The Effect of an Increase in Inotropic State and End-Diastolic Volume on the Pumping Ability of the Feline Left Heart

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SUMMARY In isolated ejecting cat hearts, the pumping ability of the left heart was described quantitatively by the relationship between mean left ventricular pressure and mean left ventricular output. This relationship was determined by making the heart eject against a series of different loads on a beat-to-beat basis. Left ventricular mean pressure—mean output relationships of control and potentiated beats (at the same end-diastolic pressure) have a common intercept on the output axis but diverge toward the pressure axis. When the mean pressure values of the potentiated beats in a given experiment are multiplied by a single factor, superposition of the two relationships is obtained. A change in left ventricular end-diastolic pressure caused a more parallel shift of the left ventricular mean pressure—mean output relationship. Here, superposition could be obtained by using one multiplication factor for the mean pressure data and one for the mean output data of the relationship found after the change in end-diastolic pressure. We concluded that, using the left ventricular mean pressure—mean output relationship, changes in cardiac pumping ability caused by given changes in inotropic state and ventricular end-diastolic volume can be quantified by one or two multiplication factors, respectively.

Methods

Preparation

For the experiments described here, we used the same isolated heart preparation as in earlier studies. An account of the apparatus is given elsewhere. Male cats (3–4.5 kg) were anesthetized with thiopental, 45 mg/kg, ip. Under artificial respiration, the hearts were prepared, isolated, and connected to the experimental apparatus shown in Figure 1. The system consists of a big reservoir containing an oxygenated mixture of bovine erythrocytes and Tyrode's solution (Na⁺, 149 mm; K⁺, 4.7 mm; Cl⁻, 138 mm; Ca²⁺, 1.35 mm; Mg²⁺, 1.05 mm; HCO₃⁻, 20.3 mm, H₂PO₄⁻, 0.42 mm; and glucose, 11.1 mm). The hematocrit was 25, pH varied between 7.39 and 7.43, Pco₂ ranged from 125 to 300 mm Hg, always providing full hemoglobin saturation, Pco₂ was found to be between 25 and 30 mm Hg. The temperature in the reservoir was controlled with the aid of a thermostirr-carbonic acid. The perfusion fluid to be within 25 and 30 mm Hg. The temperature in the reservoir was controlled with the aid of a thermistor command to pump the heated water to a glass coil suspended in the perfusion fluid. When the temperature fell below a preset value, the control is such that the heart receives fluid between 37.5°C and 38.0°C.

From the reservoir (R), fluid is passed to the left atrium through a filter (F) toward vessel SL from which the left atrial filling pressure is controlled. The reservoir containing an oxygenated mixture of bovine erythrocytes and Tyrode's solution (Na⁺, 149 mm; K⁺, 4.7 mm; Cl⁻, 138 mm; Ca²⁺, 1.35 mm; Mg²⁺, 1.05 mm; HCO₃⁻, 20.3 mm, H₂PO₄⁻, 0.42 mm; and glucose, 11.1 mm). The hematocrit was 25, pH varied between 7.39 and 7.43, Pco₂ ranged from 125 to 300 mm Hg, always providing full hemoglobin saturation, Pco₂ was found to be between 25 and 30 mm Hg. The temperature in the reservoir was controlled with the aid of a thermostirr-carbonic acid. The perfusion fluid to be within 25 and 30 mm Hg. The temperature in the reservoir was controlled with the aid of a thermistor command to pump the heated water to a glass coil suspended in the perfusion fluid. When the temperature fell below a preset value, the control is such that the heart receives fluid between 37.5°C and 38.0°C.

From the reservoir (R), fluid is passed by pressure through a filter (F) toward vessel SL from which the left atrium is filled. Left atrial filling pressure is controlled by the height of the overflow level inside this vessel. The overflow fluid is pumped back into the big reservoir.

