Introduction

It has been reported in the previous section that a deficiency of vitamin C in guineapigs leads to changes in carbohydrate metabolism, as evidenced by a diabetic type of glucose tolerance curve and depletion of the glycogen content of the liver and skeletal muscle. The changes observed have been attributed to a fall in the insulin content of the pancreas.

It is well known that adrenaline and insulin produce opposite glycemic effects. Adrenaline content of the adrenal glands were determined in normal and scorbutic guinea pigs (1). A significant increase in the adrenaline content of the adrenals of scorbutic guinea pigs was observed. The weight of adrenals of scorbutic guinea pigs was also found increased (1). The glycemic effect observed in scorbutic guinea pigs, however, was not due to increased adrenaline content of the adrenal glands of the scorbutic animals, because demedullated scorbutic guinea pigs gave a diabetic type of glucose tolerance curve, whereas demedullated guineapigs receiving ascorbic acid showed a normal tolerance to glucose (3).

Adrenal corticosteroids are also concerned with the carbohydrate metabolism. Injection of corticosteroids increases the deposition of glycogen in the liver. The diminished liver glycogen of scorbutic animals might also be due to decreased functional activity of the adrenal cortex. The urinary excretion of 17-ketosteroids and corticosteroids as measures of adreno-cortical function was determined in female guinea pigs and monkeys during the progress of scurvy. The urinary excretion of the steroids increased to a considerable extent in most of the animals when they became severely scorbutic (5). Injection of insulin to guinea pigs during the progress of scurvy did not, however, alter the pattern of urinary excretion of 17-ketosteroids although such injections in normal guinea pigs diminished the urinary...
excretion of 17-ketosteroid. The altered adrenocortical function in scurvy seemed to be a specific effect of vitamin C-deficiency and not mediated through the associated insulin insufficiency (12).

Injection of adrenocorticotropic hormone is followed by a fall in the concentration of ascorbic acid and cholesterol in the adrenals (11). Diminished adrenal cholesterol and ascorbic acid were also observed in scorbutic guinea pigs (2, 6, 9). It is well known that adrenaline stimulates the production of adrenocorticotropic hormone of the anterior pituitary which stimulates adrenal cortex leading to the depletion of adrenal ascorbic acid and cholesterol (11). Plasma content of adrenaline was, therefore, determined in scorbutic and pair fed normal guinea pigs.

Materials and methods.

Male guinea pigs weighing 250-300 g were divided into pairs according to weights. The normal control animal of the pair received an amount of a scorbutogenic diet (1) equivalent to the diet consumed by its scorbutic pair fed ad libitum on the previous day, and 5 mg ascorbic acid per day. The other animal of the pair received only the scorbutogenic diet without any ascorbic acid supplement.

At the beginning of the fourth week, all the animals not receiving supplements of ascorbic acid developed scurvy. These animals along with their pair fed normal control animals receiving ascorbic acid were fasted over night and stunned by a sharp blow on the neck next morning for the collection of blood by cardiac puncture through a syringe rinsed with a solution of heparine. The heparinised blood was taken in a centrifuge tube, centrifuged, plasma separated and stored in a refrigerator.

Spectrofluorimetric determination of adrenaline. Plasma adrenaline was determined by the trihydroxyindole reaction using a spectrofluorimeter (13). Adrenaline is oxidised to
adrenochrome and the latter is rearranged to fluorescent N-methyl-3,5,6-trihydroxyindole in alkaline solution. The unstable compound is protected from oxidation by a suitable reducing agent.

Preparation of alumina for adsorption chromatography (7). Alumina (Al₂O₃) suitable for chromatographic adsorption analysis (E. Merck), 100 g, was added to 500 ml of boiling 2N hydrochloric acid and stirred vigorously for 20 minutes. The mixture filtered on a sintered glass funnel, the residue on the filter washed with 500 ml of the hot 2N acid followed by washing with sufficient water, the washed residue transferred into a beaker of suitable size, stirred with 500 ml water, the supernatant decanted, the thick solution filtered by suction, dried in a muffle oven at 300°C for 2 hours, transferred in a watch glass to a desiccator and stored.

Treatment of plasma with alumina. Alumina thus prepared, 0.5 g, was suspended in 5 ml of 0.1M ammonium acetate in a glass stoppered 12 ml centrifuge tube, pH adjusted to 8.0 with 1M ammonia, 0.2-0.3 ml of 1N sodium hydroxide added to bring the pH of the solution to 8.4 ± 0.1, centrifuged, supernatant discarded, 3 ml plasma added to the treated alumina, the tube shaken for 5 minutes, centrifuged for 1 minute, the supernatant transferred into another 12 ml glass stoppered centrifuge tube to which 0.2 g alumina added, tube shaken for 3 minutes followed by centrifugation for 1 minute, supernatant discarded, to both the tubes 5 ml cold glass distilled water added, the tubes shaken for 1 minute, centrifuged, the supernatant discarded, the washing repeated, 5 ml of 0.33M acetic acid added to each tube, the tubes shaken for 3 minutes, centrifuged, the supernatant transferred to a glass stoppered 25 ml measuring cylinder, the elution repeated with 3 ml of acetic acid, the eluate transferred into the cylinder, the volume made up to 25 ml with water and 1 ml taken for the estimation of adrenaline.
Estimation of adrenaline (14)

Reagents: Formic acid 1M. Prepared by diluting 43.5 ml of 88% formic acid to 1000 ml with water.

