ASCORBIC ACID CONTENT OF ADRENAL AND LIVER IN CYNOMOLGUS MONKEYS SUFFERING FROM BACILLARY DYSENTERY

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SUMMARY

SUMMARY: Adrenal ascorbic acid content regarded as an indication of adrenocortical activity was determined in order to find if there is any relationship between Shigella infection and the adrenocortical function in cynomolgus monkeys that were suffering either naturally or experimentally from bacillary dysentery.

In the monkeys which died from natural dysentery, the average adrenal ascorbic acid content was about 60% lower than that in control healthy animals. In experimentally dysentery-infected monkeys sacrificed 3-4 days after oral administration of Shigella flexneri 2a, namely at the peak of disease, the average adrenal ascorbic acid content decreased to about 55% of the normal control, and the content returned to its normal value 6-8 days after the infection coinciding with the ensuing of convalescence in apparent symptomatology of experimental dysentery.

The average liver ascorbic acid level was shown to rise markedly above the healthy control 3-4 days after the experimental infection with Shigella flexneri 2a, whereas the level was almost equal to that of control monkeys 6-8 days and 13-15 days after the infection.

These results seem to prompt further studies to scrutinize the possible involvement of adrenocortical function in the course of Shigella infection in cynomolgus monkeys.

INTRODUCTION

Generally, the adrenal contains ascorbic acid at the highest concentration in the body and some possible relationship of this to adrenocortical function has been suggested (Sayers et al., 1946, Long, 1947, Sayers & Sayers, 1948, Irwin et al., 1950, Woods, 1950a,b, Elton, Zarrow & Zarrow, 1959, Duncan, 1959, Kameta, 1959, Hedner & Rerup, 1962 & Said, 1964). However, Stewart (1952) described that the function of the adrenal cortex in scurbitic monkeys was normal with occasional increases, presumably in response to stressful circumstances in scurvy.

Liver ascorbic acid content is known to increase after the administration of adrenocorticotropic hormone (Forbes and Duncan, 1954, Kameta, 1959) or of endotoxin of Salmonella enteritidis (Jefferies, 1965).

Monkeys are unable to synthesize ascorbic acid and must depend on the dietary intake to keep the physiologically constant level of ascorbic acid in the body. During the course of dysentery in cynomolgus monkeys, a decline of serum ascorbic acid level was observed (Honjo et al., 1964). From the viewpoint of pathologic physiology of dysentery, it may be of importance to examine if there is any relationship between Shigella
infection and the adrenocortical function. The present report deals with the ascorbic acid content of the adrenal and the liver in cynomolgus monkeys suffering naturally or experimentally form bacillary dysentery.

MATERIALS AND METHODS

Adrenal: For control materials, the left adrenals were taken from 24 healthy cynomolgus monkeys (Macaca irus) that had been conditioned by keeping at the animal room of our laboratory for more than 6 weeks. For test materials, 75 adrenals were removed from natural dysenteric cases that died about 3–7 days after the manifestation of bacillary dysentery which was confirmed not only clinically but also bacteriologically and pathologically (Takasaka et al., 1964, Ogawa et al., 1964). Then, the adrenals of 25 monkeys sacrificed with a lethal dose of sodium pentobarbital given intravenously at three different stages of the disease produced experimentally—at the stage of peak of disease (3–4 days after challenge), 6–8 days after challenge and the stage of recovery (13–15 days after challenge)—were analyzed as the experimental dysenteric cases. Sixteen of these 25 monkeys developed bloody mucous diarrhoea in the oral infection experiments of Shigella flexneri 2a as reported previously (Honjo et al., 1964), and the other 9 animals were dysenteric cases infected in another experiment of the same kind carried out thereafter. Six non-dysenteric cases were also analyzed for their adrenal ascorbic acid content, as reference, in the course of an experimental infection with S. flexneri 2a.

Liver: A piece of the left lateral lobe was used for analysis. Thirty-four healthy monkeys' livers served as normal controls and the livers of 25 monkeys of experimental dysentery mentioned above were analyzed.

Determination of ascorbic acid in the tissues: The adrenal and liver were analyzed immediately after the removal from the animal, otherwise the tissue was frozen at −20°C for a short time until analysis. The tissue was macerated with silica sand and a chilled, freshly prepared 5% metaphosphoric acid. The macerated tissue was allowed to stand for 15 minutes and then centrifuged at 3,000 rpm for 10 minutes. The total ascorbic acid content in supernatant fluid was determined by the method of Roe and Kuether (1943).

RESULTS

Table 1 presents the adrenal ascorbic acid content of healthy cynomolgus monkeys and of natural dysenteric cases. The average content of the control animals was about three times higher than that of the natural dysenteric cases. The table also shows that the adrenals of monkeys which died from acute lobar pneumonia had a markedly lower level of ascorbic acid than that of the healthy monkeys.