The left ventricle ejects into a hydraulic model of the arterial system of the cat. The input impedance of the model closely resembles the in vivo input impedance of the cat's aorta. The model consists of two resistances (Rc and Rp) and a capacitance chamber (C). The magnitude of the resistance representing the peripheral resistance

CHANGES in end-diastolic volume and inotropic state govern the pump function of the heart. Although it is accepted that increases in cardiac output result from a rise of end-diastolic volume, no uniform opinion is found in the literature with regard to the effects of an increase in inotropic state. Some investigators have shown an elevation in cardiac output when the inotropic state of the heart is enhanced, whereas others have demonstrated that output remains virtually the same under such conditions.

We have shown in previous studies that the pumping ability of the left heart can be described quantitatively by a graph relating mean left ventricular pressure with mean left ventricular output. Various pressures and flows are generated when the heart ejects against a series of different arterial loads. The description of pumping ability is based on the concept of the apparent source resistance of the heart. In this concept, the heart is compared with a generator that contains a finite internal resistance. The generator is useful, notwithstanding the fact that the heart, in reality, does not contain a hydraulic resistance of the expected magnitude but, rather bears more resemblance to a time-varying compliance.

In this study we report the results of experiments designed to quantify the effects of an increase in inotropic state and changes in left ventricular end-diastolic volume on the pumping ability of the left heart. In contrast to our previous investigations, we now have provided a series of different arterial loads on a beat-to-beat basis instead of producing a range of steady state load levels.

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Received March 14, 1977; accepted for publication December 21, 1977.
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FIGURE 1 Isolated ejecting cat heart set-up. The reservoir R contains an oxygenated mixture of Tyrode's solution and washed bovine erythrocytes. F is a filter, SL is a left atrial supply vessel where the fluid level is controlled by an overflow system. The left ventricle ejects into a hydraulic model of the input impedance of the cat's systemic arterial tree. This model contains two resistances (R_p and R_c) and one capacitance (C). The values of R_p and C can be controlled. An electromagnetic valve (E.M.) and an air-driven valve were used to change arterial load on a beat to beat basis. I_a0 and P_a0 are instantaneous flow and pressure in the aorta. P_priv is left ventricular pressure; I is mean aortic blood flow.

(R_p) is regulated by a slide controlled by a motor. The volume of air present in the air chamber determines the value of the capacitance modeling total arterial compliance.

Desired beat-to-beat loads were achieved by an electromagnetic valve that opened the capacitance (C) toward a bottle in which pressure could be chosen. Opening and closure of the valve could be performed at preselected moments and during chosen periods. By using a series of pressures in the bottle (and by triggering the valve), the ventricle faced different levels of arterial pressure for single beats. Arterial pressure could be changed to extremely low values by opening another air-driven valve, located at the cardiac side of the resistor, R_c, to atmospheric pressure. The timing of this valve was controlled in a similar fashion as the electromagnetic valve closing the capacitance. These sudden changes in arterial pressure were always performed late in diastole. At the outflow end of the hydraulic model, loading the left side of the heart, cardiac output could be sampled for calibration purposes. Outflow was pumped back into the big reservoir. The right side of the heart ejected only the coronary venous flow, which was discarded.

Measurements

Aortic pressure and left ventricular pressure were measured with Statham 23Db pressure transducers. Resonant frequencies of these systems were 80 and 150 Hz, respectively, the difference resulting from the difference in length of the catheters. To measure left ventricular pressure, a needle was introduced into that cavity via the apex. Aortic flow was measured electromagnetically with a Biotronex BL-610-pulsed logic flowmeter. Flowmeter and probe were adapted for this isolated heart preparation. The amplitude-frequency response of the flow-measuring system was 3 dB down at 100 Hz; time delay was 2.3 msec. One of the electrodes to measure the electrocardiogram was placed at the apex; the other lead was taken from the fluid in the "aorta."

An Elema-Schönander ink-writing system (EMT 81) and a Hewlett Packard analog tape recorder (3525 A) were used to record all data. The deviation of linearity of the EMT 81 was tested and found not to influence the results significantly (<1%). Mean values of left ventricular pressures and aortic flows of interest taken over the full cardiac cycle were obtained by planimetry. Duplicate determinations of the mean values were always done; they differed by less than 0.5 mm Hg for the pressures and less than 0.15 ml/sec for the flows.

