the per cent moisture, protein-nitrogen or total nitrogen of these infected and normal organs (Table II). Furthermore, the number of nuclei per gram of tissue is essentially the same in both normal and infected livers (Table III).

This indicates that after inoculation with herpes, there is an increase in the number of cells, in the dry weight, and in the total protein of the infected organs. In the case of liver it represents approximately a 40% increase in the total protein nitrogen. This accelerated growth rate occurs despite the fact that the total weight of the infected embryo is decidedly less than that of the normal embryo (Table I). The infected tissues appear to develop more rapidly at the expense of the rest of the chick.

Effect of virus on respiratory enzymes. Since the amount of functioning liver available to the embryo is proportionately larger after herpes infection, it was of interest to obtain some measure of the metabolic rate of the organ under these conditions. The activity of some of the respiratory enzymes of the Krebs' cycle has been shown to be lower in regenerating liver(10), and tumor tissue (6,11,12) than in the corresponding normal

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TABLE IV.
Nucleic Acid and Nuclei Content of Normal and Herpetic Embryonic Liver.

Tissue	No. of embryos	Nuclei per g liver*	DNA mg/g tissue†	PNA mg/g tissue†
Normal Liver	18	760×10^6	10.8	29.3
Herpetic Liver	18	863×10^6	13.4	36.2

* Moist wt of tissue.
† Dry wt of tissue.

tissues. The correlations previously reported between changes in the rate of growth of a tissue and the concentration of these respiratory enzymes, suggested the investigation of possible changes in the rate of oxidation of succinic and α-ketoglutaric acids. Our results show that while the succinoxidase activity is not significantly different in the infected and normal livers, there are differences in the succinoxidase activity of normal and herpetic heart tissues. The infected heart was found to have 27% less activity per unit of moist weight than the control tissue (Table III). The α-ketoglutaric oxidase activity per unit of moist weight of infected heart and liver were both found to be lower than that of normal organs by 21 and 29% respectively (Table III).

Effect of virus on nucleic acids. While the total protein of the host cell appeared to be unaffected by the virus infection, the results of the enzyme assays suggest that there might be profound changes in some of the functional proteins which occur in small amounts. Thus to obtain some information concerning the alteration in nucleoproteins, analyses for desoxyribosenucleic acid (DNA) and pentosenucleic acid (PNA) were made. The results of Table IV indicate that there is an increase of 24% in the total concentration of nucleic acid (NA) per unit of dry tissue. The increase in NA is the result of an increase in both DNA and PNA. Thus there is no appreciable change in the PNA/DNA ratio. The propagation of herpes virus appears to induce a marked stimulation in the nucleic acid synthesis of the host cell since the concentration of NA not only increases, but the total mass of the liver increases.

Discussion. Anderson describes herpetic hearts as being edematous, necrotic, enlarged and with lesions. The cells are swollen and the nuclei enlarged. The degree of inflammatory reaction appears to vary with the amount of necrosis produced by the infection (1). In tissue culture Rivers *et al.* find that there is a thickening of the epithelium, giant amitotic cells, and swollen cells(2). These findings would indicate that in the embryo the enlargement of the infected organs is due to an inflammatory reaction and edema. Our observations indicate that the enlargement of the liver and heart is due to an accelerated rate of proliferation which accompanies the herpes infection. This is borne out by the facts that the mass of the herpetic liver is greater than that of the normal, while the percentage composition is the same with regard to moisture, protein nitrogen and total nitrogen. In addition, the number of nuclei found per unit moist weight of tissue is approximately the same in viral and normal liver. There are simply more cells, protein and dry weight in the former than the latter.

The amount of functioning liver and heart in the herpetic embryo is proportionately larger than in the normal because firstly, these organs are larger and secondly, the embryo is smaller. However, the α-ketoglutaric oxidase activity per unit weight of heart and liver was found to decrease to such an extent that the calculated activities of both the total enlarged heart and enlarged liver were no greater than the total activities of the smaller normal organs. This is also true of the succinoxidase activity of the heart. Thus in the herpetic organs there is some loss of metabolic activity which appears to be compensated for by an increase in the size of the organ. The succinoxidase activity per unit weight of liver, however, does not

decrease; hence, the total liver succinoxidase available to the embryo increases by nearly 50%.

It is believed that both of these enzymes are localized in liver in the mitochondrial particles of the cell(13,14,15). A measure of their activity in the assay procedures used requires the presence of intact mitochondria in the homogenate. Thus if the loss of α-ketoglutaric oxidase activity in the cell were due to necrosis of the cell and mitochondrial particles, one would expect an equal loss in both respiratory enzymes. The loss of α-ketoglutaric acid oxidase activity relative to the succinoxidase would appear to be a specific effect representing a change in the enzyme pattern of the tissue. The possibility remains that the change in activity might not be in the dehydrogenase enzymes proper but in some part of the hydrogen transport mechanism (which they do not use in common).

One of the most striking changes accompanying the herpetic infection is the accelerated rate of nucleic acid synthesis. The total nucleic acid of the herpetic liver when calculated on the basis of the total dry weight is 70% greater than normal.

There is little change in the ratio of DNA to PNA. If this has any significance with regard to the composition of the virus, as suggested by other workers(16,17,18), it would be that herpes virus contains both DNA and PNA. However, it has also been reported that during that period of embryonic development when protein metabolism is most conspicuous, there is a rise in the concentration of PNA and DNA(19). Thus, the rise in NA might as easily be correlated with the accelerated growth rate of the infected liver.

Summary. 1. A description is given of some of the biochemical changes which occur in tissues infected with herpes virus. 2. Evidence is presented which indicates that herpes virus causes accelerated proliferation in the host cells of embryonic liver and heart. 3. The propagation of the herpes virus appears to induce a marked stimulation in the nucleic acid synthesis of the host cell. 4. It is shown that in herpetic liver there is a change in the normal ratio of succinoxidase to α-ketoglutaric acid oxidase activity. Herpetic infection of the heart is accompanied by a decrease in succinoxidase and α-ketoglutaric acid oxidase activities.

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Response of Man to Egg-Adapted Colorado Tick Fever Virus.* (17830)

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In a previous note(1) the results of immunization of 4 human volunteers with an egg-adapted strain of Colorado Tick Fever

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