The Effect of ACTH on Experimental Myocardial Infarcts

By William B. Wartman, M.D., Linn A. Campbell, M.D. and Robert L. Craig, M.D.

With the collaboration of Robert B. Jennings, M.D.

Measurable myocardial infarcts of predictable size and location were produced in dogs by low ligation of the left descending coronary artery just proximal to the origin of the apical branch. Two groups of dogs with such infarcts were studied, one group being treated intramuscularly with two daily doses of 20 units of Acthar Gel for 9 to 11 days and the other group being untreated.

At the end of the experimental period there was no demonstrable difference between the two groups with respect to the size, site or histologic appearance of the infarcts.

Although several papers have been written concerning the effects of cortisone upon experimental myocardial infarcts in dogs, no similar studies of the action of ACTH have been published and therefore, the experiments described in this paper were performed. Since these experiments necessitated a comparison of the infarcts produced in two different groups of animals, namely an untreated control group and an experimental group treated with ACTH, it was essential to know the limits of variation of the infarcts in the control as well as in the experimental group with respect to size, site and histologic appearance. A search of the published papers on experimentally produced infarcts in dogs showed that the authors frequently overlooked this important consideration and that a method for producing a standard infarct was not available.

Therefore, the first part of the study was devoted to finding a way of making a standard infarct with predictable characteristics. A detailed account of this work will be reported separately, but because it is essential for the reader to have some knowledge about the method, in order to evaluate the results of the experiments, a brief account will be given of the technic of producing a standard infarct.

**Experimental**

**Animals.** Healthy, adult, mongrel dogs selected at random were used. They were fed dog mash once a day and as much water as they wanted. All of them ate well and maintained their weight throughout the period of the experiments.

**Surgical Methods.** The dogs were anesthetized intravenously with 60 mg. of sodium pentobarbital per five pounds of body weight. The heart was exposed in a one-stage operation through incisions in the left fourth intercostal space and pericardium using positive intra-alveolar pressure and an aseptic technique. The anterior descending branch of the left coronary artery was identified and a silk ligature placed on it immediately above the origin of the branch going to the apex of the left ventricle. Since this branch did not already have a name, we have called it the apical branch of the left anterior descending coronary artery. It was present in about 75 per cent of the dogs, arising a little more than half way down the left anterior descending coronary artery. Once the heart had been exposed the apical branch was easily seen, but occasionally it was absent or lay entirely within the myocardium and could not be seen. Such dogs were discarded, because in them it was impossible to be certain of the location of the ligature or the size of the infarct.

After ligation, the wound was closed and the animals were given a daily intramuscular dose of 300,000 units of procaine penicillin for three days. Infections did not occur, there was no mortality, and the animals had an uneventful convalescence.

**Method of Examination of the Heart.** Both control and experimental groups of animals were killed by injecting lethal doses of sodium pentobarbital intravenously on the 11th day after operation. The heart was removed immediately, the location of the ligature and its adequacy were verified, and the chambers were washed free of blood with tap water.

The heart was weighed and then fixed for 24 hours.
in about 400 cc. of Zenker's formulin solution, care being taken to fill the chambers with fixative. After washing for 24 hours in running tap water the heart was stored in 70 per cent ethyl alcohol.

The location and volume of the infarct were determined in the following manner. The fixed heart was placed in a commercial meat slicer in such a way that the posterior surface rested on the platform and the apex against the knife blade. Transverse slices 2.7 mm. thick were cut and laid out serially on trays for inspection. The infarct was clearly demarcated in these slices and its location with respect to the thickness of the ventricular wall easily recognized. The entire infarct from each slice was blocked separately in paraffin and sections 8 microns thick were cut, mounted on large slides, and stained with hematoxylin and eosin and with Mallory's aniline blue collagen stain. The first section from each block was placed in a 35 mm. photographic enlarger and the image of the section projected onto paper using a constant magnification. The area of the infarct in each section was measured with a planimeter, and since the slices were of constant thickness the volume of the entire infarct could be calculated.