Experiments

Ten experiments were performed on nine different hearts. All hearts were paced from the left atrium at a frequency of 120 beats/min. This was possible only after
destruction of the SA node. The heart was paced with a Digitimer (Devices, type 3296) which triggered a pacemaker. At a selected delay after the pacing pulse, an amplifier was triggered, opening either of the valves in diastole for a chosen period. Aortic pressure dropped or rose at the selected moment to the value of the pressure in the bottle. The mean values of left ventricular pressure and aortic flow of the first beat following this intervention provided the data for the left ventricular mean pressure-mean output relationship.

The effect of an increase in inotropic state on the left ventricular mean pressure-mean output relationship was studied in five experiments using potentiated beats as the inotropic intervention. Such potentiated beats were obtained by introducing an extra cardiac contraction at a constant interval after a control beat. This interval was 240 msec in four experiments and 250 msec in the fifth. The occurrence of the potentiated beat immediately following the extra contraction was timed in such a way that end-diastolic pressure of the potentiated beat was the same as that during control beats. This timing was fixed over one experiment. The level of aortic pressure during the potentiated beat was varied in the same way as described above for beats studied at a constant rate, to obtain the relationship sought.

The highly amplified diastolic part of the left ventricular pressure curve was monitored on an oscilloscope. Errors estimated were less than 0.3 mm Hg and inconsistent in direction for high and low loads. Pacing at a rate of 120 beats/min was resumed immediately after the potentiated beat was obtained.

The analysis resulted in a left ventricular mean pressure-mean output relationship for beats with a higher inotropic state but starting their contraction from the same end-diastolic pressure. Experimental design was such that the sudden load changes were applied in alternating order for high and low loads. Pacing at a rate of 120 beats/min with that found for potentiated beats having the same left ventricular end-diastolic pressure.

These two graphs per experiment are shown in the five upper subpanels of Figure 3. The lines through the points are drawn by hand. It can be seen that the two relationships have a common intercept on the mean output axis and diverge toward the mean pressure axis. The values measured during the steady state at the start and at the end of the experiment are shown as two dots surrounded by a circle. That these values appear to be so close demonstrates the stability of the preparation during the period of the experimental measurements.

When the mean left ventricular pressure values from the potentiated beats in a given experiment were multiplied by a factor, full superposition of the two relationships was obtained. This phenomenon is demonstrated in the five lower subpanels of Figure 3. The factors were found empirically and were not the same for the different hearts (range, 0.62–0.80).

In four experiments, the left ventricular mean pressure-mean output relationship was obtained at three levels of end-diastolic pressure, whereas in one experiment, only two levels were studied. The common intercept on the mean output axis was not found in these experiments (Fig. 4, upper subpanels). Now a more parallel shift of the relationship was obtained. This shift could be quantified using two factors, one for the mean left ventricular pressure data and the other for the mean left ventricular output values (Fig. 4, lower subpanels). The factors were found empirically and provided superposition of the relationships. In four of the five experiments, steady state values obtained in the control situation at the start and at the end of the experiment (dots) are indicated by a circle (Fig. 4, upper subpanels). Table 1 gives values of the end-diastolic pressures used in these experiments.

In one experiment the two interventions studied, i.e., changing left ventricular filling and inotropic state, respectively, were combined (Fig. 5). In the left panel, four curves are shown which represent the pumping ability of the left heart at two end-diastolic pressures and at two levels of inotropic state (control and potentiated beats). The middle panel shows that the shifts of the relationships brought about by the potentiated beats were the same at the two degrees of ventricular filling (factor 0.8 for both relationships). In the right panel, all four relationships are superimposed by using two (different) factors to characterize the shift resulting from the different end-diastolic pressures.

