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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Received April 10, 1950. P.S.E.B.M., 1950, v74.

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

HILARY KOPROWSKI, HERALD R. COX, MABEL STEWART MILLER, AND LLOYD FLORIO.
*From the Section for Viral and Rickettsial Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., and from the University of Colorado School of Medicine, Denver, Colo.*

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

* The authors wish to express their grateful appreciation to the volunteers who participated in this study, and to Miss Maja Platzer for her helpful suggestions in the preparation of the manuscript.

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TABLE I.
Presence of Neutralizing Antibodies in Sera of Volunteers Inoculated with Egg-adapted Colorado Tick Fever Living Virus.

| No. | Serum specimen Days post-inoculation | Mortality ratio of mice inoculated intracerebrally with serum-virus dilutions: 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 | 10-7 | $LD_{50}$ virus titer | $LD_{50}$ doses neutralized |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | | 6/6 | 3/5 | 2/6 | 0/6 | | | 10-3.45 | 140* |
| | 15 | 6/6 | 4/6 | 2/6 | 0/6 | | | | 10-2.50 | 1260 |
| | 31 | 5/6 | 5/6 | 5/6 | 2/6 | 0/6 | | | 10-3.40 | 160 |
| | 45 | 6/6 | 3/6 | 1/6 | 0/5 | | | | 10-2.15 | 2820 |
| 2 | 0 | | | 6/6 | 6/6 | 6/6 | 1/6 | 0/6 | 10-5.60 | 0 |
| | 15 | | 6/6 | 5/5 | 3/6 | 0/5 | 0/5 | | 10-4.00 | 40 |
| | 45 | | 4/4 | 4/6 | 0/5 | 0/4 | 0/6 | | 10-3.25 | 225 |
| 3 | 0 | | 6/6 | 4/5 | 0/6 | 0/6 | 0/6 | 0/6 | 10-3.40 | 160* |
| | 15 | 6/6 | 6/6 | 2/6 | 1/6 | 0/6 | 0/6 | | 10-2.90 | 500 |
| | 31 | 6/6 | 4/6 | 4/6 | 0/6 | 0/6 | | | 10-3.00 | 400 |
| | 45 | 6/6 | 3/5 | 0/6 | 0/5 | 0/6 | | | 10-2.20 | 2510 |
| 4 | 0 | | | 6/6 | 5/5 | 5/6 | 1/5 | 0/6 | 10-5.60 | 0 |
| | 15 | | 6/6 | 5/5 | 3/6 | 1/5 | 0/6 | | 10-4.50 | 15 |
| | 31 | 6/6 | 6/6 | 3/5 | 1/6 | 0/5 | 0/5 | | 10-3.30 | 200 |
| | 45 | 5/5 | 1/6 | 0/6 | 0/6 | 0/6 | | | 10-1.60 | 10000 |
| 10 | 0 | | | | 6/6 | 6/6 | 2/6 | 2/6 | 10-6.00 | 0 |
| | 15 | | 5/6 | 6/6 | 6/6 | 1/6 | | | 10-4.50 | 30 |
| | 47 | | 6/6 | 6/6 | 4/6 | 1/6 | | | 10-4.35 | 45 |
| 12 | 0 | | | | 6/6 | 4/6 | 0/6 | 0/6 | 10-5.25 | 0 |
| | 15 | | 6/6 | 6/6 | 6/6 | 2/6 | | | 10-4.75 | 3 |
| | 47 | | 6/6 | 5/6 | 1/6 | 1/6 | | | 10-3.55 | 50 |
| 15 | 0 | | | | 6/6 | 5/6 | 0/6 | 0/6 | 10-5.40 | 0 |
| | 15 | | 6/6 | 5/6 | 5/5 | 0/6 | | | 10-4.40 | 10 |
| | 30 | | 6/6 | 6/6 | 5/6 | 0/6 | | | 10-4.40 | 10 |
| | 54 | | 6/6 | 4/6 | 1/6 | 0/6 | | | 10-3.35 | 110 |
| 17 | 0 | | | | 6/6 | 6/6 | 2/6 | 1/6 | 10-5.80 | 0 |
| | 15 | | 5/6 | 6/6 | 5/7 | 1/6 | | | 10-4.30 | 30 |
| | 30 | | 3/5 | 1/4 | 6/6 | 0/6 | | | 10-4.10 | 50 |
| | 54 | | 2/4 | 0/6 | 0/6 | 0/6 | | | 10-2.00 | 6310 |

* Based on average $LD_{50}$ virus titer of $10^{-5.6}$ in normal serum.