Experimental and Control Groups of Animals. Table 1 sets forth the data for both groups of animals. The 13 animals in the control group received no ACTH (dogs No. 1 to 13). Eight animals in the experimental group (dogs No. 14 to 21) were given 20 Armour units of H.P. Acthar Gel intramuscularly twice daily. This dose was sufficient to cause a prompt drop in the number of eosinophiles in the peripheral blood. H.P. Acthar Gel is a highly purified preparation of ACTH in gelatin and one Armour unit provides a physiologic response in the human equivalent to 1 U.S.P. unit (1 U).* Five of the dogs received the drug for 11 days, beginning on the day of operation, and three of them for nine days, beginning on the second postoperative day. Since the results were the same in both groups they have been combined in table 1.

RESULTS

Control Group. The site and histologic appearance of infarcts in the 13 control animals were remarkably uniform and the volume of the infarcts varied little. All infarcts were located in the left ventricle, extending from about 2 mm. above the ligature to the apex, and included approximately 1 cm. of the lateral wall of the left ventricle and a similar amount of the anterior interventricular septum. The right ventricle was never involved. The infarcts were subendocardial involving only the inner half of the ventricular wall (fig. 1).

- The H.P. Acthar Gel was kindly supplied by Armour Laboratories.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>ACTH (Days)</th>
<th>Body Weight (Kg.)</th>
<th>Heart Weight (Gm.)</th>
<th>Infarct Volume (cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>13.0</td>
<td>127.8</td>
<td>3.50</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>15.0</td>
<td>125.0</td>
<td>3.33</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>19.0</td>
<td>124.7</td>
<td>3.27</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>14.0</td>
<td>124.4</td>
<td>3.51</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>12.5</td>
<td>109.8</td>
<td>3.11</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>13.6</td>
<td>102.0</td>
<td>2.33</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>13.6</td>
<td>98.9</td>
<td>2.57</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>12.9</td>
<td>97.7</td>
<td>2.19</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>14.7</td>
<td>93.6</td>
<td>2.04</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>12.0</td>
<td>83.5</td>
<td>1.94</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>11.7</td>
<td>83.3</td>
<td>3.22</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>12.5</td>
<td>81.5</td>
<td>2.36</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>11.2</td>
<td>80.0</td>
<td>2.33</td>
</tr>
</tbody>
</table>

| ACTH Animals |
|--------------|-------------|------------------|------------------|
| 14          | 11          | 14.6             | 112.0            | 2.90                |
| 15          | 11          | 14.1             | 122.5            | 0.65                |
| 16          | 11          | 12.7             | 112.9            | 3.86                |
| 17          | 9           | 10.9             | 108.1            | 4.07                |
| 18          | 11          | 12.0             | 93.6             | 2.03                |
| 19          | 9           | 9.6              | 77.0             | 2.38                |
| 20          | 9           | 6.9              | 56.5             | 0.61                |
| 21          | 11          | 7.1              | 41.7             | 1.75                |

Average Controls: 13.51 102.47 2.75
Average ACTH: 10.98 92.01 2.31
S.D. Controls: 1.99 18.18 0.59
S.D. ACTH: 2.94 32.50 1.27
p test: >0.4 1.30 1.70
p value: >0.1

* The H.P. Acthar Gel was kindly supplied by Armour Laboratories.

The average volume of the infarcts was 2.75 ± 0.59 cc. and was thus fairly constant from dog to dog, as Table 1 shows.

All control infarcts showed the same microscopic appearance, which corresponded closely with Karsner and Dwyer's² description of 11 day old infarcts in dogs and with Mallory, White and Salcedo-Salgar's³ for infarcts of three weeks' duration in humans. Destruction of myocardium and removal of necrotic tissue had taken place and healing by organization had begun. Numerous fibrocytes, capillaries and small mononuclear cells, including occasional plasma cells, were present, but only a few
lymphocytes and no giant cells. A moderate amount of collagen had been laid down in many areas. Fresh hemorrhage was inconspicuous, but there were moderate amounts of hemosiderin most of which had been phagocyted by mononuclear cells. There was no evidence of myocardial regeneration.

