The absence of plasma free fatty acid response to epinephrine in vitamin-C-deprived guinea pigs

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SUMMARY

Plasma concentrations of free fatty acid (FFA) and blood glucose were measured in normal and 25-day scorbutic guinea pigs 15 minutes after injection of either saline or epinephrine. The normal group showed a large increase in FFA after epinephrine and a smaller increase after saline as compared to no injection at all. The scorbutic animals had no increase of FFA either after saline or epinephrine. The injection of 50 mg ascorbic acid intraperitoneally into 25-day scorbutic guinea pigs 5 hours before sacrifice restored to this group the normal response to epinephrine and saline. The mean plasma 17-OH corticosteroid concentration was 206.5 μg/100 ml for six 25-day scorbutic guinea pigs and was 71.5 μg/100 ml for six normal guinea pigs. Scorbutive animals had marked increases of glucose both after saline and epinephrine injections as compared to uninjected scorbutive animals.

METHODS

Twelve male, NIH stock, mixed-color guinea pigs were fed a normal diet of greens and chow for one week and then injected intraperitoneally with 25 μg of epinephrine in saline at approximately 3:45 PM after a 5-hour fast. They were then sacrificed in groups of three at intervals of 5, 10, 15, and 20 minutes to determine the time of peak effect of epinephrine on blood glucose and FFA. Blood was collected from the severed necks for 30 seconds through silicone-coated funnels into silicone-coated test tubes, each containing three drops of heparin (10,000 U. S. P. units/ml). Blood samples were immediately placed in crushed ice and analyzed for blood glucose and FFA.

Additional guinea pigs, weighing from 245 to 310 g, were fed a commercial powdered ascorbic acid-free diet (Nutritional Biochemicals, Inc.) and tap water ad libitum as in the previous study (1). Every morning each animal was given a 0.10-ml intraperitoneal injection. Scorbutive animals received normal saline; normal animals received 50 mg of ascorbic acid in solution. Twenty-five days after the beginning of the experiment, food was removed from all cages at 11 AM. The animals were injected intraperitoneally with either 25 μg of epinephrine in 0.25 ml saline or 0.25 ml normal saline at approximately 3:45 PM. Exactly 15 minutes later the animals were sacrificed and blood was collected as before. The order of injection and sacrifice was alternated between normal and scorbutive animals as well as between saline and epinephrine injections. One group of 25-day scorbutive animals (25-day scorbutive plus vitamin C) received injections of 50 mg of ascorbic acid...
instead of the usual saline immediately after food was withdrawn—5 hours before sacrifice.

Twelve guinea pigs, six normal and six 25-day scorbutic, were fasted for 5 hours and then anesthetized with 15 mg of pentobarbital intraperitoneally. Their abdomens were opened and as much blood as possible was removed from the abdominal aorta with a heparinized syringe and needle. The plasma was removed after centrifugation and frozen immediately. At a later date, 17-OH corticosteroids were determined on these plasmas by the method of Silber and Porter (9).¹

Plasma free fatty acid concentrations were determined by extraction of 1 ml of plasma for 24 hours in a mixture of 2,2,4-trimethyl-pentane (isooctane), acetic anhydride, and glacial acetic acid, followed by two washings and titration against approximately 0.02 N NaOH (10). Blood glucose was determined by the Glucostat ²% method (11).

Statistical methods employed included the standard "t" test and the standard correlation coefficient.

RESULTS AND DISCUSSION

Figure 1 shows the effect of 25 µg epinephrine intraperitoneally on FFA and glucose in relation to time. The zero determinations were made at a previous date. Large increases in FFA and smaller increases in glucose follow epinephrine with the peak reached at 15 minutes. Accordingly, the 15-minute period between injection and sacrifice was chosen for the subsequent studies.

The effect of epinephrine and saline injections upon FFA and glucose is shown in Table 1. The normal guinea pig FFA after a 5-hour fast without any injection has been determined to be 0.41 ± 0.12 mEq per liter (1). Apparently saline injections alone cause enough distress to increase FFA to 0.72 mEq per liter in 15 minutes. Epinephrine, however, increases FFA further to a mean 1.19 mEq per liter, which is significantly greater (p < 0.01) than the mean FFA after saline.

The injection of vitamin C five hours prior to sacrifice into the 25-day scorbutic guinea pigs apparently restores their ability to increase FFA both after saline and epinephrine. The FFA means of 0.81 mEq per liter after saline and 1.15 mEq per liter after epinephrine are very similar (p > 0.1) to those observed in the normal animals. Three of the seven plasmas obtained from the guinea pigs receiving epinephrine were lipemic.

The mean blood glucose in uninjected normal and

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² Worthington Biochemical Corporation.
TABLE 2. PLASMA 17-OH CORTICOSTEROID CONCENTRATIONS IN NORMAL AND SCORBUTIC GUINEA PIGS (µg/100 ml)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>X ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>70</td>
<td>59</td>
<td>52</td>
<td>63</td>
<td>98</td>
<td>87</td>
<td>71.5 ± 18</td>
</tr>
<tr>
<td>25-day scorbutic</td>
<td>116</td>
<td>280</td>
<td>199</td>
<td>134</td>
<td>315</td>
<td>195</td>
<td>206.5 ± 78</td>
</tr>
</tbody>
</table>

The plasma 17-OH corticosteroid concentration noted by Done et al. in controls was 301.9 ± 43.03 µg/100 ml and 32.9 ± 4.60 µg/100 ml in stocks. Therefore, the mechanism of abolition of increased FFA after epinephrine appears to be apart from the normal 17-OH corticosteroid, either in exact chemical structure or in its role of potentiating FFA release with epinephrine. The work of Burstein and associates (8) would make this possibility highly unlikely, as they identified in urine by chromatographic methods only normally appearing corticosteroids in high quantities in scurvy pigs. The almost threefold increase of plasma 17-OH corticosteroids in the 25-day scorbutic guinea pigs agrees well with the findings of Burstein et al. (8) and Done et al. (7). The mean plasma 17-OH corticosteroid concentration noted by Done et al. in scurvy was 301.9 ± 43.03 µg/100 ml and 32.9 ± 4.60 µg/100 ml in controls. Therefore, the mechanism of abolition of increased FFA after epinephrine appears to be apart from adrenal insufficiency in scurvy. The possibility remains, however, that there may be an increase of a blood constituent in scurvy that reacts to the Porter-Silber test as 17-OH corticosteroid but that is not the same as the normal 17-OH corticosteroid, either in exact chemical or in its role of potentiating FFA release with epinephrine. The work of Burstein and associates (8) would make this possibility highly unlikely, as they identified in urine by chromatographic methods only normally appearing corticosteroids in high quantities in scurvy pigs.

REFERENCES