EFFECT OF DIGITOXIN AND k-STROPHANTIN ON THE ADRENALS AND TESTES OF THE GUINEA-PIG

By

Kalevi Pyörälä

Glycosides of the digitalis group and steroids of the adrenal cortex are known to have somewhat similar effects on the electrolyte balance and carbohydrate metabolism of the organism (for references, see Pyörälä & Eränkö, 1957). There are also investigations, according to which digitalis glycosides influence the function of the adrenal cortex. Zwemer et al. (1940) and Kinsell et al. (1942) observed that treatment with glycosides causes a decrease in the lipid content of the adrenal cortex. Gardner et al. (1954), on the other hand, found in rats treated with g-strophantin an enlargement of the adrenal glomerulosa with an increase in its lipid content. According to Metzler (1949, 1951, 1952) glycoside treatment in guinea-pigs causes an adrenal hypertrophy, increases the adrenal hypertrophy caused by exercise, and prevents the adrenal atrophy caused by desoxycorticosterone acetate. Kuschinsky (1947) and Moskopf & Sarre (1953, 1954, 1955) found that digitalis glycosides increase the performance of animals in stress experiments and prevent the adrenal hypertrophy caused by exercise. Metzler & Greeff (1954) observed that relatively small doses of k-strophantin cause an adrenal hypertrophy in rats, even after hypophysectomy. They conclude that digitalis glycosides have a specific adrenal-stimulating effect. Pyörälä & Eränkö (1957) found that large doses of digitoxin, k-strophantin, and lanatoside C in rats cause both an adrenocortical and adrenomedullary stimulation as indicated by increases in the adrenal weight and adrenomedullary volume, by increases in the absolute amounts and/or concentrations of adrenal cholesterol and ascorbic acid, and by the disappearance of the sudanophobe zone between the zona glomerulosa and zona fasciculata. A smaller dose of digitoxin, however, did not cause any significant changes in the adrenal cortex and medulla.

The purpose of this work was to study the effect of digitoxin and k-strophantin on the adrenal weight, on the content of adrenal cholesterol and ascorbic acid, as well as on the distribution of lipids in the adrenal cortex in guinea-pigs. In addition, the effect of glycosides on some other organs, especially on the testes and accessory male sex organs, was studied.
Digitoxin (Merck) was dissolved in a mixture containing 50 per cent (v/v) ethyl alcohol, 30 per cent glycerol and 20 per cent distilled water. The concentration of digitoxin was 0.4 mg./ml. K-strophanthin (Boehringer & Soehne) was dissolved in 20 per cent ethyl alcohol in a concentration of 0.1 mg./ml. The solvent for digitoxin was used as a control solution.

Twenty-eight male guinea-pigs were used in the experiments. They were fed on the standard laboratory diet. Animals were divided into three groups:

1. **Control group**: 8 guinea-pigs were injected with the control solution.
2. **Digitoxin group**: 8 guinea-pigs were injected with digitoxin 0.2 mg./kg. body weight.
3. **Strophanthin group**: 12 guinea-pigs were injected with k-strophanthin 0.05 mg./kg. body weight.

Injections were given subcutaneously daily for 28 days. Syringes graded to 0.005 ml. were used in order to get the doses in proportion to the animal’s weight as accurately as possible.

14 days after the beginning of the experiment electrocardiograms were taken under light ether anesthesia. Standard and unipolar limb leads were recorded with Mingograph (Elema).

29 days after the beginning of the experiment the animals were decapitated with sharp scissors without anesthesia. The adrenals were removed as quickly as possible, dissected free from adipose tissue and weighed on a torsion balance.

Left adrenals were immediately frozen fresh with solid carbon dioxide and kept in a frozen state until the chemical analyses were made. Each frozen left adrenal was homogenized in a glass homogenizer in one ml. of distilled water. Water was then added to make 6 ml. One ml. of the homogenate was used for the determination of total cholesterol which was performed in duplicate by the method of Sperry & Webb (1950). Four ml. of the homogenate was used for the determination of total ascobic acid which was performed in duplicate by a method slightly modified from that of Schaffert & Kingsley (1955).

Right adrenals were immersed in 4 per cent solution of neutral formalin for 48 hours. They were then sectioned at 10 μ with a freezing microtome. For the demonstration of cortical lipids, sections were stained with Sudan IV and mounted in glycerol.

The hypophysis, thyroid, testes, thymus, spleen, heart, kidneys, and liver were weighed. The testes, prostate, seminal vesicles, and mammary glands were fixed in neutral formalin, embedded in paraffin, sectioned at 5 μ and stained with hemalum-eosin and Weigert-van Gieson stain.

Differences between the control and glycoside groups were tested with Student’s t-test (Fisher, 1950).