Experimental Group. The infarcts produced in the dogs treated with ACTH were nearly identical with those occurring in the control animals. The location in the left ventricle, and the thickness of the infarcts with respect to the thickness of the ventricular wall were the same. Histologically they were indistinguishable. The average volume of the experimental infarcts was $2.31 \pm 1.27$ cc. Thus, the average infarct volume was nearly the same for the two groups of animals, although the range of values was greater in dogs receiving ACTH than in the controls. Analysis of the data, however, disclosed no significant difference in the size of the infarcts in the two groups (table 1).
DISCUSSION

Critique of Methods. The methods used for producing a standard predictable infarct, and for the estimation of its size, contain several sources of error which should be discussed.

It is possible that improper selection of animals for the two groups—control and ACTH—may have biased the results. However, analysis of the body weights and heart weights given in table 1 indicates that this did not happen, and that the chance that the 2 groups of animals came from the same population was greater than 40 per cent (p value > 0.4). If they had come from different populations, with respect to body and heart weights, the chance would have been less than 0.5 per cent (p value < 0.05). Thus the evidence indicates that similar animals were used in both the control and ACTH groups.

We have already mentioned that the apical branch of the left descending coronary artery may be absent or deviate from its usual course. However, a special study of this artery showed that the origin and distribution of the vessel were constant in 75 per cent of the dogs examined and that deviations from the normal could usually be detected by direct visual observations and the dogs excluded from the experiments. Nevertheless, it is probably true that in a small proportion of animals an inconspicuous variation in the pattern of the apical branch was not discovered, and if such animals were used, infarcts of unpredictable size were unintentionally produced.

An error may have been introduced into the planimeter measurements of the area of the infarct, and consequently into the calculation of its volume, by the intermingling of dead and living tissue in the infarcts and the difficulty of recognizing their boundaries. Experience, however, showed that this source of error was not great and could be adequately controlled by making the planimeter tracings from an enlarged, projected image of sections stained with Mallory’s connective tissue stain, and by careful microscopic checking on the extent of the infarcts.

The external appearance of the infarct was found to be an unreliable indication of its size. Usually the epicardium was grey, opaque and slightly thickened in a roughly triangular area at the apex of the left ventricle, but the area and exact location of this triangle varied considerably in different animals and, of course, gave no indication of the thickness of the infarct, nor of its relation to the endocardium. For these reasons we believe conclusions about the size of an infarct that are based only on its external appearance are likely to be false.

Unequal shrinkage of tissue during its preparation for examination may also cause an error. This was controlled, insofar as it was possible to do so, by the use of a rigid routine of fixation, slicing, blocking, embedding and staining of all specimens.

Significance of Results. The results of these experiments show that, under the conditions which prevailed, ACTH caused no constant change in the size, location or histological appearance of infarcts examined by ordinary histological methods 11 days after the infarct occurred. The lack of effect upon healing by fibrosis is at variance with certain other experimental work in which it has been claimed that ACTH delays healing by inhibiting fibrosis. However, the results of the published reports are by no means in agreement and there seems to be need for a critical reevaluation of the subject. ACTH had no effect upon the mortality rate of the animals.

The effect of cortisone upon experimentally produced myocardial infarcts has been studied by four groups of investigators. Chapman and associates found that cortisone given to dogs with experimentally produced myocardial infarcts had no effect on the size of the infarcts nor on the rate and quality of healing. They were also unable to demonstrate changes in electrolyte balance. Opdyke and co-workers confirmed these findings. On the other hand, Johnson and associates reported a marked reduction in the size of experimentally produced infarcts in dogs, with increased vascularity, decrease in local fibroblastic proliferation and delay in healing. They also found a marked reduction in the mortality rate of the experimental animals as compared to the controls. The idea of Johnson and associates, that cortisone produces increased vascularity in the heart has also been studied by Eckstein using
more refined methods of measurement including post occlusion retrograde flow determinations. No increase in vascularity was observed.

**Summary**

ACTH, given in the form of Armour's H.P. Acthar Gel intramuscularly twice a day in doses of 20 Armour units for 9 to 11 days, had no effect upon the size, site or histologic appearance of standard infarcts when examined 11 days after operation.

A method is described for producing a standard myocardial infarct of predictable size, site and histologic appearance.

**REFERENCES**


The Effect of ACTH on Experimental Myocardial Infarcts
WILLIAM B. WARTMAN, LINN A. CAMPBELL and ROBERT L. CRAIG

Circ Res. 1955;3:496-500
doi: 10.1161/01.RES.3.5.496

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/3/5/496

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/