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# Morphometric Observations on the Rat Heart After High-Dose Treatment with Cortisol***

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**Summary.** Male Wistar rats were treated with high cortisol doses for 1 week. The dose administered daily was 15 mg per animal in group 1 (7 animals) and 30 mg in group 2 (7 animals). 7 rats served as control group. After cortisol treatment the body weights decreased due to skeletal muscle catabolism and the heart weights increased. Morphometric analysis of the left ventricular posterior papillary muscles gave evidence that the increased heart weights resulted from an increased number of mitochondria and an increased volume of the cytoplasm, whereas the myofibrillar mass was not affected. The surface area of inner mitochondrial membranes (+cristae mitochondriales) per myofibrillar unit volume increased from 15.7 $\mu^2/\mu^3$ to 21.3 $\mu^2/\mu^3$ in group 1 and 21.4 $\mu^2/\mu^3$ in group 2. Ultrastructural changes indicating myocardial cell damage were absent. Similar quantitative results have been reported to occur in the early phase of cardiac overload. For elucidating the hemodynamic effects of glucocorticoid a second experiment was performed: 7 Wistar rats were treated with cortisol in the same way as group 1, 7 others of the same body weight served as control. The systolic arterial pressure was significantly elevated in the cortisol group. Though myocardial tissue is known to be able to accumulate large quantities of glucocorticoids our results indicate that the application of high cortisol doses for a short time does not produce myocardial cell damage and does not suppress the myocardial adaption to the glucocorticoid-induced hypertension, i.e. hypertrophy. On the contrary, it seems to be possible that the adaption process is itself facilitated or accelerated by the presence of high cortisol concentrations in the heart. This thesis is supported by the considerably higher relative heart weights in the cortisol groups and is in agreement with observations reported by other authors.

**Key words:** Cortisol – Myocardium – Ultrastructure – Morphometry.

* Dedicated to Professor Dr. W. Doerr on the occasion of his 65th birthday

** The results have been partially reported in 1977 (cf. G. Mall and H. Reinhard, Verh. Dtsch. Ges. Path. 61, 445)

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## Introduction

Glucocorticoids are thought to act on myocardial cells. H. Selye (1961, 1969) found that corticoids having both glucocorticoid and mineralocorticoid properties can produce cardiac necroses if sensitization by a sodium salt, for example $Na_2HPO_4$, is performed. An ultrastructural study was published by D'Agostino (1964). On the other hand there are many investigations which support a protective glucocorticoid effect on ischaemic myocardial cells (Libby et al., 1973; Spath et al., 1974; Spath and Lefer, 1975; Mueller et al., 1977; Busuttil and George, 1978; Kloner et al., 1978). The application of glucocorticoids alone and their effect on normal myocardium was examined by several groups. Nienhaus et al. (1963) treated rats with cortisol for three weeks. They found increased muscle fibre diameters and some ultrastructural changes which they regarded as the result of cortisol-induced arterial hypertension rather than of direct cortisol effects. Ketelsen et al. (1974) studied rabbit hearts after treatment with a single dose of glucocorticoids (methylprednisolone and triamcinolone). 19 to 60 days later they found extremely attenuated myofibrils, 35 to 60 days later megamitochondria and mitochondrial inclusions of glycogen and at the 19th day a great number of sarcoplasmic lipid droplets. Between the 3rd and the 12th day there were only slight changes to be seen using conventional electron microscopic methods. The freeze etch technique, however, showed an increased number of plasmalemmal membrane particles. This was explained as the morphological equivalent of activated cardiac metabolism. In performing a morphometric study we hoped to recognize quantitative structural changes which might contribute to the understanding of the early cortisol effects on the heart.

## Material and Methods

21 male Wistar rats (body weight: $123 \pm 1$ g) were randomly divided into three groups of seven animals each. The first group (group 1) was treated with 15 mg cortisol (Hydrocortison Hoechst) per animal per day, the second group (group 2) with 30 mg per animal per day and the third group (control group) with Ringers solution. The daily dose was applicated by one single intraperitoneal injection between 4 p.m. and 5 p.m. At the seventh day all rats were fixed by perfusion between 8 a.m. and 1 p.m. After weighing the hearts the left ventricular papillary muscles were removed for electron microscopic morphometry and embedded in Araldite. The procedure is described in detail elsewhere (Mall et al., 1978).

