THE EFFECT OF UNDERNOURISHMENT ON THE SUSCEPTIBILITY OF THE RABBIT TO INFECTION WITH VACCINIA*

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It is generally believed that the healthy, well nourished person is more resistant to disease than the individual who is already diseased or poorly nourished. That such is not the case, however, has long been recognized in certain instances of viral infection. Jenner (1) found that individuals with another disease were sometimes immune to vaccination. Underwood (2) in 1799 pointed out that poliomyelitis attacked the finest children. Rous (3) in 1911 reported that healthy fowls are more susceptible to infection with sarcoma virus than unhealthy ones. Rivers (4) reviewing these and similar observations with viruses, has suggested that such behavior is not wholly unexpected and may be related to the extreme type of parasitism exhibited by viruses, namely, the dependence of viral disease on the multiplication of virus in susceptible cells.

At the present little is known concerning the factors upon which susceptibility of a cell to virus infection depends. However, it is reasonable to suppose that a poorly nourished cell may be lacking in some of the materials necessary for the formation of new virus particles. With this possibility in mind, it was decided to study quantitatively the effect of undernourishment on the susceptibility of the skin to infection with vaccinia. In spite of the fact that the rabbit is an extremely unsatisfactory animal for metabolic experiments, it was chosen for this work because it is well suited for precise quantitative studies with vaccinia.

The first experiments reported here confirmed fully the previously cited observations, in that they showed that simple undernourishment decreases the susceptibility of the rabbit's skin to infection with vaccinia. However, it became apparent from other experiments that simple reduction of available nutrient materials is not the sole factor involved in this decreased susceptibility. One other factor shown by the present experiments to be important is the amount of interstitial fluid present. In the presence of an increase of this

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Fluid the virus particles tend to remain localized at the site of injection; this localization, as shown in an earlier paper (5), suggests that the virus comes into contact with fewer susceptible cells and the number of lesions is thereby reduced. The opposite is true where the amount of interstitial fluid is either normal or reduced.

The amount of interstitial fluid increases if animals are deprived of food but are permitted to drink water freely. This increase is not the result of lowered plasma proteins, as these are unchanged. It is prevented, however, if no water is administered by mouth or if physiological saline solution is administered parenterally. It is therefore apparent that experimental techniques are at hand for studying the effects both of undernourishment and of variations in the amount of interstitial fluids. This paper is a report of the influence of these factors on vaccinia infection.

Methods and Materials

Animals.—Normal white adult male rabbits weighing between 2 and 3 kilos were used.

Virus.—The virus employed in the first 3 experiments was provided by Dr. J. W. Beard. This strain was derived from vaccine lymph originally obtained from the North Carolina Laboratory of Hygiene, Raleigh, and has been described in detail (6). This virus had been passed 50 times in rabbit skin. It was purified as described by Craigie and Wishart (7) and Parker and Rivers (8). Measured amounts were rapidly lyophilized and sealed in a vacuum until used in these experiments.

The virus used in the other experiments was a strain of vaccinia supplied by Dr. T. M. Rivers. This strain had been derived through serial passage through rabbit skin for a number of years and was well adapted to this animal. This strain was purified as was the other strain. It was not lyophilized but was kept frozen in dry ice until used.

Conditions of Fasting.—The rabbits to be fasted were kept in cages with wire mesh bottoms sufficiently large to prevent the eating of feces. Three different regimes of fasting were observed in these experiments. In the first, the rabbits received no food but were given free access to water. In the second, neither food nor water was given. For the third, the animals were fasted with access to water and in addition were injected intraperitoneally with 50 cc. of physiological saline solution twice daily. These regimes were maintained for 10 days before virus inoculation and for 48 hours afterward. The animals were then allowed such small amounts of food that their weights remained stationary. The rabbits of the group deprived of both food and water were given free access to water as well as some food 48 hours after vaccination. Estimations were made of plasma proteins in most of the fasted animals. No significant changes were noted, so that this factor could be ruled out as an influence in the production of hydration.

Seven days after virus inoculation all the rabbits were killed and necropsied. The animals listed in Table I as fasted were practically free of any subcutaneous, mesenteric, perirenal, or any other deposits of fat. Six of the 21 rabbits which were
fasted with water were found to have some fat and have been listed as incompletely fasted.

Controls.—The control animals were kept on the usual stock diet. Normal deposits of fat were seen in all of them. Certain of the fasted rabbits which failed to lose fat served as additional controls. A few of the rabbits developed diarrhea during the course of the experiment, some before and some after vaccination. These were excluded from the experiments, since this complication had been seen to affect susceptibility to viral infection. The incidence of this complication was as follows: 3 of 21 control rabbits, 3 of 21 rabbits which were fasted but given water, 3 of 9 rabbits which were fasted and given isotonic saline solution, and none of the 9 rabbits which were given neither food nor water. Two of this last group, however, died of inanition.