(CTF) virus were reported. In view of the good immunological response obtained in the inoculated individuals and because of the relative innocuousness of the procedure, it was decided to repeat the experiments with a larger group of volunteers.

*Strain of virus.* The Florio egg-adapted strain of CTF virus was employed(1-3). The inoculum was prepared in the same manner as described previously(1). One batch represented the 37th egg-passage(1) and the other the 74th egg-passage of the virus. Both batches were distributed in lyophilized form. At the time of inoculations enough physiological salt solution was added to make a 10% concentration of the embryo suspension. Prior to inoculation both preparations were tested for their immunogenic potency in mice (1) and were found to give a high degree of protection to mice challenged subsequently with mouse-brain adapted virus. Twenty individuals, 17 males and 3 females 21 to 60 years of age, volunteered for the inoculations. Individuals 1 to 12 received a subcutaneous injection in the region of the biceps muscle of 1.0 ml of a suspension representing the

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TABLE II.
Neutralization Tests on Sera of Volunteers Inoculated with Egg-adapted Colorado Tick Fever Living Virus.

| No. | Serum specimen Days post-inoculation | Mortality ratio of mice inoculated intracerebrally with serum-virus dilutions: 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 | 10-7 | $LD_{50}$ virus titer | $LD_{50}$ doses neutralized |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 0 | | | | 5/5 | 5/5 | 1/4 | 0/6 | $10^{-5.65}$ | 0 |
| | 47 | 6/6 | 6/6 | 4/6 | 1/5 | | | | $10^{-3.35}$ | 200 |
| 6 | 0 | 5/5 | 6/6 | 2/6 | 1/5 | 0/6 | | | $10^{-2.85}$ | 1415* |
| | 48 | 3/4 | 2/6 | 1/6 | 0/6 | | | | $10^{-1.75}$ | 17780 |
| 7 | 0 | | | | 6/6 | 4/6 | 0/6 | 1/5 | $10^{-5.35}$ | 0 |
| | 47 | 5/5 | 4/6 | 4/6 | 0/6 | | | | $10^{-3.00}$ | 225 |
| 8 | 0 | | | | 6/6 | 6/6 | 4/6 | 2/6 | $10^{-6.50}$ | 0 |
| | 48 | | 6/6 | 6/6 | 4/6 | 1/6 | 0/5 | | $10^{-4.35}$ | 140 |
| 9 | 0 | | | | 6/6 | 6/6 | 4/6 | 2/6 | $10^{-6.50}$ | 0 |
| | 47 | | 6/6 | 5/5 | 1/6 | 0/6 | 0/6 | | $10^{-3.60}$ | 795 |
| 11 | 0 | | | 5/5 | 6/6 | 2/6 | 0/5 | | $10^{-4.75}$ | 0 |
| | 47 | 6/6 | 5/6 | 1/6 | 0/6 | | | | $10^{-2.50}$ | 175 |
| 13 | 0 | | | | 6/6 | 6/6 | 2/6 | 1/6 | $10^{-5.90}$ | 0 |
| | 56 | | 5/6 | 5/6 | 1/6 | 0/6 | | | $10^{-3.40}$ | 315 |
| 14 | 0 | | | | 6/6 | 6/6 | 4/6 | 1/6 | $10^{-6.35}$ | 0 |
| | 54 | | 6/6 | 4/6 | 0/6 | 0/6 | | | $10^{-3.25}$ | 1260 |
| 16 | 0 | | | | 6/6 | 6/6 | 4/6 | 0/6 | $10^{-6.25}$ | 0 |
| | 54 | | 6/6 | 4/6 | 2/6 | 1/6 | | | $10^{-3.60}$ | 445 |
| 18 | 0 | | | | 6/6 | 6/6 | 5/6 | 2/6 | $10^{-6.65}$ | 0 |
| | 55 | | 6/6 | 6/6 | 3/6 | 0/6 | | | $10^{-4.00}$ | 445 |
| 19 | 0 | | | | 6/6 | 6/6 | 3/6 | 2/6 | $10^{-6.30}$ | 0 |
| | 54 | | 5/6 | 2/6 | 0/5 | 0/6 | | | $10^{-2.60}$ | 5010 |
| 20 | 0 | | | | 6/6 | 5/5 | 2/6 | 0/6 | $10^{-5.75}$ | 0 |
| | 54 | | 6/6 | 3/6 | 2/6 | 0/6 | 0/6 | | $10^{-3.30}$ | 280 |