### *Morphometry*

The morphometric analysis was performed on sections cut at an angle of 32,4° to the longitudinal axis of the left ventricular posterior papillary muscle. This angle has been demonstrated recently to be optimal in the case of geometrically anisotropic surfaces (Mall et al., 1978). The following quantitative variables were evaluated: Volume densities of myocardial cells, interstitial tissue ("extramyocardiocytic tissue"), sarcoplasm, myofibrils, mitochondria and cytoplasm, surface densities of myocardial cells and of outer and inner mitochondrial membranes (+cristae mitochondriales). The morphometric procedure is described and discussed elsewhere (Mall et al., 1978). Using this method it is not necessary to analyze more than 1 UD section per animal.

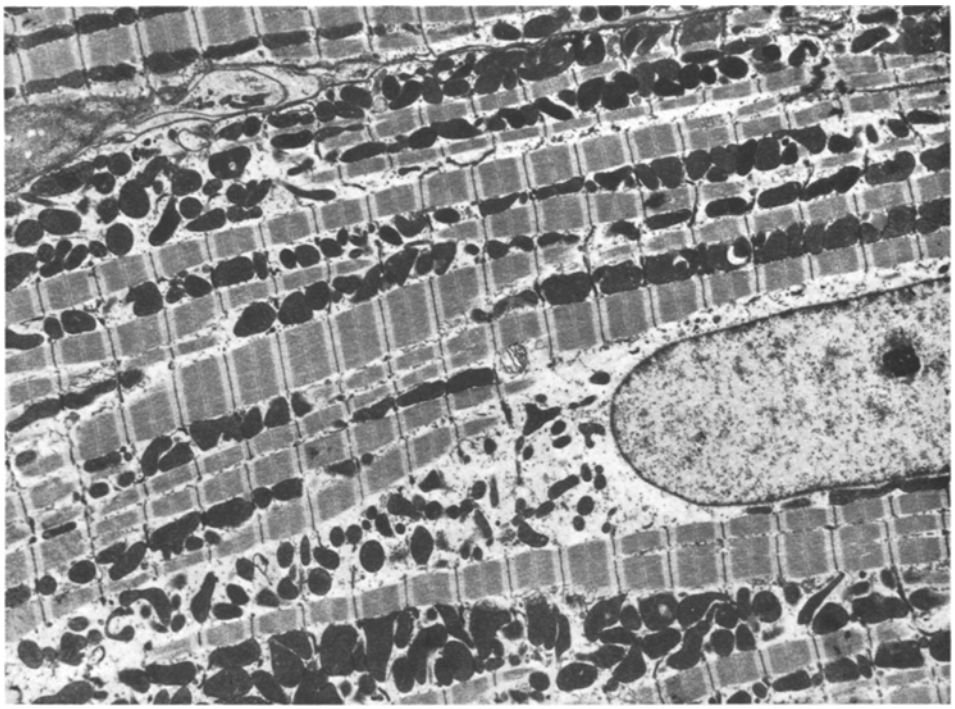


**Fig. 1.** Magnification 4,300:1. Micrograph from a cortisol treated rat (group 2). Longitudinal section of the left ventricular anterior papillary muscle. Only slight changes. Increased sarcoplasmic matrix (ground plasm). In the middle a single vacuolated mitochondrium

## Results

The electron microscopic examination of the left ventricular papillary muscles showed a nearly normal ultrastructure in both the experimental groups and in the control group (Fig. 1). Only slight changes could be seen. In group 1 small lipid droplets adjacent to the mitochondria seemed to be slightly increased, particularly in mitochondrial invaginations. In group 2 the mitochondrial matrix was slightly less electron dense indicating a slight swelling (Fig. 2). The ground sarcoplasm was increased (Figs. 1 and 2) in both groups.