The results obtained from the experimental dysenteric cases are illustrated in Figure 1. The average content of adrenal ascorbic acid of 15 animals sacrificed 3–4 days after the experimental infection with S. flexneri 2a decreased to approximately 55% of the healthy control. On the other hand, 3 non-dysenteric cases showed a constant level of adrenal ascorbic acid within the normal range even 3–4 days after infection. In 10 cases sacrificed 6–8 days or 13–15 days after the administration of the bacilli, the average adrenal ascorbic acid content returned to the normal value, although the fluctuation of the level was relatively wide. Three non-dysenteric cases killed 6–8 days after challenge clearly showed normal values.
Table 1. Average ascorbic acid content of the adrenal

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Animals which died from natural dysentery</th>
<th>Animals which died from natural pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases analyzed</td>
<td>24</td>
<td>75</td>
<td>39</td>
</tr>
<tr>
<td>Average (mg/100 g)</td>
<td>112.4</td>
<td>34.2*</td>
<td>24.6*</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±35.6</td>
<td>±17.8</td>
<td>±9.1</td>
</tr>
</tbody>
</table>

* Statistically different from the value of healthy controls at 95% level of significance.

![Graph of adrenal ascorbic acid content](image)

Fig. 1. Adrenal ascorbic acid content of animals suffering from experimental dysentery.
As shown in Table 2, the average content of the liver was about 65% higher in the monkeys suffering from experimental dysentery than in healthy controls after 3-4 days of infection, while the values were not significantly different 6-8 days or 13-15 days after the administration of Shigella bacilli.

Table 2. Average ascorbic acid content of the liver

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>3-4 days after infection</th>
<th>6-8 days after infection</th>
<th>13-15 days after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases analyzed</td>
<td>34</td>
<td>15</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Average (mg/100 g)</td>
<td>14.9</td>
<td>24.6*</td>
<td>16.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Standard deviation (mg/100 g)</td>
<td>±6.1</td>
<td>±9.1</td>
<td>±6.3</td>
<td>±6.2</td>
</tr>
</tbody>
</table>

* Statistically different from the value of healthy controls at 95% level of significance.

DISCUSSION

The finding that the ascorbic acid content of the adrenal of monkeys which died from natural dysentery was much lower than that of the healthy controls seems to suggest that the adrenal cortex of the former animals was exhausted probably after hyperfunctional elaboration of a large amount of corticosteroids in the course of disease. From the viewpoint of non-specific adrenocortical response to infections of some pathogens it would be interesting that a similar finding was obtained in the monkeys which died from acute lobar pneumonia. Moreover, these results are in accordance with those by Jungeblut and Feiner (1937) who found that the amounts of ascorbic acid present in the nervous tissue and the adrenal of rhesus monkeys paralyzed as a result of poliomyelitis infection were below the normal average when examined at the height of paralysis.

The results obtained from the experimental dysenteric cases may indicate that the exacerbation of corticosteroid secretion and the conspicuous consumption of ascorbic acid may have resulted from the experimental dysentery during the first 3-4 days following the administration of Shigella flexneri 2a and then the adrenocortical function recovered to the normal level by the 7th-9th day.

It is noticeable that the degree of diminution of adrenal ascorbic acid of the monkeys which died from natural dysentery exceeds that of monkeys sacrificed at the height of experimental dysentery. This may be interpreted as suggesting that in such a serious clinical state that the monkey died spontaneously within a week the cortical hyperfunction may have continued and the resistance to Shigella infection may have been reduced perhaps by excessive elaboration of corticosteroids and then the adrenal cortex was severely exhausted resulting in a marked decline of its ascorbic acid level; on the other hand, in the experimental dysenteric cases the excitement of adrenocortical activity may have been so moderate even at the peak of disease that the cortex was not exhausted.

The increase of liver ascorbic acid content at the height of experimental dysentery which was found in the present study seems to be similar as observed by Forbes and Duncan (1954), Kameta (1959) and Jefferies (1965), because this increase may have resulted from either some direct toxic action of the infected Shigella to the liver tissue or indirect action through hypophyseal adrenocortical axis. If the latter indirect process actually exists, the increase of liver ascorbic acid content in dysentery-infected monkeys
should substantiate the abnormally fluctuating state of adrenocortical function expressed by the decrease of ascorbic acid.

Since the adrenal ascorbic acid content, regarded as an indicator of adrenocortical function, apparently fluctuate corresponding to the severity of dysentery, it seems proper to conclude that either the natural or the experimental infection of monkeys with *S. flexneri* 2a may entail the abnormal fluctuation of the adrenocortical function. At any event, we consider that it would rather be reasonable to comprehend the present results as indicating a patho-physiological response of the host in the course of dysentery. Therefore, further studies should be carried out to determine whether or not the monkeys in the state of hypo- or hyperadrenal corticalism induced experimentally would develop dysentery when administered with *Shigella*. And then, further work not resorting to such an indirect method as the measurement of adrenal ascorbic acid but to a more direct measurement of adreno-corticosteroids content or analyses of concentration of corticoids in the plasma would be necessary to scrutinize more precisely the relationship between *Shigella* infection and adrenocortical function in the course of dysentery in the monkey.

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