Cupric acetate 0.01M. 0.2% solution of cupric acetate in water.

Potassium ferricyanide 0.25% in water.

Sodium hydroxide 10N. Prepared by dissolving 40 g sodium hydroxide pellets in water to make 100 ml.

Sodium sulphite 20%. in water.

Mercaptoethanol reagent. Equal volumes of 1% mercaptoethanol in 20% sodium sulphite and 10N sodium hydroxide mixed immediately before use.

Acetic acid 10N. Prepared by dissolving 57.5 ml glacial acetic acid in water to make 100 ml.

Stock standard adrenaline solution. Prepared by dissolving 181.9 mg 1-adrenaline bitartarate in 0.1N hydrochloric acid to make 100 ml. (1 mg adrenaline base / ml).

Dilute standard. Stock standard is suitably diluted with 0.01N hydrochloric acid to make 1 μg or 10 μg / ml.

Procedure: 1 ml of diluted eluate was taken into each of three centrifuge tubes marked A (sample), B (standard), and C (blank). 1 ml of dilute standard adrenaline (1 μg / ml) was added to B and 1 ml water was added to A and C. To each of the three tubes the following solutions were added in the following order: 0.6 ml of formic acid, 0.1 ml of 0.01M cupric acetate and 0.1 ml of 0.25% potassium ferricyanide. After 5 min 0.6 ml of 10N sodium hydroxide-mercaptop ethanol sulphite reagent was added to tubes A and B followed by the addition of 0.6M acetic acid after 4 minutes. To tube C 0.3 ml of 10N sodium hydroxide and after
10 minutes 0.6 ml 10M acetic acid followed by 0.3 ml of mercaptoethanol-sulphite reagent were added. All the tubes were centrifuged for 5 minutes at 1000g and the fluorescence was read at an extinction wavelength of 415 mp and an emission wavelength of 500 mp.

**Additions.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted eluate</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Adrenaline standard (μg/ml)</td>
<td>0</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Water</td>
<td>1.0 ml</td>
<td>0</td>
</tr>
<tr>
<td>Formic acid 1M</td>
<td>0.6 ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Cupric acetate 0.01M</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Potassium ferricyanide (0.25%)</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Sodium hydroxide 1N</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mercaptoethanol reagent</td>
<td>0.6 ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Acetic acid 10M</td>
<td>0.6 ml</td>
<td>0.6 ml</td>
</tr>
</tbody>
</table>

**Results and Discussion.**

Results are given in Table 1.

There was significant increase in the plasma level of adrenaline in the scorbutic guinea pigs. Adrenaline content of adrenal glands also increased in scorbutic guinea pigs (1,3). The increase in the plasma level of adrenaline along with the increased content of adrenaline in the adrenals of the scorbutic animals indicated hypersecretion of adrenaline in the scorbutic condition. A condition of stress is produced in the extreme stage of scurvy. Increased adrenaline secretion seems to be possibly due to stress which leads to stimulation of the adrenal cortex through the adrenocorticotropic hormone. This is supported by the report of markedly elevated plasma levels of adrenaline, noradrenaline and cortisol in lambs kept in a slaughter house environment as compared with the basal hormone levels (10). After injection of ascorbic acid to the normal guinea pigs injection of adrenocorticotropic hormone produced a decrease in the adrenaline level in the adrenal...
Table 1. Adrenaline levels in plasma of guinea pigs.

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Normal</th>
<th>Scorbutic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microgram adrenaline per ml of plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.08</td>
<td>8.58</td>
</tr>
<tr>
<td>2</td>
<td>7.75</td>
<td>7.92</td>
</tr>
<tr>
<td>3</td>
<td>8.50</td>
<td>9.92</td>
</tr>
<tr>
<td>4</td>
<td>8.17</td>
<td>9.17</td>
</tr>
<tr>
<td>5</td>
<td>6.58</td>
<td>10.75</td>
</tr>
<tr>
<td>6</td>
<td>7.50</td>
<td>8.17</td>
</tr>
<tr>
<td>7</td>
<td>8.92</td>
<td>10.83</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>7.79 ± 0.31</td>
<td>9.33 ± 0.45</td>
</tr>
</tbody>
</table>

\[ t = 2.85 \]
\[ p \leq 0.02 \]
gland with a rise in the level of 11-hydroxycorticosteroids (15). Vitamin C excess, 300 mg per guinea pig per day for 3 days before experiments prevented the development of endocrine stress syndrome in the animals. The decrease in adrenal ascorbic acid and cholesterol content and number of blood eosinophils which were present during cold water (15°C) swimming was not observed when high doses of vitamin C were given (8).

Although decreased adrenal ascorbic acid and cholesterol content were observed in scorbutic guinea pigs (2,6) liver glycogen values of these animals were not only decreased but they were found markedly low as compared to normal guinea pigs. This might be due to diminished supply of glucose-6-phosphate as a result of diminished hexokinase activity of the liver of the scorbutic animal (4).

Summary.

Plasma level of adrenaline was determined by the spectrofluorometric method in scorbutic and pair-fed normal guinea pigs.

A significant increase in the plasma level of adrenaline was observed in scorbutic guinea pigs (p < 0.02).

The increased plasma adrenaline in scorbutic guinea pigs seemed to be due to the condition of stress produced in the animal during scurvy.

References.