### *Quantitative Results*

The body weights of the control rats increased from 122 g on average at the begin of the experiment to 146 g at the end, whereas the body weights of the cortisol treated rats decreased from 122 g to 111 g in group 1 and from 125 g to 106 g in group 2 (Fig. 3). In contrast, the heart weights of the cortisol treated rats were higher than the weights of the control, 0.72 g in group 1, 0.77 g in group 2, and 0.62 g in the control (Fig. 4). The morphometric results are illustrated in table 1. The volume densities of mitochondria (Fig. 5) and

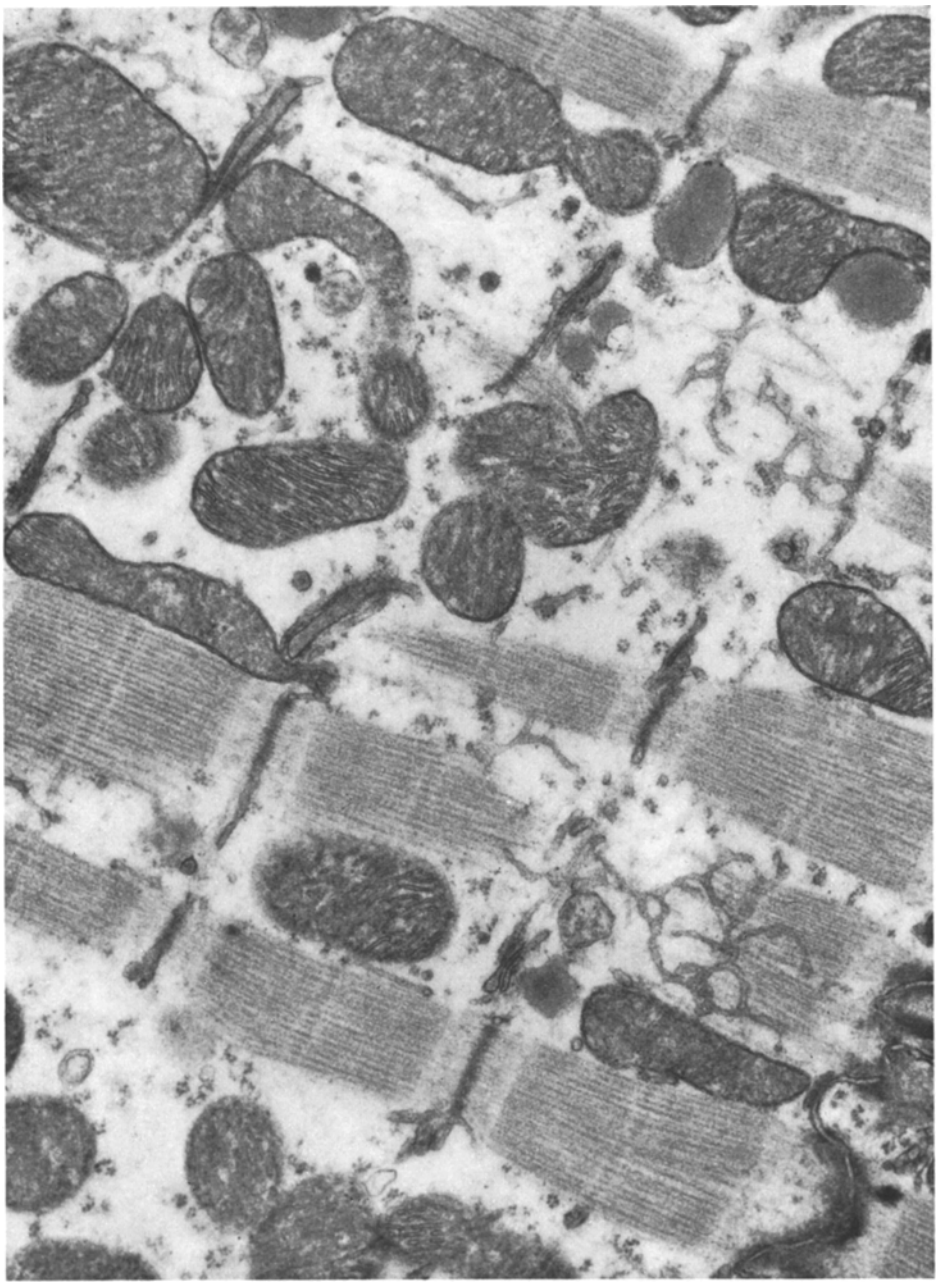
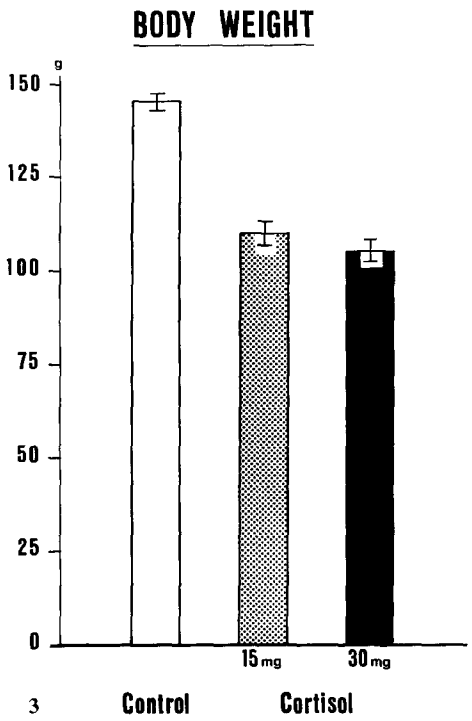