Virus Inoculation.—Tests for susceptibility to infection were made by intradermal injection of 0.25 cc. of 7 fourfold dilutions of virus. Each rabbit received 7 inoculations of each dilution or a total of 49 inoculations in each animal. The 50 per cent points were calculated by the accumulation positive and negative method of Muench and Reed (9).

Spread of Materials.—This was studied by the intradermal injection of 0.5 cc. of an India ink suspension. The spread of India ink was measured with a planimeter immediately after injection and at 1, 2, and 4 hours afterward. Only the 4 hour readings are given. The technique of this procedure is given in detail elsewhere (10).

Throughout this paper the expression, fasted animals, will mean those which were deprived of food but given free access to water.

EXPERIMENTAL

The Effect of Undernourishment.—An experiment was done on the effect of the deprivation of food but not water on the susceptibility of the rabbit to viral infection. Food was withheld from rabbits, but they were given access to water, and 3 were kept as controls. The results of inoculation of graded quantities of virus in this group are given under Experiment 1 in Table I. In the 3rd column are the number of inoculations of each dilution injected and in the 4th the per cent change in weight occurring during fasting prior to inoculation. Here it is seen that while the controls lost only 1 per cent of their weight the loss in the fasted animal was 19 per cent. In the 5th column are given the 50 per cent points. The logarithm of the virus dilution corresponding to the 50 per cent point for the control animals is 6.85 (dilution 1:7,080,000). In contrast, that for the fasted animals was 5.88 (1:759,000). The difference in logarithms was 0.97, the antilogarithm of which is 9.3. Therefore, the fasted animals may be thought of as being more than 9 times as resistant to viral infection as the controls. This result is well within the limits of the method of infectivity measurement and therefore indicates a definite increase in resistance related to fasting.

Viewed solely in the light of response as measured by the respective 50 per cent points the data obtained in this experiment might well be construed as
indicative of increased resistance due solely to poor nutrition. That this was not necessarily the case, however, was evident in associated findings. For one thing, it was observed that the blebs raised by the injections of the virus preparations subsided much more slowly in the fasted animals than did those in the controls. In earlier work (11) it was found that when blebs subside slowly the spread of particulate matter is correspondingly less than in instances where the blebs disappear rapidly. This was of particular interest in the present problem for it has been observed (5) that localization of injected virus due to failure to spread is associated with diminished likelihood of infection. A study was then made to learn whether or not the spread of India ink is altered in animals fasted as in the present experiment.

Comparison of Susceptibility and India Ink Spread in Undernourished Rabbits.—An experiment was carried out to find whether deprivation of food but not water actually localized the virus as the foregoing observations suggested. This question is important for it has been shown (5) that localization of a virus diminishes the chance of infection. Therefore, in the present experiment both virus and India ink were injected into the skin of 3 fasted animals and 2 controls. As might have been expected from Experiment 1, the results (Table I, Experiment 2), indicate that the spread of India ink was considerably less in the fasted animals than that in the controls. In the latter the mean area of spread was 7.84 cm² as compared with 5.68 cm² in the fasted animals. In the same group, the logarithm of the respective dilution of virus was 6.86 (dilution 1:7,244,000) for the controls and 5.64 (1:437,000) for the fasted animals. It thus appears that the decreased spread in the fasted rabbits parallels the increase in resistance.

Effect of Undernourishment with and without Increase in Interstitial Fluid.—Since the chance of viral lesions is decreased by localizing the virus (5), it was thought likely that some, at least, of the above results might be due to a reduction in spread of virus rather than to a change of the susceptibility in the individual cell directly referable to undernourishment. To determine this point a method was needed by which rabbits might be brought to a poorly nourished condition without any change in the spread of materials in the skin. Taylor and Sprunt (12) have shown that the spread is reduced by an increase in interstitial water and aided by the opposite. It was therefore possible that the failure of particulate matter to spread, as noted in the last experiment, was due to an increase in interstitial fluid. If present, such an increase would have to be dependent on some factor other than decreased plasma proteins, for these were normal. Regardless of the mechanism responsible for the accumulation of interstitial fluid, edema does not develop if the animal is deprived of water. The observation was also made that increases in interstitial fluid can be prevented if the fasting animal is injected intraperitoneally with physiological saline solution. It is beyond the scope of this paper to attempt
TABLE I