In our previous studies, we could describe only a part of the left ventricular mean pressure-mean output relationship because of the limitations of the experimental technique. Based on those results, we thought the relationship to be more or less linear. This study shows the relationship to be curved. We have determined the degree of curvature by normalizing the intercepts with the two axes, connecting them with a straight line and determining the ratio between the area outside (shaded area, A1) and inside (A2) this straight line (Fig. 6). No curvature gives zero for this index. The area A1 in the experiments (Figs. 3 and 4) was determined by calculating the distance from
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Arterial load on the left heart is changed suddenly during diastole. This is done for normal beats (a) as well as for potentiated beats (b). Decreases (upper panels) and increases (lower panels) in load were used. Pressures are given in mm Hg, flow in ml/sec. The first derivative of the left ventricular pressure curve (dP/dt) is given in mm Hg/sec. When early ejection occurs (low aortic pressure values) peak dP/dt does not reach its full isovolumic value. In each of the four panels, the first tracing = electrocardiogram; second = aortic pressure; third = left ventricular pressure; fourth = left ventricular end-diastolic pressure; fifth = aortic flow; sixth = left ventricular dP/dt.

Each point of measurement to the straight line, and multiplying the mean value by the length of the straight line. Table 1 gives the results of the calculations.

To ensure that the values of the points obtained in the left ventricular mean pressure-mean output plot were the same when the release technique described here was used, instead of using steady states of different loads, as in previous studies, in one experiment we compared the results obtained with the two different techniques (Fig. 7). The dots in this graph indicate the values obtained from three different steady state levels of the load, and the data represented by squares were obtained with the technique used in this study.

Discussion

We have shown that simple quantitative measures can be obtained for the change in pumping ability of the left heart that results from an increase in inotropic state and from changes in end-diastolic pressure. We found that an increase in inotropic state rotates the left ventricular mean pressure-mean output relationship so that only the mean pressure values increase by a factor while the mean output...
values remain unaltered. The value of this factor may provide a quantitative measure of the change in pumping ability of the left heart that results from the increase in inotropic state found in potentiated contractions at the same end-diastolic pressure. These results differ essentially from the changes in pumping ability brought about by changes in end-diastolic pressure, where a more parallel shift of the left ventricular mean pressure-mean output relationship was found. For a given change in end-diastolic pressure, this shift could be characterized by two factors, one for the mean left ventricular pressure and one for the mean left ventricular output values.

In our study, a series of different loads were obtained by sudden changes in arterial pressure. Although the changes in aortic pressure were so rapid that coronary insufficiency could not have occurred during the beats studied, we have tested the possibility that this experimental procedure would lead to different results compared with the investigations in which steady states of different loads were used. We did this by applying both tech-

Figure 3: The five upper subpanels show mean left ventricular mean pressure-mean output relationships for control (■) and potentiated beats (★). When the mean pressure values of the potentiated beats are multiplied by a factor, the two relationships superimpose (lower panels). Multiplication factors are indicated.
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niques in one experiment (see Fig. 7). It appeared that
the values obtained were independent of the experimental
procedure over the range studied. This range is a limited
one for the steady state values. To evaluate further a
possible effect of the experimental technique on the
measured values, we compared the slopes of the relation-
ships obtained in our last study with the ones found in the
experiments reported here. From the latter group, only
data over the same limited range were taken. The slopes
were not significantly different (Student's t-test; \( P >
0.05 \)). We therefore conclude that, as far as we can
measure, the experimental procedure has not affected our
results; applying sudden load changes during diastole or
using steady state load levels to measure the relationship
between mean left ventricular pressure and mean left
ventricular output leads to the same results.

The left ventricular mean pressure-mean output rela-
tionships in Figure 3 shows that the effect of an increase

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** Left ventricular mean pressure-mean output relationships obtained at three different levels of left ventricular end-diastolic pressures (upper subpanels): low (▲), control (■), and high (▲). In one of the five experiments, only two levels were studied. Multiplying the mean pressure values and the mean output values of the lower and the higher relationships by the factors indicated in the lower subpanels resulted in superposition of the graphs.
### Table 1  The Degree of Curvature