**Fig. 2.** Magnification 22,000:1. Micrograph from a cortisol treated rat (group 2). Longitudinal section of the left ventricular anterior papillary muscle. Slight mitochondrial swelling, increased sarcoplasmic matrix, some lipid droplets. Normal structure of T tubules and sarcoplasmic reticulum

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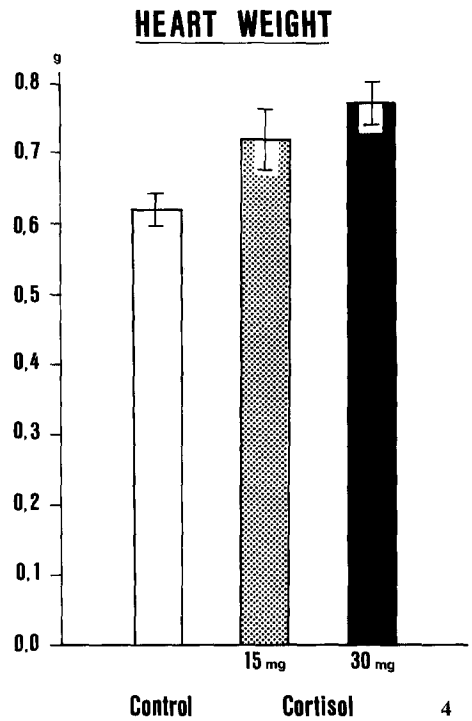


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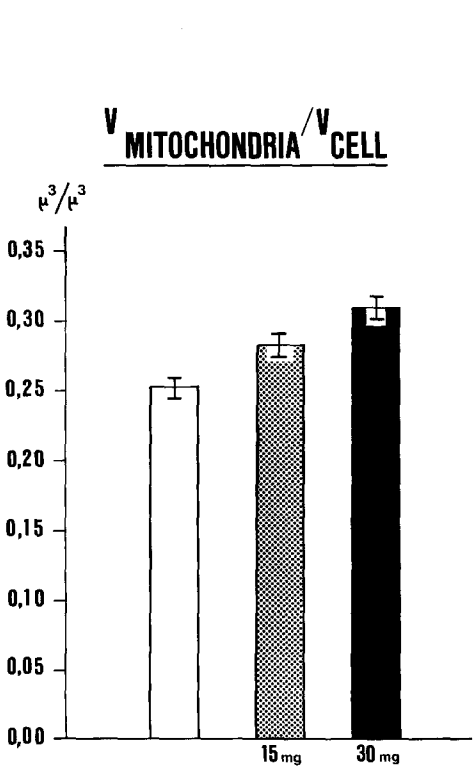


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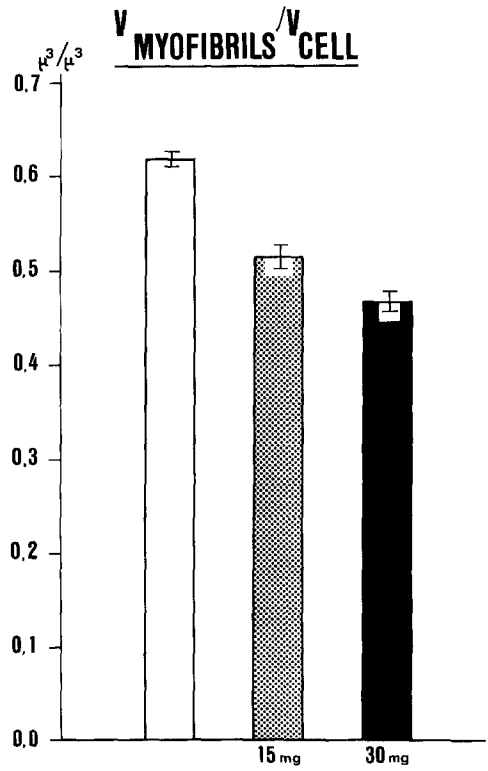