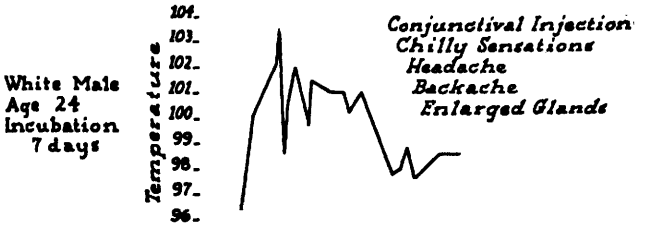
* Based on average $LD_{50}$ virus titer of $10^{-6.00}$ in normal serum.

37th egg-passage, whereas individuals 14, 16, 19 and 20 were given subcutaneously 1.0 ml and volunteers 13, 15, 17 and 18 received 0.1 ml intradermally of the 74th egg-passage material. Blood specimens were secured from all of the individuals prior to and at various intervals after the inoculations.

*Neutralization tests.* Serum samples obtained from the individuals before and after inoculations were submitted to neutralization tests against mouse-brain adapted Condon(2) strain of CTF virus. The only deviation in the technique described previously(1) was that the serum-virus mixtures were incubated for 26 hours prior to inoculation in mice instead of 2 and 26 hours.

*Results. Clinical observations.* Of the 12 individuals (1 to 12) who were inoculated with the 37th egg-passage material, 2 (nos. 3 and 6, Tables I and II) had a history of a prior laboratory infection with CTF virus(4) and one (no. 1, Table I) had lived in an endemic area for many years. These 3 individuals showed no clinical reaction whatsoever to the inoculation. The remaining 9 individuals in this group developed enlarged, tender axillary lymph glands on the side of the injection which subsided within 2 to 7 days after inoculation. Four of these volunteers had a mild attack of an illness which

4. Florio, L., Stewart, M. O., and Mugrage, E. R., *J. Exp. Med.*, 1944, v80, 165.




| Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Leucocytes in Thousands per c.mm. | 6.55 | 3.63 | 3.08 | 4.08 | 4.65 | | 7.22 |
| Segmenters % | 58 | 39 | 29 | 24 | 37 | | 47 |
| Band Forms % | 10 | 30 | 25 | 23 | 15 | | 4 |
| Lymphocytes % | 19 | 24 | 36 | 38 | 39 | | 45 |
| Monocytes % | 11 | 7 | 10 | 14 | 8 | | 3 |
| Eosinophils % | 1 | | | 1 | 1 | | 1 |
| Basophils % | 1 | | | | | | |

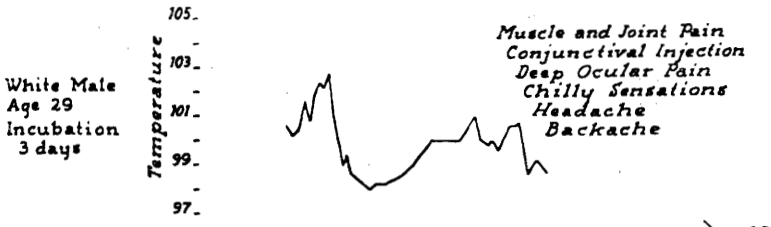
FIG. 1.
Clinical findings in a volunteer inoculated with the egg-adapted strain of Colorado tick fever virus.