**RESULTS**

**General observations.** No significant difference in weight gain was observed during the experiment between the digitoxin and control groups (Table 1). Animals treated with strophanthin showed a marked loss of weight during the first week of the experiment and three of them died. Thereafter the weight of the animals began to increase. The final weight gain, however, was smaller.
Table 1.
Changes in body weight.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Digitoxin group</th>
<th>Strophantin group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 9</td>
</tr>
<tr>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
</tr>
<tr>
<td>Initial body weight (gm.)</td>
<td>328 ± 40.7</td>
<td>315 ± 30.9</td>
<td>318 ± 16.8</td>
</tr>
<tr>
<td>Final body weight (gm.)</td>
<td>429 ± 52.7</td>
<td>425 ± 50.0</td>
<td>361 ± 19.6</td>
</tr>
<tr>
<td>Change in body weight (gm.)</td>
<td>+101 ± 29.2</td>
<td>+110 ± 30.0</td>
<td>+43 ± 24.7</td>
</tr>
</tbody>
</table>

n = number of animals
2) P ~ 0.01
3) P < 0.001

in the strophanthine group than in the control group (Table 1). The difference was highly significant.

No definite pathological changes were observed in electrocardiograms taken on the 14th day of the experiment.

Adrenals. The mean absolute and relative weights of the adrenals and the mean absolute amounts and concentrations of cholesterol and ascorbic acid in the left adrenals are given in Table 2.

Table 2.
Adrenal weight, adrenal cholesterol, and adrenal ascorbic acid.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
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<tr>
<td></td>
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<td>n = 8</td>
<td>n = 9</td>
</tr>
<tr>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
<td>S. D.</td>
</tr>
<tr>
<td>Adrenal (fresh) weight (mg.)</td>
<td>200 ± 29.6</td>
<td>189 ± 29.0</td>
<td>177 ± 28.4</td>
</tr>
<tr>
<td>(mg./100 gm. final body weight)</td>
<td>47.1 ± 8.1</td>
<td>45.3 ± 8.8</td>
<td>49.1 ± 8.1</td>
</tr>
<tr>
<td>Left adrenal cholesterol (mg.)</td>
<td>5.30 ± 0.73</td>
<td>4.84 ± 0.93</td>
<td>4.44 ± 0.77</td>
</tr>
<tr>
<td>(per cent of adrenal weight)</td>
<td>5.97 ± 0.56</td>
<td>4.78 ± 0.51</td>
<td>4.85 ± 0.37</td>
</tr>
<tr>
<td>Left adrenal ascorbic acid (mg.)</td>
<td>0.206 ± 0.038</td>
<td>0.203 ± 0.021</td>
<td>0.181 ± 0.045</td>
</tr>
<tr>
<td>(per cent of adrenal weight)</td>
<td>0.197 ± 0.037</td>
<td>0.204 ± 0.035</td>
<td>0.194 ± 0.038</td>
</tr>
</tbody>
</table>

n = number of animals
1) P ~ 0.05
No significant differences were observed in the mean absolute or relative weights of the adrenals, in the mean concentration of adrenal cholesterol, or in the mean absolute amount or concentration of adrenal ascorbic acid between the glycoside-treated groups and the control group. The mean absolute amount of adrenal cholesterol was smaller in the strophanthin than in the control group. The difference was significant at the 5 per cent level. There was no significant difference in the absolute amount of adrenal cholesterol between the digitoxin and the control group.

No clear differences were observed in the distribution or amount of Sudan-positive lipids in the adrenal cortex between the glycoside-treated and the control group.

Testes and accessory male sex organs. In the digitoxin group no significant difference was observed in the mean relative weight of the testes as compared with the control group (Table 3). The sizes of the prostates and seminal vesicles did not differ from those of the control group. In a detailed microscopical examination no differences between the digitoxin and control groups were observed in the testes, prostates, and seminal vesicles.

A marked involution of the testes was observed in the strophanthin group as compared with the control group (Table 3). The difference was highly significant. The sizes of the prostates and seminal vesicles were about one half of those of the control group. A detailed microscopical examination of the testes of the strophanthin group revealed narrow seminiferous tubules with atrophied

**Table 3.**
Relative organ weights (mg./100 gm. final body weight).

<table>
<thead>
<tr>
<th></th>
<th>Control group n = 8</th>
<th>Digitoxin group n = 8</th>
<th>Strophantin group n = 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S. D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Hypophysis</td>
<td>3.2</td>
<td>0.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Thyroid</td>
<td>11.4</td>
<td>2.1</td>
<td>12.2</td>
</tr>
<tr>
<td>Testes</td>
<td>681</td>
<td>89</td>
<td>590</td>
</tr>
<tr>
<td>Thymus</td>
<td>95</td>
<td>15</td>
<td>92</td>
</tr>
<tr>
<td>Spleen</td>
<td>208</td>
<td>74</td>
<td>193</td>
</tr>
<tr>
<td>Heart</td>
<td>393</td>
<td>60</td>
<td>389</td>
</tr>
<tr>
<td>Kidneys</td>
<td>809</td>
<td>89</td>
<td>849</td>
</tr>
<tr>
<td>Liver</td>
<td>4890</td>
<td>740</td>
<td>4680</td>
</tr>
</tbody>
</table>

n = number of animals
¹) P < 0.05
³) P < 0.001
germinal epithelium. Epithelial cells of the prostate and seminal vesicles showed signs of diminished secretory activity as compared with the controls.