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**Figs. 3–6.** Body weights, heart weights, volume densities of mitochondria, volume densities of myofibrils. Graphic demonstration of the differences between the animal groups (+standard errors)

**Table 1.** Morphometric parameters

| Component | Parameter | Reference volume | Dim | Control | | Group 1 | | Group 2 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| Myocardial cells | Volume | Total tissue | $\mu^3/\mu^3$ | 0.8089 | 0.0121 | 0.8619* | 0.0065 | 0.8532* | 0.0135 |
| Interstitial space | Volume | Total tissue | $\mu^3/\mu^3$ | 0.1911 | 0.0121 | 0.1381* | 0.0065 | 0.1468* | 0.0135 |
| Sarcoplasm | Volume | Total tissue | $\mu^3/\mu^3$ | 0.7961 | 0.0117 | 0.8487* | 0.0072 | 0.8397* | 0.0138 |
| Mitochondria | Volume | Sarcoplasm | $\mu^3/\mu^3$ | 0.2529 | 0.0044 | 0.2863*** | 0.0061 | 0.3129*** | 0.0057 |
| Myofibrils | Volume | Sarcoplasm | $\mu^3/\mu^3$ | 0.6120 | 0.0062 | 0.5091*** | 0.0107 | 0.4666*** | 0.0079 |
| Ground plasm (+tubules) | Volume | Sarcoplasm | $\mu^3/\mu^3$ | 0.1351 | 0.0098 | 0.2046*** | 0.0118 | 0.2205*** | 0.0082 |
| Myocardial cells | Surface | Myocardial cells | $\mu^2/\mu^3$ | 0.3180 | 0.0149 | 0.2635** | 0.0056 | 0.2403*** | 0.0076 |
| Outer mitochondrial membranes | Surface | Mitochondria | $\mu^2/\mu^3$ | 7.2614 | 0.2020 | 7.1645 | 0.2238 | 6.4221* | 0.2585 |
| Inner mitochondrial membranes (+cristae)[a] | Surface | Mitochondria | $\mu^2/\mu^3$ | 38.22 | 0.86 | 37.86 | 1.53 | 31.88* | 1.93 |

For statistical evaluation the *t* test has been used. The experimental groups were compared with the control. $*p<0.05$, $**p<0.01$, $***p<0.001$.

[a] Corrected values (cf. Mall et al., 1977, and Mall et al., 1978).

cytoplasm were raised in the cortisol groups, whereas the volume density of myofibrils (Fig. 6) was lowered. The surface to volume ratio of the cardiac myocytes decreased ($S_V$ ratio). The mitochondrial $S_V$ ratio and the surface area of inner mitochondrial membranes (+cristae mitochondriales) per mitochondrial volume were the same in group 1 and in the control, whereas they were slightly decreased in group 2. The interstitial space ("extramyocardiocytic tissue") decreased.

## Discussion

Before discussing the results it is necessary to emphasize the moderate growth of the control rats during the experiment. Taking in consideration the final heart weights of 0.62 g on average ($4.2^0/_{00}$ of the body weight) in the control we suppose that at the begin of the experiment the heart weights were about 0.52 g. Therefore, we have to postulate accelerated growth of the hearts in the cortisol treated groups.

Morphometric data are relative, they are related to a reference volume. In order to understand quantitative structural alterations one has to interprete the relative data with respect to the absolute myocardial mass which depends upon the heart weights. Assuming that the response of the papillary muscles to cortisol treatment is similar to that of the chamber walls and the septum we conclude that there was an accelerated growth of mitochondria and cytoplasm in the experimental groups, because both the heart weights and the volume densities were increased. On the other hand the decreased myofibrillar volume densities are nearly inversely proportional to the increased hearts weights (in other words: myofibrillar volume per volume total myocardial tissue multiplied by heart weights is constant in the three groups) which indicates that the myofibrillar growth was not significantly affected by cortisol treatment. If this is true, we can calculate that the total mitochondrial volume is about 35% higher in group 1 and about 60% higher in group 2 than in the control group. An increase of the total mitochondrial mass could theoretically be caused by mitochondrial swelling or by an increased number or by both. In our case the $S_V$ ratios of mitochondria in the control group and in group 1 were the same indicating an increased number rather than swelling.

