The Question of Vascular Hyper-Responsiveness in Hypertension

By PAUL D. REDLEAF, M.D., and LOUIS TOBIAN, M.D.

After inhibition of spontaneous tone, the contraction of spirally cut strips of aorta in response to norepinephrine was studied quantitatively in normotensive rats and in 4 different types of hypertensive rats. In no instance did the aortas of hypertensive rats show hyporesponsiveness to norepinephrine. The majority of responses were entirely normal; in a few instances there was decreased responsiveness. In vivo studies which fail to consider the critical effect of an altered “base-line” state in hypertension may demonstrate “hyper-responsiveness” which is more apparent than real.

The mechanism which produces arteriolar narrowing in essential hypertension remains obscure. Three main possibilities exist. First, there might be an alteration in the normal balance of circulating pressor and depressor substances, to which the arterioles respond in normal fashion. Secondly, the physiology of the blood vessels might be altered so that the muscle fibers of the arteries and arterioles of hypertensive subjects might respond to normal amounts of circulating vasopressor material and normal amounts of norepinephrine released at sympathetic nerve endings with a greater than normal amount of shortening. Thirdly, there might be neither an imbalance of vasoactive humoral substances nor vascular hyper-responsiveness, but the increased vascular resistance might be entirely passive, occurring as a result of “water-logging” or hypertrophy. 1 Folkow’s studies, 1 as well as those of Mendlowitz and Meyer, 2 show that such a mechanism occurs in human hypertension.

The present study is concerned with testing the possibility of vascular hyper-responsiveness. Greisman, 3 who studied the reactivity of the capillary bed of the nailfold to circulating norepinephrine in patients with normal blood pressure and others with essential hypertension, showed a lower threshold; for ischemia of the nailfold in the latter group. Visible contraction of the metarterioles of the conjunctiva is more readily elicited in hypertensive than in normotensive individuals when epinephrine is applied topically. 4 Patients with hypertension demonstrate greater than normal decreases of blood flow through the hand during intra-arterial infusion of epinephrine. 5 Along the same line, an enhanced pressor response to norepinephrine in hypertensive patients has been observed by some investigators, 6 although Judson, Epstein, and Wilkins could not confirm this. 7

Such in vivo experiments cannot distinguish between “real” and “apparent” hyper-responsiveness, however. By real hyper-responsiveness we mean an increased per cent shortening of arteriolar smooth muscle after a given dose of pressor agent. By apparent hyper-responsiveness we mean a greater than normal change in blood pressure, peripheral resistance, or flow accomplished by a normal per cent shortening of arteriolar smooth muscle. In this regard Tobian and Binbin, 8 and more recently Folkow, have postulated that in vessels with an increase in the thickness of the arterial wall due either to “water-logging” or to hypertrophy, the same degree of shortening of circular smooth muscle...
would cause a greatly increased resistance to flow. Furthermore, leaving out the possibility of a thickened arterial wall, a decrease in lumen size per se as a result of already shortened circular muscle might tend to produce apparent hyper-responsiveness. In this situation an exaggerated increase in arteriolar resistance could occur with a normal amount of additional muscle shortening. This is discussed below.

An in vitro approach to the question of altered vascular reactivity in hypertension might meet some of these objections. By studying the behavior of helically cut strips of arterial tissue, effects dependent upon the initial diameter of the vascular lumen or upon the thickness of the vessel wall may be eliminated. Such a preparation would thereby exclude the possibility of apparent hyper-responsiveness without real hyper-responsiveness. Furthermore, reflex effects and direct effects of sympathomimetic substances on cardiac output are obviated. Thus the actual contractile properties of the arterial muscle might be more clearly elucidated.

