Conditioning by Cortisol for the Production of Acute Massive Myocardial Necroses During Neuromuscular Exertion

By Hans Selye, M.D., D.Sc.

Following pretreatment with cortisol and NaHPO₄, a brief period of neuromuscular stress regularly produces massive myocardial necroses in the rat. Pretreatment with desoxycorticosterone and the phosphate is less effective in this respect.

Previous observations have shown that rats, pretreated with 2-methyl-9a-chlorocortisol (Me-Cl-COL) and an excess of dietary phosphate, develop massive myocardial necroses within a few hours following exposure to various stressor agents. Me-Cl-COL is an artificial synthetic steroid which does not occur in nature; it possesses rather unusual pharmacologic properties in that it is not only extraordinarily active as a mineralocorticoid, but it also exhibits some glucocorticoid effects. It was therefore important to determine: (1) whether cortisol (a predominantly glucocorticoid substance known to be produced in excess during stress in man) could take the place of Me-Cl-COL in conditioning the rat for the production of acute myocardial necroses by neuromuscular exertion and (2) whether desoxycorticosterone (a virtually pure mineralocorticoid) exhibits similar effects.

Materials and Method

One hundred twenty female Sprague-Dawley rats, with a mean initial body weight of 96 gm. (range 90 to 108 gm.), were subdivided into 12 equal groups and treated as indicated in table 1.

Monobasic sodium phosphate (NaHPO₄·H₂O) was given in the form of a 15 per cent aqueous solution at the dose level of 2 ml., twice daily by stomach tube. This was done because our previous observations had shown that, for optimal sensitization to the production of myocardial necroses by stress, both phosphate and steroid treatment are necessary. Both cortisol acetate (COL-Ac) and desoxycorticosterone acetate (DOC-Ac) were administered in the form of aqueous microcrystal suspensions in dosage of 5 mg. in 1 ml. of water, subcutaneously, once daily.

A neuromuscular stress situation was established, 72 hours after initiation of treatment with the steroids and NaHPO₄, by restraining the rats in the prone position on a wooden board with strips of adhesive tape for a period of 7 hours. During this time, the animals struggled in an effort to free themselves and hence were under considerable stress.

Ninety-six hours after initiation of the conditioning with steroids and the phosphate (that is, 24 hours after the initiation of the 7 hour restraint period), all animals were killed with chloroform. Their hearts were carefully explored throughout for signs of necroses with a dissecting microscope. Under these conditions, the necrotic lesions are easily visible as yellowish or grayish patches between the darker healthy muscle tissue. The severity of the cardiac changes was expressed in an arbitrary scale of 0 to 3. The hearts were then fixed in Susa solution for subsequent histologic study of 2 hematoxylin-phloxin-stained sections through each heart. It was found, however, that the lesions can be graded at least as accurately by mere inspection of the whole heart with the dissecting microscope as by the study of a few histologic sections through each specimen. The incidence (per cent positive animals within the group) and the severity of the cardiac necroses (the latter with the standard error of the mean grade), as well as the mortality rates, are summarized in table 1.

In agreement with the recommendation of C. W. Shoppee, the term "cortisol" is used instead of "hydrocortisone," since it eliminates confusion with the 4, 5-hydrocortisone and obviates the possible implication that "hydrocortisone" (the principal natural glucocorticoid) is merely a derivative of cortisol.
TABLE 1.—Conditioning for Production of Myocardial Necroses by Neuromuscular Exertion

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Incidence (%)</th>
<th>Severity (Scale: 0 to 3)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>NaH$_2$PO$_4$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>COL-Ac</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>DOC-Ac</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Restraint</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>NaH$_2$PO$_4$+COL-Ac</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>NaH$_2$PO$_4$+DOC-Ac</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>NaH$_2$PO$_4$+Restraint</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>9</td>
<td>COL-Ac+Restraint</td>
<td>30</td>
<td>0.3±1.4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>DOC-Ac+Restraint</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>NaH$_2$PO$_4$+COL-Ac+</td>
<td>100</td>
<td>1.4±0.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Restraint</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>NaH$_2$PO$_4$+DOC-Ac+</td>
<td>20</td>
<td>0.3±0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

* Mean and standard error.

RESULTS

In confirmation of our earlier work, cardiac necroses did not occur in any of the untreated rats (group 1) nor in those treated only with NaH$_2$PO$_4$ (group 2), COL-Ac (group 3), DOC-Ac (group 4) or restraint (group 5). Even treatment with NaH$_2$PO$_4$ combined with COL-Ac (group 6), DOC-Ac (group 7) or restraint (group 8) failed to produce any detectable cardiac lesion. On the other hand, COL-Ac given alone sufficed to sensitize the rats so that subsequent restraint induced just detectable foci of cardiac necroses in 30 per cent of the animals (group 9). This was not the case in the rats pretreated for the same short period with a similar dose of DOC-Ac or restraint (group 10). Cardiac necroses occurred in all the animals that were restrained following pretreatment with NaH$_2$PO$_4$ plus COL-Ac (group 11). This is the only group in which the necrotic foci were so large that they could readily be seen to the naked eye; two of the animals in this group succumbed with evident signs of cardiac failure, shortly after the period of restraint (fig. 1). Restraint after conditioning with NaH$_2$PO$_4$ plus COL-Ac produced myocardial necroses of very moderate size in 20 per cent of our animals (group 12) but only one rat succumbed.