In contrast the slightly lowered $S_V$ ratio in group 2 and the slightly decreased surface density of the inner mitochondrial membranes are the quantitative correlates of the slightly less electron dense mitochondrial matrix described above, i.e., there was slight mitochondrial swelling.

McCallister and Page (1973) proposed the index: surface area of inner mitochondrial membranes per myofibrillar volume, for comparing the structures mainly involved in the ATP production and consumption. This ratio ammounts to 15.7 $\mu^2/\mu^3$ in the control group, 21.3 $\mu^2/\mu^3$ in group 1 and 21.4 $\mu^2/\mu^3$ in group 2. The increased heart weights correspond to the decreased $S_V$ ratios of the myocardial cells. In the case of cylindrical structures the radius r can be calculated from the $S_V$ ratio ($S_V = 2/r$; Weibel, 1969). We derive the following diameter estimates: 12.5 $\mu$ in the control group, 15 $\mu$ in group 1 and 16.5 $\mu$ in group 2. The raised muscle fibre diameters are the cause of the decreased volume densities of the interstitial tissue, the interstitium itself is not affected by cortisol treatment.

Comparing our quantitative structural changes with the plasmalemmal changes confirmed by Ketelsen et al. we find that different methods – morphometry and freeze etching – lead to the same conclusion: in the early phase of glucocorticoid application an activation of the myocardial metabolism may be assumed. Our main finding was an increased mitochondrial mass and an increased surface area of the inner mitochondrial membranes, the main finding of Ketelsen et al. was an increased number of membrane particles on the plasmalemma. An increased mitochondrial volume density in myocardial cells has been reported by several groups which have investigated the early stage of cardiac pressure overload (Meerson et al., 1964; Hatt et al., 1978; Hatt, 1977) and volume overload (Bózner and Meesen, 1969).

McCallister and Page (1973) observed the same effect after thyroxin application to thyroidectomized rats, Bózner et al. (1969) after feeding a thiamine deficient diet to rats. A decreased mitochondrial volume density was detected after long-standing pressure overload (Meerson et al., 1964; Wollenberger and Schulze, 1962; Poche, 1968; Anversa, 1971; Page et al., 1972; Page and McCallister, 1973). Recently Warmuth et al. (1978) found a decreased volume fraction of mitochondria in human subjects with severe hypertrophy. Tate and Herbener (1976) demonstrated the same effect in aging mice and Smith and Page after hypophysectomy in rats. Looking for a structural correlate of function it is evident that the surface area of inner mitochondrial membranes is more important than the mitochondrial mass. Not all the authors mentioned above measured the cristal surface density, but in most cases the mitochondrial mass seems to be correlated with the surface area of inner mitochondrial membranes.

Smith and Page (1976) considered that the index: surface area of inner mitochondrial membranes per myofibrillar volume was related to myosin ATPase activity. This idea is supported by the finding that this index is higher in ventricular myocardial cells from rats than in those from rabbits. This finding correlates with the observation of Delcayre and Swynghedauw (1975) who found that the ATPase activity of myosin from rat heart muscle is 4.5 times greater than from rabbit heart muscle. Furthermore, this concept is compatible with the fact that in cardiac hypertrophy both the myosin ATPase activity (Jacob et al., 1977; Swynghedauw et al., 1977) and the volume density of mitochondria – as described above – are depressed. Our results conclusively indicate an increased myosin ATPase activity i.e., an elevated ATP consumption by the myofibrils. Which factors could be responsible for the increased ATP consumption? As shown above our results are similar to those of acute cardiac overload. Studies on hemodynamic glucocorticoid effects have been reported, with controversal results. Sambhii et al. (1965) in normal human subjects, found an increase in cardiac output after large doses of glucocorticoids administered intravenously, but no change in arterial pressure. Similar changes were described by Hofmann and Emmrich (1959) during the first days following oral medication of glucocorticoids. Cortisone and ACTH administered for a period exceeding 2 weeks caused an increase in arterial pressure but no change in cardiac output resulted (Albert et al., 1955). V. Kuegelgen et al. (1959) in the rat found elevated arterial pressure in the first hours after glucocorticoid application, whereas the cardiac output was depressed because of an increased peripheral resistance. Friedman

et al. (1952) found a hypertensive effect of compound F (17-OH-Corticosterone-21-acetate) in the rat. As we were using high cortisol doses in our experiment we thought it necessary to examine the haemodynamic effects under our experimental conditions. 7 rats initially weighing 125 g were treated with cortisol in the same way as group 1 (15 mg cortisol/day), others of the same weight served as control.