Such an approach at present necessitates the use of large arteries rather than the arterioles which are of paramount importance in determining peripheral vascular resistance. Our studies have utilized the aorta of the rat because of the ease with which hypertension may be produced in this species. The well-developed musculature of this vessel justifies its use as an index of the contractile properties of arterial muscle in large arteries. It is of course by no means certain that the behavior of large arteries parallels that of the arterioles. Nevertheless, if an alteration of the contractile properties of the larger vessels could be demonstrated in hypertension, it would support the possibility that similar changes may occur in the arterioles. Failure to find real hyper-responsiveness in the larger arteries would not exclude entirely its possible presence in the arterioles. However, it would reinforce our scepticism concerning such an interpretation based on data available at present and demonstrate the need for more direct evidence on this matter.

**METHODS**

**Measurement of "Spontaneous Tone" and Strength of Contraction**

Adult male Wistar rats were used throughout this study. After exsanguination, the thoracic aorta was rapidly dissected free, and placed in Krebs-Henseleit solution, containing glucose 0.01 M and calcium disodium versenate at a concentration of 10⁻³, warmed to 37 C. (The use of a chelating agent was suggested by Furchgott, to bind heavy metal ions, which otherwise catalyze the oxidation and deterioration of norepinephrine.) Starting from the distal end of the thoracic aorta, a helical strip approximately 1.5 mm. wide and ranging from 25 to 52 mm. in length was cut in the manner described by Furchgott and Bhadra-kom. (Variations in the length of strips tended to cancel out when groups of rats were compared, and in any case, did not appear to account for variations in the responses observed.) The strip was transferred to a muscle bath containing 25 ml. of Krebs-Henseleit solution at 37 C. which was aerated with a gas mixture of 95 per cent O₂ and 5 per cent CO₂. One end of the strip was anchored in the bath with a Nylon loop and the other end attached similarly to a Grass strain gage which was mounted on a laboratory jack that could be adjusted to put any desired tension on the muscle. For each experiment the strip was stretched just enough to produce a tension of 0.5 Gm. before any drugs were added.

In preliminary studies, only 2 of 12 consecutive strips contracted upon addition of norepinephrine to the bath after a previous incubation period of 3 hours. Unlike the aorta of the rabbit, that of the rat exhibits considerable spontaneous tone, which usually does not disappear after a 3 hour period of incubation. That spontaneous tone is indeed present is evidenced by the relaxation which occurs upon addition of nitrite. Because such tone might prevent the full change of tension in response to norepinephrine, we attempted to depress or abolish it by the addition of nitrite at a 10⁻⁴ concentration (as NaNO₂) to the bath. Approximately 15 min. after the aorta had been dissected free, it was exposed to nitrite for exactly 10 min. After recording relaxation during this period, the strip was transferred to a 250 ml. Erlenmeyer flask containing fresh, warmed, oxygenated Krebs-Henseleit solution. It was again placed in the muscle bath 2½ hours later, and a stretching force of 0.5 Gm. reapplied. Although nitrite had been removed in the successive changes of bath fluid, spontaneous tone remained almost completely inhibited. In 3 specimens re-exposed to nitrite after the incubation period, no additional relaxation occurred during the second exposure to nitrite.
Norepinephrine was selected to test responsiveness to a pressor agent since it is thought to be the physiologic neuro-effector substance which is released at the sympathetic nerve endings.

Fresh dilutions of norepinephrine bitartrate in isotonic saline were prepared immediately before use such that the addition of 0.1 ml. of solution would make the final concentration of norepinephrine base in the bath $10^{-4}$, $10^{-5}$, or $10^{-6}$ respectively. The arterial strip was exposed to these concentrations of norepinephrine successively at 3 min. intervals without flushing. The final tension was recorded as that developed 9 min. after exposure to a concentration of $10^{-4}$.

In contrast to our earlier experience, with this method a contraction occurred on exposure to norepinephrine in every case. Hence, unless full relaxation of the arterial spiral is induced by some means such as nitrite, we believe that the full response to norepinephrine cannot be measured with validity.

