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# THE INFLUENCE OF ROLITETRACYCLINE UPON SOME METABOLIC PARAMETERS OF RABBIT HEART MUSCLE

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**Abstract**—The effect of rolitetracycline (*N*-pyrolidin-methyl-tetracycline) on the consumption of $O_2$ and $P_i$ by heart muscle mitochondria on the glycolytic activity of heart muscle homogenates, on the glycogen concentration in the heart muscle and glycaemia in rabbits after administration of 20 mg/kg body wt daily for 6 or 12 days was studied. No significant changes were found in any of the parameters after 6 days of treatment. Treatment for 12 days resulted in hypoxia, increased consumption of inorganic phosphate, increased glycaemia, increased glycolytic activity due to hypoxia and decreased glycogen concentration in the heart muscle.

Antibiotics of the tetracycline series cause adverse metabolic effects. The inhibitory effects on cell respiration and phosphorylation of many organs and tissues [1–6], including the heart muscle [6, 7], and on cardiac function [8–10] have been described by a number of authors.

The present investigation aimed to clarify the mechanism of the undesirable effects of rolitetracycline (RTC) upon the heart muscle by examining some principal metabolic parameters. $O_2$ and $P_i$ consumption by heart muscle mitochondria, glycolytic activity of heart muscle homogenates, glycogen concentration in heart muscle and glycaemia in rabbits after 6 or 12 days' treatment with a daily dose of 20 mg RTC/kg have been investigated.

## MATERIAL AND METHODS

*Chemicals.* POD, GOD, *O*-dianizidinhydrochloride, glucose (Boehringer); NAD (Koch–Light); $Na_2ATP$ (Reanal); rolitetracycline (Spofa). The other chemicals used were obtained from Lachema.

*Methods.* Female chinchilla rabbits with an average weight of 2·5 kg were given 20 mg/kg of rolitetracycline daily for 6 or 12 days. During the experiment the animals were fed a standard diet and allowed water *ad lib.* Animals which did not receive antibiotics served as controls.

Immediately after sacrifice the heart was removed from the body, cleaned of fat and other tissue remnants. The heart muscle was homogenized in 10 vol. 0·1 M phosphate buffer, pH 7·4. In the preparation of mitochondria [11], 1 g of tissue was homogenized in 3 ml of 0·25 M sucrose containing 1·85 g $K_2HPO_4$/100 ml. All the procedures were carried out at a temperature of 0°–4°.

The consumption of oxygen by heart mitochondria was measured manometrically using an incubation medium containing 0·65 ml 1 M sucrose, 0·05 ml 1 M phosphate buffer, pH 7·2, 0·1 ml 0·05 M $Na_2ATP$, 0·005 ml 0·1 M $MgSO_4$, 0·1 ml 1·0 M glucose, 0·1 ml 0·5 M malate, 0·1 ml 0·5 M pyruvate, 0·05 ml 1% hexokinase, 0·45 ml mitochondrial suspension at a protein concentration of 13 mg/ml and 0·4 ml distilled water. The phosphorus concentration before and after 20 min incubation was determined according to Goldenberg and Fernandez [12]. Protein concentration was measured by Hartee's [13] modification of Lowry's method.

Glycolytic activity was measured by the method of Reeves [14] in an incubation medium containing 0·2 ml 0·25 M histidine, 0·2 ml 0·17 M NaCl, 0·2 ml 0·55 M KCl, 0·2 ml 0·03 M $MgCl_2$, 0·2 ml 0·07 M glucose, 0·2 ml 9 mM $NAD^+$, 0·2 ml 0·025 M ATP, 0·2 ml $H_2O$ and 0·5 ml of heart muscle homogenate. Glycolytic activity is expressed in $\mu$moles of lactate/mg protein per hr [15].

The glycogen concentration was measured according to Krisman [16] in mg/g of tissue and the glycaemia by means of the GOD method in mg/100 ml of blood. The difference between the control and experimental group of animals was evaluated by the Student's *t*-test.

## RESULTS AND DISCUSSION

As indicated in Table 1, RTC administration for 6 days did not induce any change in the oxygen consumption by cardiac mitochondria, whereas administration for 12 days caused a significant decrease in oxygen consumption. Consumption of inorganic phosphate increased in comparison with the control group after 6 days as well as after 12 days, administration of RTC. Glycolytic activity showed the same pattern (Table 2); no significant changes occurred after the administration of antibiotics for 6 days, whereas after 12 days glycolytic activity increased significantly and glycogen concentration in the heart muscle decreased correspondingly (Table 3). Compared with the controls glycaemia appeared to increase in both experimental groups.