In a microscopical examination no clear differences in the glandular structure of the mammary glands could be observed between the glycoside-treated groups and the control group.

Weights of some other organs. The relative organ weights are given in Table 3.

There were no significant differences in the mean relative weights of the hypophysis, thyroid, testes, thymus, spleen, heart, kidneys, or liver between the digitoxin and the control group.

The mean relative weights of the hypophysis and thyroid were increased, whereas the mean relative weight of the spleen was decreased in the strophanthin group as compared with the control group. The differences were significant at the 5 per cent level. There were no significant differences in the mean relative weights of the thymus, heart, kidneys, or liver between the strophanthin and control groups.

DISCUSSION

In the present work digitoxin and k-strophanthin in the dosage used caused no significant changes in the adrenal weight, in the concentration of adrenal cholesterol, and in the absolute amount or concentration of adrenal ascorbic acid in guinea-pigs. K-strophanthin caused a slight decrease in the absolute amount of the adrenal cholesterol. Glycosides caused no changes in the amount or distribution of Sudan-positive lipids in the adrenal cortex. These results are not in agreement with those of Metzler (1951, 1952), who observed a marked adrenal hypertrophy in guinea-pigs, given 0.045 mg./kg. body weight of k-strophanthin daily for 21 days. In the present work the dose of k-strophanthin (0.05 mg./kg. body weight) was 1/9 and that of digitoxin (0.2 mg./kg. body weight) i.e. 1/7 of the lethal dose for the strain of guinea-pig used (Kuusisto, 1952). These doses may be considered rather large. The dose of k-strophanthin was somewhat toxic, as was indicated by its effect on the weight of the animals. These doses of digitoxin and k-strophanthin could be expected to cause changes in the adrenals, providing glycosides had a specific adrenal-stimulating effect. A previous investigation (Pyörälä & Eränkö, 1957) has shown that large doses of digitoxin, k-strophanthin, or lanatoside C in rats cause both an adrenocortical and adrenomedullary stimulation, whereas a smaller dose of digitoxin caused no changes in the adrenal cortex or medulla.

Strophanthin treatment caused a marked involution of the testes and accessory male sex organs. Siliquini & Durando (1954) have shown that digitalis glycosides have an oestrogen-like effect on the endometrium and vaginal epithelium.
of castrated rats. Some cases of gynecomastia (LeWinn, 1950, 1953, Olmer et al., 1954) or mammary hypertrophy (Calov & Whyte, 1954) occurring during clinical digitalis therapy have been described. In this investigation no signs of hypertrophy could be observed in the mammary glands of male guinea-pigs treated with digitoxin or k-strophantin. The involution of the testes and accessory male sex organs of the strophantin-injected animals may also be due to a toxic effect of the drug. According to Hueper & Ichniowski (1941) single lethal doses of digitalis glycosides cause a degeneration of the spermatogenic epithelium in guinea-pigs.

Strophantin also caused a slight hypertrophy of the hypophysis and thyroid. Kuusisto (1952) found that digitalis glycosides have an inactivating effect on the thyroid.

According to some previous studies digitalis glycosides cause hypertrophy of the heart in guinea-pigs (Metzler, 1951, 1952) and in rats (Metzler & Greeff, 1954). In the present work digitoxin and k-strophantin failed to bring about any changes in the heart weight of guinea-pigs.

**SUMMARY**

Daily subcutaneous injections of 0.2 mg./kg. body weight of digitoxin or 0.05 mg./kg. body weight of k-strophantin given for 28 days, in guinea-pigs had no effect on the adrenal weight, on the concentration of adrenal cholesterol, and on the absolute amount or concentration of adrenal ascorbic acid. Strophantin caused a slight decrease in the absolute amount of adrenal cholesterol. Glycosides caused no changes in the amount or distribution of Sudan-positive lipids in the adrenal cortex.

Strophantin injections caused a marked involution of the testes and accessory male sex organs. A slight hypertrophy of the hypophysis and thyroid, and also an involution of the spleen were observed in the strophantin-injected guinea-pigs.

**REFERENCES**