lasted 2 to 5 days and resembled CTF except for the following aspects. The onset was 6 to 7 days after inoculation, as compared to 3 to 4 days in the individuals infected with "human" or "hamster" passaged strains of CTF virus(4). There was a single instead of a dual episode of illness and the onset and disappearance of symptoms were gradual, rather than relatively abrupt. In each of the 4 individuals a low white blood cell count and differential blood picture characteristic of CTF was found(4). Three of the volunteers (nos. 2, 4 and 8), 2 of whom had reacted with enlarged lymph glands and 1 who had become ill after the inoculation, were submitted to intravenous challenge inoculations with a human strain of CTF virus 10, 6 and 6 months, respectively, after inoculation with the egg-passage material. No signs of illness were noted in any of these 3 individuals following the challenge inoculation with the human strain.

In the second group of 8 volunteers (13 to 20) inoculated with the 74th egg-passage virus, all became ill after an incubation period of 6 to 7 days, and 7 had enlarged lymph glands near the site of injection. The disease lasted from 3 to 4 days and again only a single attack occurred in contrast to the typical CTF infection(4,5). As in the previous group, the onset and subsidence of symptoms were gradual but the course of the disease seemed to be more severe, particularly in those volunteers who had been inoculated by the intradermal route. Fig. 1 depicts the clinical findings in a volunteer of this group which may be compared to the findings in Fig. 2 of a typical case of naturally acquired CTF infection. Volunteers 13 and 19 (Table II) were challenged by the intravenous route with a human strain of CTF virus 3½ and 5 months, respectively, after immunization with the egg-passage virus. As with the 3 individuals challenged in the first group of volunteers, no signs or symptoms of illness were observed following the challenge inoculation.

*Serological data.* In order to determine, on a preliminary basis, when the highest level of circulating antibodies would be encountered

5. Cox, H. R., Colorado Tick Fever, Chap. 27, Viral and Rickettsial Infections of Man, edited by T. M. Rivers. J. B. Lippincott Co., Philadelphia, 1948.




| Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|
| Leucocytes in Thousands per c.mm. | 8.30 | 3.23 | 4.65 | 3.40 | 4.23 | 2.30 | 2.55 | 2.88 | 3.40 | 7.30 |
| Segmenters % | 58 | 59 | 25 | 21 | 25 | 19 | 19 | 15 | 16 | 39 |
| Band Forms % | 4 | 11 | 20 | 20 | 22 | 32 | 37 | 28 | 31 | 6 |
| Lymphocytes % | 31 | 18 | 44 | 47 | 40 | 40 | 37 | 45 | 45 | 51 |
| Monocytes % | 2 | 10 | 11 | 11 | 12 | 7 | 7 | 12 | 6 | 3 |
| Eosinophils % | 4 | 2 | | 1 | 1 | 1 | | | 2 | 1 |
| Basophils % | 1 | | | | | 1 | | | | |

FIG. 2.
Clinical findings in a typical case of naturally acquired Colorado tick fever infection.

after immunization, sera from 6 individuals of the first group and from 2 individuals in the second group were submitted to neutralization test. It is observed in Table I that the pre-inoculation sera obtained from individuals 1 and 3 showed significant neutralizing properties when compared to the pre-inoculation sera of the other volunteers, who showed an average $LD_{50}$ virus titer of $10^{-5.6}$. This is not surprising since individual 3 had become infected previously in the laboratory with CTF virus and volunteer 1 had lived in an endemic CTF area. Antibody titers determined on the 15th and 31st post-inoculation days indicated that these 2 individuals showed a somewhat more rapid serological response, a fact compatible with their previous history. All volunteers, regardless of previous history, showed their highest levels of neutralizing antibodies at the 45th to 54th day after inoculation. The serological response varied, however, from individual to individual, as shown by the fact that serum samples from 10 and 12 neutralized only 45 and 50 $LD_{50}$ of virus, whereas serum samples from 4 and 17 neutralized 10,000 and 6,310 $LD_{50}$ of virus, respectively.