The Effect of Undernourishment on Susceptibility to Infection with Vaccinia (As Indicated by the 50 Per Cent Point)

<table>
<thead>
<tr>
<th></th>
<th>No. of rabbits used</th>
<th>No. of injections of each dilution</th>
<th>Change in weight before vaccination</th>
<th>Log of 50 per cent point</th>
<th>Mean spread of India ink in 4 hrs.</th>
<th>Increased resistance over controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3</td>
<td>21</td>
<td>-1.1</td>
<td>6.85</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Fasted with water</td>
<td>3</td>
<td>21</td>
<td>-19.1</td>
<td>5.88</td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

Experiment 2

| Controls         | 2                   | 14                                 | +4.4                               | 6.86                     | 7.84                              | 9                                |
| Fasted with water| 3                   | 21                                 | -11.7                              | 5.64                     | 5.68                              | 11                               |

Experiment 3

| Controls         | 3                   | 21                                 | -0.6                               | 6.23                     | 8.91                              | -                                |
| Fasted with water| 2                   | 14                                 | -27.4                              | 5.35                     | 5.38                              | 7                                |
| Fasted with saline injected | 1   | 7                                 | -18.6                              | 6.07                     | -                                 | 1                                |

Experiment 4

| Controls         | 2                   | 14                                 | -1.8                               | 8.86                     | 8.63                              | -                                |
| Incompletely fasted with water | 2   | 14                                 | -17.2                              | 8.44                     | 7.51                              | 3                                |
| Fasted with saline injected        | 2   | 14                                 | -24.4                              | 8.86                     | 8.71                              | 1                                |

Experiment 5

| Controls         | 3                   | 21                                 | -2.8                               | 8.07                     | 7.38                              | -                                |
| Fasted with water| 1                   | 7                                 | -18.5                              | 6.67                     | 5.38                              | 12                               |
| Fasted without water | 3     | 21                                 | -22.6                              | 7.98                     | -                                 | 0                                |
| Incompletely fasted with water     | 1   | 7                                 | -20.0                              | 8.14                     | 7.40                              | 0                                |

Experiment 6

| Controls         | 3                   | 21                                 | -1.7                               | 7.70                     | 8.07                              | -                                |
| Fasted with water| 1                   | 7                                 | -18.2                              | 7.07                     | 5.74                              | 4                                |
| Fasted without water | 2      | 14                                 | -23.1                              | 7.69                     | -                                 | 0                                |
| Incompletely fasted with water     | 2   | 14                                 | -20.3                              | 7.56                     | -                                 | 1                                |

Experiment 7

| Controls         | 2                   | 14                                 | -2.5                               | 7.85                     | 7.27                              | -                                |
| Fasted with water| 1                   | 7                                 | -16.0                              | 7.06                     | 7.93                              | 6                                |
| Fasted without water | 2     | 14                                 | -29.4                              | 7.75                     | -                                 | 0                                |
| Incompletely fasted with water     | 1   | 7                                 | -17.6                              | 7.71                     | 7.41                              | 0                                |
| Fasted with saline injected        | 3   | 21                                 | -21.5                              | 7.45                     | -                                 | 2                                |

to explain these peculiarities in the metabolism of the rabbit. A series of experiments (Nos. 3 to 7) was undertaken in which, by utilization of these two facts, it was possible to determine the effects of increased interstitial fluids and of simple undernourishment and to relate the former with the spread of India ink.
The results of these experiments are shown in Table I. This table shows conclusively that the fasted animals with increased interstitial fluid required from 3 to 12 times more virus to infect than did the controls. Moreover the spread of India ink was also reduced. This reduction of spread was certainly associated with, if not caused by, accumulation of excessive water in the tissues, for it was observed that the animals in this group (with the exception of those in Experiment 3) always lost less weight than the ones which were deprived of water or given physiological saline solution intraperitoneally. This difference is apparently due to the fact that, if allowed to drink water, the fasted rabbits store it in the interstitial spaces.

If the animals were deprived of both food and water (dehydrated), essentially the same amount of virus was needed to infect as in the controls. The same was true in the case of those animals which received intraperitoneal injections of isotonic saline solution. Unfortunately, in all but one instance, in which the spread was slightly larger than in the controls, measurements of the India ink spread in these 2 groups were unsatisfactory, and correlation of susceptibility with spread of particulate matter is therefore impossible. However, in both groups, the blebs raised by the injection of the India ink suspension disappeared very rapidly, most of the ink remaining near the point of injection. The identical behavior of the blebs in these 2 groups indicates that dehydration of the interstitial tissues characterizes both groups. Apparently, the tissues were so dry that they allowed the menstruum of the ink to pass easily through the skin, while the large ink particles remained where they were injected. Experiments are now under way to determine the amount of interstitial water by direct measurement.