The systolic arterial pressure was significantly elevated after seven days ($153.75 \pm 2.95$ mm Hg) compared with the control ($116.25 \pm 2.95$ mm Hg) ($p < 0.001$, $t$ test). Therefore, we conclude that pressure overload probably was the cause of increased myofibrillar ATP consumption. This conclusion is compatible with the results of Nienhaus et al. (1963). Our quantitative structural pattern relates to the early phase of myocardial hypertrophy as described above.

It should be noted that the adaption of the myocardial cells has been realized without focal disturbances of metabolism, because we did not observe any alteration of myofibrillar structure or severe mitochondrial alterations. Furthermore, it is likely that the increased mitochondrial mass following thyroxin application to thyroidectomized rats reported by McCallister and Page (1973) and the lowered mitochondrial mass following hypophysectomy reported by Smith and Page (1976) are also influenced by haemodynamic factors; it is well known, for example, that hypophysectomy depresses the arterial blood pressure (Fizel and Fizelova, 1972; Beznák, 1954).

On the other hand, some biochemical results indicating a direct action of glucocorticoids on the heart have been published. Seleznev et al. (1978) detected rat heart glucocorticoid-binding-proteins; binder I is a intramyocardial transcortin-like protein, binder II a heart cytoreceptor which might participate directly in the transfer of glucocorticoids into cell nuclei. Binder II is similar to cytoplasmic glucocorticoid proteins of typical target tissues for glucocorticoids which are considered at present as glucocorticoid cytoreceptors. Funder et al. (1973) described specific glucocorticoid receptors in the heart which are probably analogous to the binder II of Seleznev. The existence of specific myocardial glucocorticoid receptors supports the assumption of a direct glucocorticoid action on cardiac function (Funder et al., 1973). Myocardial cells have the capacity to accumulate large quantities of glucocorticoids with a significant fraction bound to cell membranes (Beardsley et al., 1976; Okuda et al., 1976). The working heart takes up cortisol much more avidly than does the resting skeletal muscle (Kolanowski and Lammerant, 1973). Bullock et al. (1972) tested various steroids in rats investigating the function of muscle ribosomes during the early phase of steroid induced catabolism. The loss of body weight was paralleled by loss in muscle weight. Ribosomal incorporation activity was decreased in skeletal muscles. However, heart weights were not affected by steroid administration and heart ribosomes maintained normal activity although concentrations of steroids in the heart 5 min after administration were 2–3 times that in skeletal muscle. The catabolic effects of glucocorticoids on skeletal muscles are well documented (Mayer et al., 1976; Shoji and Pennington, 1977). In our experiment the low body weights are related to severe skeletal muscle catabolism.

Nearly 3 decades ago Metzler (1952) postulated a hormonal cause for cardiac hypertrophy. He found that swimming rats develop higher heart weights, if

cortisol is administered. The cortisol was much more effective than the mineralocorticoid Percorten. On the other hand, adrenalectomized rats do not develop arterial hypertension after constriction of the aorta abdominalis (Beznák, 1954). Therefore, it seems to be possible, but not certain, that adrenocortical hormones modify the metabolic processes of the heart in the early stage of hypertrophy (Fizel and Fizelova, 1972). In our experiment the greatly increased relative heart weights (150% in group 1, 170% in group 2) may be the result of a facilitated or accelerated development of hypertrophy under high cortisol concentrations.

We conclude that in the early phase of cortisol administration myocardial cell damage does not occur, rather the glucocorticoid-induced hypertension stimulates an accelerated growth of the myocardial cells, i.e. hypertrophy. Therefore, it is probable that cortisol in the early phase does not induce catabolic processes in the heart as it does in skeletal muscles. Alternatively the adaption may be modified by the presence of high cortisol concentrations.

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