<table>
<thead>
<tr>
<th>Experiment</th>
<th>End-diastolic pressure</th>
<th>Inotropic state</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (3.8 ± 0.8 mm Hg)</td>
<td>Control (6.6 ± 0.8 mm Hg) High (11.6 ± 1.0 mm Hg)</td>
</tr>
<tr>
<td>15-7-76</td>
<td>0.62</td>
<td>0.53</td>
</tr>
<tr>
<td>20-8-76</td>
<td>0.35</td>
<td>0.27</td>
</tr>
<tr>
<td>24-8-76</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>2-9-76</td>
<td>0.39</td>
<td>0.34</td>
</tr>
<tr>
<td>7-9-76</td>
<td>0.39</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control (0.30)</td>
<td>0.33</td>
</tr>
<tr>
<td>1-7-76</td>
<td>0.49</td>
<td>0.41</td>
</tr>
<tr>
<td>12-7-76</td>
<td>0.49</td>
<td>0.55</td>
</tr>
<tr>
<td>14-7-76</td>
<td>c.n.c.</td>
<td>c.n.c.</td>
</tr>
<tr>
<td>29-6-76</td>
<td>0.53</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The degree of curvature is given as the ratio A1/A2 (see Fig. 6); e.n.d. = experiment not done; c.n.c. = curvature not computed.

**Figure 5**  In one experiment a given increase in inotropic state was studied at two levels of left ventricular end-diastolic pressure. Using the factors given in the figures, the four relationships were superimposable. Note that just one factor (0.80) was needed for the enhanced inotropic state at two degrees of ventricular filling.

**Figure 6**  The degree of curvature was determined by connecting the intercepts with the two axes by a straight line, normalizing these values, and determining the ratio between the areas A1 and A2.

in inotropic state on stroke volume depends largely on the pressure level at which the heart functions. At high pressures, stroke volume can increase easily by a factor of two, whereas, in the low pressure range, the change in mean output is 10% or even less. The increase in stroke volume with an increase in inotropic state not only depends on the pressure level at which the heart beats but on the contractile state at which the heart functioned before the inotropic intervention as well. Figure 8 shows that the left ventricular mean pressure-mean output relationship is steeper at normal pressure levels (mean left ventricular pressure of about 50 mm Hg) when the heart operates at a higher contractile state. This implies that when the inotropic state is increased the same amount by starting from a higher level of contractility, the effect on stroke volume will be smaller. For example, in Figure 8, compare the difference between curves 1 and 2 (X) with the difference between curves 2 and 3 (Y). This conclusion is in line with the findings of Noble et al., who found no apparent increase in stroke volume when the inotropic...
The pumping ability of the left heart was increased in normal conscious dogs, whereas increases in stroke volume under these conditions were found when the heart was depressed.

Thus, the effect of an increase in inotropic state on stroke volume depends on the initial contractile state of the heart and on the pressure level at which it functions. This may explain the difference of opinion found in the literature concerning the presence\(^1\) or absence\(^2\) of a rise in stroke volume with an increase in inotropic state. A change in inotropic state of the heart does not cause a change in the intercept of the relationship under study with the mean output axis (Fig. 3). However, a change in left ventricular end-diastolic pressure does cause a change in the maximal left ventricular output which is obtained at zero pressure (Fig. 4). We think, therefore, that under control conditions the ventricle empties maximally when the pressure is very low. Only a given volume remains in this cavity. It does not become smaller when inotropic state is enhanced. This constant rest volume probably is identical to the extrapolated remaining volume \(V_d\) shown by Suga et al.\(^7\) which seemed also to be independent of inotropic state. Increasing left ventricular end-diastolic volume will probably not cause a change in the rest volume at zero pressure. However, the ventricle can have a higher output because ejection starts at a higher degree of ventricular filling. Therefore it is likely that the change in left ventricular end-diastolic volume can be estimated from the shift of the intercept with the mean output axis.

The shift of the left ventricular mean pressure-mean output relationship, resulting from a change in end-diastolic pressure, seems to be parallel. This is in line with the results obtained in an earlier study,\(^8\) using steady state load changes. The multiplication factors used to characterize the shift often are very close for both mean pressure and mean output (Fig. 4). Nevertheless, one should realize that this is not a true parallel shift, because the two variables plotted are not related to one another in such a simple manner.