In contrast to the skeletal muscle the heart muscle is known to utilize lactate for its energy needs, provided the oxygen supply is sufficient. This ability to

Table 1. $O_2$ and $P_i$ consumption by heart muscle mitochondria after rolitetracycline administration

| Period of administration (days) | | n | μmoles $O_2$/mg protein/min | ±% | P | μmoles $P_i$/mg protein/min | ±% | P | P:O |
|---|---|---|---|---|---|---|---|---|---|
| 6 | control | 5 | 0·052 ± 0·006 | — | — | 0·039 ± 0·004 | — | — | 1·63 |
| | experimental | 12 | 0·055 ± 0·003 | +5 | >0·05 | 0·043 ± 0·007 | +11 | >0·05 | 1·59 |
| 12 | control | 16 | 0·068 ± 0·004 | — | — | 0·043 ± 0·005 | — | — | 1·27 |
| | experimental | 17 | 0·053 ± 0·003 | −21 | <0·05 | 0·049 ± 0·006 | +16 | >0·05 | 1·98 |

Results are expressed as mean ± S.E.M. n, number of individuals. Significance was determined by Student's *t*-test.

oxidase lactate is lost under conditions of hypoxia and the lactate concentration in the heart muscle increases [17] as a result of the breakdown of its own glycogenic supplies. RTC administration for 12 days results in an increased lactate concentration (Table 2) during the experimental period and decreased glycogen concentration (Table 3).

The results obtained in the present studies can be interpreted on the basis of some data in the literature. De Jonge [6] does not give an unambiguous explanation of inhibitory effects caused by antibiotics of the tetracycline series on the oxidation processes. He admits the possibility of a loss of $Mg^{2+}$ ions as a result of the formation of a chelate with tetracyclines, or a toxic effect on proteosynthesis and thus a quantitative decrease of some enzyme systems. According to our experiments, in which tetracycline administration decreased oxygen consumption and increased both phosphorylation and lactate formation, the mechanism of so-called side-effects upon the activity of the heart muscle is not likely to be effected only by the interaction with $Mg^{2+}$ ions. It seems more probable that this effect is caused by the inhibition of proteosynthesis. This may result in overall structural changes of the mitochondrial membranes, with consequent disturbances in electron transfer i.e.

Table 2. Glycolytic activity of the heart muscle homogenate after rolitetracycline administration

| Period of administration (days) | | n | μmoles of lactate/mg protein/hr | ±% | P |
|---|---|---|---|---|---|
| 6 | control | 12 | 6·95 ± 0·06 | — | — |
| | experimental | 12 | 7·17 ± 0·04 | +3 | >0·05 |
| 12 | control | 15 | 8·63 ± 0·31 | — | — |
| | experimental | 14 | 9·73 ± 0·28 | +13 | <0·05 |

Table 3. Glycogen concentration in the heart muscle after rolitetracycline administration

| Period of administration (days) | | n | Glycogen (mg/g of wet tissue) | ±% | P |
|---|---|---|---|---|---|
| 6 | control | 16 | 7·74 ± 0·26 | — | — |
| | experimental | 15 | 7·13 ± 0·16 | −8 | >0·05 |
| 12 | control | 16 | 6·32 ± 0·17 | — | — |
| | experimental | 16 | 3·90 ± 0·02 | −38 | <0·05 |

Table 4. Glycaemia after rolitetracycline administration

| Period of administration (days) | | n | Glucose concn. (mg/100 ml of blood) | ±% | P |
|---|---|---|---|---|---|
| 6 | control | 12 | 118·92 ± 1·32 | — | — |
| | experimental | 12 | 131·23 ± 2·30 | +10 | <0·05 |
| 12 | control | 10 | 106·86 ± 1·60 | — | — |
| | experimental | 10 | 114·22 ± 1·50 | +7 | <0·05 |

when tetracyclines act as pseudocarriers of electrons.

Heart muscle is supposed to compensate hypoxia induced by rolitetracycline by obtaining the needed energy anaerobically. This explains the increased depletion of glycogen, the increased consumption of inorganic phosphate and the increased concentration of lactate. This is in agreement with the findings of Bing [17] and Hochrein [118] who found that lactate is formed from the glycogen stored in the heart muscle under hypoxic conditions. Our results indicating increased glycaemia (Table 4) as a result of increased glycogen depletion fit into the general picture.

In conclusion, prolonged administration of RTC to rabbits under our experimental conditions inhibited the respiration of cardiac muscle, causing increased phosphorylation of glycogen. This manifests itself by both the decreased glycogen concentration in the heart and increased lactate concentration which accumulates under hypoxic conditions. This effect becomes more pronounced as the period of antibiotic administration is extended.

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