In addition to the above changes it was also noted that the animals deprived of both food and water and those that received physiological saline solution (dehydrated) had lesions which were relatively much smaller and which appeared about 24 hours later. Their lesions regressed somewhat sooner than did those in the controls. The lesions in the rabbits which received no food but were given water (increased interstitial fluid) were also smaller than in the controls but this difference was not as marked as with the above groups.

In other words, depriving rabbits of food increases the resistance of the rabbit to infection with vaccinia; this resistance is accentuated by increase of interstitial fluids, and is offset by dehydration of the interstitial spaces.

**DISCUSSION**

These experiments show that the response of rabbits to viral infection is affected in two ways by undernourishment. One is the depletion of the necessary intracellular elements for virus multiplication. As pointed out earlier in this paper, a susceptible cell must contain, regardless of its other requirements, the necessary constituents for virus multiplication. At present little is
known as to what these requirements are, but Hoagland and associates (13) have shown that biotin is always present in purified vaccinia and is probably a necessary constituent of the virus. Other substances of which the body has been depleted by undernourishment are probably equally necessary for virus multiplication. According to this concept increased resistance to viral infection depends in some part at least on the absence of materials required for synthesis of the virus.

The other influence is more striking and is associated with a change in the amount of interstitial fluids. It has been shown previously in this laboratory (5, 12) that alteration in the amount of these fluids modifies the extent of spread of particulate matter in the tissues and that this spread can be correlated with the capacity of a virus to produce lesions. Increase in interstitial fluids decreases the spread of particulate matter and reduces the incidence of viral lesions, while decrease in these fluids has the opposite effects. It is quite likely that this relationship can be explained simply on the basis of number of cells exposed to the action of the virus, for as shown in this laboratory (5) and by Olitsky and Schlesinger (14), the larger the area of dissemination of a virus the greater the likelihood of its coming into contact with a susceptible cell and hence the greater the chance of a lesion. The experiments reported here are entirely compatible with such an hypothesis. However, an alternative possibility is that the susceptibility of the individual cell may be modified by changes in the amount of interstitial fluid. The whole question of the mechanism of water balance on susceptibility to infection is being studied further.

It seems likely from the present experiments that both mechanisms are involved in determining the incidence of lesions. Deprivation of food resulted in either fewer or smaller lesions. This effect was accentuated in the presence of accumulation of interstitial fluids but was partially nullified if the interstitial tissues were dehydrated.

In this laboratory it has been observed that, generally speaking, rabbits do not react as well to infection with vaccinia in summer as in winter. This may be the result of increased fluid in the tissues consequent upon changes in food and water intake in hot weather. If this is so, then experimental work with vaccinia and perhaps some other viruses might be facilitated in the summer by the injection of suitable amounts of isotonic salt solution.

The possibility that invasion of the central nervous system by the virus of poliomyelitis may be aided by dehydration is also suggested. De Rudder and Petersen (15) noted that poliomyelitis occurred among athletes in a boys' school after strenuous exercise and thought that the virus was already present and gained access to the nervous system because the nerve cells had been rendered more susceptible. Perhaps the dehydration associated with the exercise may have increased the spread of the virus in the nervous tissue and thus led to infection.
SUMMARY

Experiments are reported in which it is shown that if rabbits are deprived of food, the lesions resulting from injection of vaccinia are either fewer or smaller; presumably this is partially explainable on reduction of available nutrients in the cell. The number and character of the lesions are also modified by the state of hydration of the interstitial tissues: If the amount of interstitial fluid is increased by permitting the animal to drink water, the lesions are even less numerous; but if the interstitial tissues are dehydrated either by withholding water or by injecting physiological saline solution into the peritoneal cavity, then the lesions are more numerous.

The increase in interstitial fluids in these experiments was not due to decreased plasma proteins, for these were normal. In this respect, therefore, the rabbit differs from man, for unless the plasma proteins are reduced, simple starvation in man results in dehydration rather than edema of the tissues.

From these experiments it is concluded that the virus is less able to multiply in the poorly nourished cell than in the well nourished one, and that hydration of the tissues increases the resistance of the tissue to infection while dehydration has the opposite effect. It is suggested that this is because hydration tends to localize the virus in situ, with result that fewer cells are exposed to it, while dehydration has the opposite effect. However, actual changes in cell susceptibility consequent upon altered water balance may be responsible for the effect.

BIBLIOGRAPHY