In previous studies in which a limited range of load values was used because of the differences in experimental techniques,\(^5\) we thought the left ventricular mean pressure-mean output relationship to be approximately linear. The full relationship appears to be curved. The curvature is in the same direction as sometimes was found using a series of steady state loads to obtain the relationship.\(^9\) The amount of curvature seems to be different for different hearts (Table 1). For a given heart the variations in curvature are small, regardless of the intervention. This is in accord with the fact that superposition of the relationships can be obtained by only one or two multiplication factors (Figs. 3–5). It seems to us that a change in inotropic state affected the amount of curvature even less than changes in end-diastolic pressure. Table 1 shows that low end-diastolic pressures were related to somewhat greater curvatures than high end-diastolic pressures. However, as stated above, the degree of curvature seems to be determined mainly by what heart is studied.

In one experiment, we combined post-extrasystolic potentiation with changes in ventricular filling (Fig. 5). We found that the multiplication factor needed to characterize the change of the pressure-output relationship with a change in inotropic state was the same for two different values of the end-diastolic pressure (0.80). This indicates that the change in pumping ability resulting from a given change in inotropic state may be independent of the end-diastolic volume at which the left heart functions.

References

SUMMARY Nonhistone nuclear proteins (NHNP) were isolated from the hearts of hamsters in the myolytic, hypertrophic, and failing phases of cardiomyopathy and from paired controls. These proteins were solubilized in phenol and fractionated by polyacrylamide gel electrophoresis. One-dimensional gel electrophoresis, using either isoelectrofocusing or sodium dodecyl sulfate, showed quantitative differences between the dystrophic hearts and the controls. A high resolution of NHNP was achieved by two-dimensional gel electrophoresis, revealing both quantitative and limited qualitative differences between the two groups. Proteins focusing from pH 5.0 to 5.6 with molecular weights of 55,000 (P7) and 100,000 (P4) were strikingly increased in the cardiomyopathy. A protein (P2) with an isoelectric point of 5.1 and some NHNP from dystrophic hearts in the entire region from pH 7.0 to 9.0 with molecular weights ranging from 35,000 (P3) to 68,000 (P1) were markedly reduced or absent. Differences in NHNP could be detected during the myolytic phase of cardiomyopathy but were most striking during the terminal phase of the disease. There were no detectable differences between the profiles of proteins derived from whole heart homogenates of dystrophic hamsters and controls. There were no significant differences at the failing phase between NHNP isolated from a purified preparation of myocardial cells and those isolated from whole heart. Therefore, the differences in NHNP appear to be reflections of alterations in nuclear composition of the dystrophic myocardial cell. Some of these observations may represent changes secondary to heart disease. However, if NHNP interacting with DNA play a major role in genetic expression, some of the manifestations of hamster cardiomyopathy could be due to a different constitution of NHNP in the dystrophic heart.

THE CARDIOMYOPATHIES may be defined as disorders of heart muscle in which the pathological process originates in the myocardium itself rather than in associated structures such as the coronary arteries, heart valves, lungs, or peripheral vessels. In some varieties of cardiomyopathy, genetic factors play a significant role. The group of cardiomyopathies associated with the degenerative heredofamilial neuromyopathic diseases, notably Duchenne’s progressive muscular dystrophy, myotonic muscular dystrophy, and Friedrich’s ataxia, have a definite genetic basis.1 In a very high percentage of the cases of idiopathic hypertrophic cardiomyopathy, a heart muscle disorder characterized by an excessive growth of ventricular myocardium, the trait is transmitted as an autosomal dominant.2,3 The cardiomyopathic hamster is a reproducible, spontaneous model of cardiac hypertrophy, dilation, and congestive failure and is thus an important paradigm of myocardial disease.4 Homburger et al.5 have shown this defect to be transmitted by an autosomal recessive gene. Many investigators have described changes in catecholamine metabolism, oxidative phosphorylation, enzyme activity, and membrane composition.6-8 As it is known that genetic expression in eukaryotic cells may be regulated primarily by DNA-associated proteins,9-10 we decided to isolate and characterize the nuclear proteins of the hearts.
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doi: 10.1161/01.RES.42.5.620

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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