In view of the fact that the serum samples obtained 45 to 54 days after inoculation seemed to contain more antibodies than the samples obtained at shorter intervals after inoculation, it was decided to submit only 2 serum samples from each individual to the neutralization test; one pre-inoculation specimen and one obtained at 47 to 56 days after inoculation. It may be observed in Table II that the pre-inoculation serum of individual 6 showed significant neutralizing antibodies when compared with the sera of the other volunteers, who showed an average virus titer of $10^{-6.0}$. Since volunteer 6 had contracted a laboratory infection with CTF virus prior to the inoculation, this enhanced serological response conforms with the history of prior infection. The serological response of all of the other volunteers was good, with the possible exception of individual 8, as judged by the neutralization index of 100 or more obtained with the serum samples after inoculation.

From 3 of the 5 volunteers who had been challenged with the human strain of CTF virus, serum samples were available for determining their neutralizing potency. It may be seen in Table III that the sera obtained after the challenge inoculation indicate a

TABLE III.
Comparison of Neutralization Titers in Sera of Three Volunteers Inoculated with Egg-adapted Colorado Tick Fever Living Virus and Challenged with Human Strain of Colorado Tick Fever Living Virus.

| No. | Serum specimen Days post-inoculation | Mortality ratio of mice inoculated intracerebrally with serum-virus dilutions: 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 | 10-7 | $LD_{50}$ virus titer | $LD_{50}$ doses neutralized |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0 | | | 6/6 | 6/6 | 6/6 | 1/5 | 0/6 | $10^{-5.65}$ | 0 |
| | 31 | | | 6/6 | 6/6 | 5/6 | 1/6 | 0/6 | $10^{-4.50}$ | 15 |
| | 20 days after challenge* | 5/5 | 3/6 | 3/6 | 0/6 | 0/6 | | | $10^{-2.50}$ | 1415 |
| 8 | 0† | | | | 6/6 | 6/6 | 3/6 | 0/6 | $10^{-6.00}$ | 0 |
| | 15 | | | 6/6 | 6/6 | 6/6 | 2/5 | 1/5 | $10^{-5.75}$ | 2 |
| | 64 days after challenge‡ | 6/6 | 1/6 | 1/6 | 0/6 | 0/6 | | | $10^{-1.70}$ | 19950 |
| 19 | 0† | | | | 6/6 | 6/6 | 3/6 | 0/6 | $10^{-6.00}$ | 0 |
| | 31 | | 6/6 | 6/6 | 3/6 | 0/5 | 0/6 | | $10^{-4.00}$ | 100 |
| | 65 days after challenge§ | 1/6 | 0/6 | 0/6 | 0/6 | | | | $<10^{-1.00}$ | $>100000$ |

* Challenged with human strain 10 months after inoculation.
† Pre-inoculation serum sample of volunteer 20 used because of insufficient amount of serum.
‡ Challenged with human strain 6 months after inoculation.
§ Challenged with human strain 5 months after inoculation.

marked increase in their neutralizing indices and show neutralizing antibody titers as high or higher than those shown in Tables I and II. Since the volunteers were not bled immediately prior to the challenge inoculation, it cannot be assumed with certainty that the rise in circulating antibody occurred solely as a result of the challenge inoculation. On the basis of the results summarized in Tables I and II which show that the serological response of normal individuals increased with the duration of time following inoculation, it is apparent that this fact must be considered also before attributing the higher neutralizing values to the challenge dose.

*Summary.* Twenty volunteers were inoculated with the egg-adapted strain of CTF living virus. Reactions ranged from enlarged, tender axillary lymph glands to single-episode attacks of illness resembling Colorado Tick Fever. Five of the inoculated individuals were subsequently challenged with a human strain of CTF living virus but they showed no reactions whatsoever to this second inoculation. All of the individuals developed neutralizing antibodies, but the 3 who presumably were immune prior to the inoculations showed a comparatively earlier and greater response. Sera obtained from the 3 individuals after they had been challenged with a human strain likewise showed a high neutralizing titer.

Received April 11, 1950. P.S.E.B.M., 1950, v74.