The question arose whether prolongation of the preliminary conditioning period could increase the sensitization to the production of myocardial necroses by restraint. To establish this, 2 groups of 10 female rats, with a mean initial body weight of 100 Gm. (range 96 to 109 Gm.), were injected as in groups 11 and 12, but here the 7 hour period of restraint was imposed after 96 hours of pretreatment with the steroid and phosphate and the animals were killed one day later than in the principal series. Under these circumstances, the incidence of necrosis in the DOC-Ac group rose to 90 per cent and the severity, to 1.3 ± 0.25; while in the COL-Ac group the incidence was 100 per cent and the severity 2.7 ± 0.2 (which comes very close to the theoretical maximum of 3.0 that could be attained using our scale).

DISCUSSION

It is evident that cortisol, presumably the most important glucocorticoid secreted by the human adrenal during stress, is capable of conditioning the myocardium for the production of necroses by a subsequent comparatively short period of restraint. It is true that, in this respect, 5 mg./day of COL-Ac proved to be somewhat less effective than 50 µg. of Me-Cl-COL had been under similar conditions. Nevertheless, it must be kept in mind that COL-Ac is comparatively slowly absorbed. In fact, dissection of the injection sites revealed that considerable amounts of the compound were still detectable in the subcutaneous tissue at the time of autopsy. It is possible, therefore, that treatment with the more soluble free cortisol alcohol would be even more effective. However, the object of our work was not to establish the minimum effective dose, but to demonstrate that a natural glucocorticoid can condition the heart for the production of these acute massive myocardial necroses.

Without NaH$_2$PO$_4$ pretreatment, restraint elicited only mild necroses in a limited number of our COL-Ac conditioned rats; however, this observation proves that an excess of phosphate, though very helpful, is not indispensable for the sensitizing effect of COL-Ac.
Fig. 1. Sections from the heart of a rat in which infarct-like, multiple massive necroses have been precipitated by neuromuscular effort after conditioning with cortisol and NaH2PO4. A. General view on a cross-section through the entire heart. The dark regions correspond to necrotic muscle. Note characteristic predisposition of the subendocardial regions and the papillary muscles, although necroses are also seen in other parts of the myocardium (X 10). B. High magnification of the borderline between necrotic and healthy muscle. The intact muscle cells are large and gray, the necrotic ones dark and in the process of dissolution (X 1000). C. Early subendocardial necroses (dark fibers), surrounded and partially invaded by histiocytes and a few polymorphonuclear cells (X 100). D. Somewhat older lesion, in which the necrotic fibers are almost completely phagocytosed by the invading histiocytes (X 120).
It has long been known that potassium-deficient diets can cause myocardial necroses in rats. Occasional microscopic foci of myocardial necrosis have also been obtained in rats after chronic treatment with desoxycorticosterone, a fact which has been attributed to the hypokalemic action of this compound. However, these lesions are often absent even after long-lasting heavy overdosage with DOC-Ac, perhaps because they are so largely dependent upon the phosphate content of the diet. It should also be kept in mind that other steroids, particularly those of the digitalis group and the toxic vitamin D derivatives, can also produce myocardial necroses in the rat, although they are not endowed with mineralocorticoid potency. The fact that in the present experimental series COL-Ac, a glucocorticoid, was so much more active than DOC-Ac further emphasizes the lack of a close relationship between the cardiotoxicity and the mineralocorticoid activity of steroids.

Nothing is known as yet about possible relationships between the acute myocardial necroses produced by our technique and the spontaneous cardiac infarcts that occur in man. The experimental necroses do not exhibit any close relationship with the arborizations of the vascular tree, nor have we seen arterial occlusions in our animals. It is noteworthy, however, that the experimental necroses that we produce are sharply localized, massive foci (not diffuse lesions that affect many isolated small groups of fibers) and that they can be precipitated by a stressful situation; in both these respects they resemble the cardiac infarcts of man. It is not impossible that humoral changes within the myocardium, such as are induced by treatment with steroids and a phosphate, might predispose the heart to the development of acute necroses in vascular territories in which blood circulation is impeded, for example, as a result of chronic atheromatosis. It should be pointed out, however, that at the present time this possibility is not yet supported by any objective evidence.

SUMMARY
Experiments on rats indicate that, following conditioning with cortisol acetate (COL-Ac) and NaH₂PO₄, a brief period of restraint (induced by strapping rats to a board with adhesive tape) results in the development of acute massive cardiac necroses. Conditioning with NaH₂PO₄ and desoxycorticosterone acetate (DOC-Ac) is less effective in this respect. Even without the administration of an excess of the phosphate, COL-Ac pretreatment can sensitize the rat to the production of such acute myocardial necroses, but phosphate supplements considerably increase the incidence and severity of the lesions.

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REFERENCES
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HANS SELYE

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