**Production of Hypertension**

We have studied the responses of the aorta after producing hypertension by 4 distinct methods:

1. **Partial Constriction of One Renal Artery.** One renal artery was constricted by a silver ribbon clip and excision of the opposite kidney results in the almost uniform development of hypertension within 2 months. Rigid restriction of the dietary intake of sodium, begun early, will reduce the incidence of hypertension about 50 per cent. This was done by placing these rats on distilled water and feeding a special diet low in sodium,* beginning 4 days before operation. Aortas from rats which had become hypertensive and from rats which had remained normotensive were studied 4 months after operation.

2. **Post-Desoxycorticosterone** Hypertension. The hypertension which occurs with the administration of desoxycorticosterone and supplementary sodium may persist in some cases after absorption of desoxycorticosterone has ceased.* In several respects this form of experimental hypertension is closely analogous to human essential hypertension. There has been no operative procedure involving the kidneys; there is no obvious excess of circulating vasopressor substance; and the structural changes which occur in the kidney are slight, and insufficient in themselves to allow differentiation between rats remaining hypertensive and rats which again become normotensive after the administration of desoxycorticosterone is stopped.”

3. **Partial Constriction of One Renal Artery and Removal of the Contralateral Kidney.** Constriction of one renal artery by a ribbon clip and excision of the opposite kidney results in the almost uniform development of hypertension within 2 months. Rigid restriction of the dietary intake of sodium, begun early, will reduce the incidence of hypertension about 50 per cent. This was done by placing these rats on distilled water and feeding a special diet low in sodium,* beginning 4 days before operation. Aortas from rats which had become hypertensive and from rats which had remained normotensive were studied 4 months after operation.

4. **Hypertension Resulting from Desoxycorticosterone Plus Renal Ischemia.** One renal artery was constricted by a clip. Simultaneously 125 mg. of desoxycorticosterone trimethylacetate was administered subcutaneously. Four weeks later an additional 125 mg. of desoxycorticosterone trimethylacetate was given. The rats consumed a “synthetic” diet* with sodium chloride added to give a final concentration of 1 per cent, and drank only 1 per cent saline. When sacrificed 6 weeks after operation, all the rats had been hypertensive for at least 10 days.

This form of hypertension represents a mixture of two influences which we cannot separate entirely. Hypertension developed uniformly in every rat in this group at about the same time expected in rats given desoxycorticosterone alone (unpublished observations). Since hypertension has developed in not more than 70 per cent of rats subjected to “clipping” alone in several previous experiments, we attribute at least part of the hypertension which developed in this group to the effect of desoxycorticosterone plus supplementary sodium.

**Controls.** Aortas from normotensive rats were studied concurrently. These rats were of two general types. “Operated normotensive controls” had received treatment identical to that of the corresponding group of hypertensive rats. “Un-operated controls” received the same diet as the corresponding hypertensive rats, but had had neither surgical manipulation nor desoxycorticosterone.

Normal Wistar rats have a blood pressure of $107 \pm 7$ mm. Hg, as determined by the micro-
Threshold Studies

Small differences in sensitivity to minute amounts of circulating norepinephrine might be important in accounting for increased peripheral vascular resistance in hypertension. Our basic procedure was therefore modified in order to determine accurately the threshold for norepinephrine of aortic spirals from 12 hypertensive and 6 normotensive rats. The hypertensive group included 6 rats with "post-desoxycorticosterone" hypertension, 3 rats in which one renal artery had been constricted 2½ months earlier, and 3 other rats in which one renal artery had been constricted 9 months earlier. Normotensive controls included 4 rats in which desoxycorticosterone pellets were implanted 15 months earlier, and 2 rats in which one renal artery was constricted 9 months earlier.

After the usual exposure to nitrite and an incubation period for 2½ hours as described above, these aortas were exposed to successively higher concentrations of norepinephrine, beginning with a concentration of \(2 \times 10^{-7}\). Successive doses (given in 0.1 ml. of isotonic saline at intervals of 1 min.) doubled the concentration of norepinephrine in the bath. Since the response at threshold concentrations was a minute deflection of the baseline, 1 or 2 doses above threshold were given. When a true threshold was found, succeeding doses gave progressively stronger contractions. After the threshold had thus been determined, the concentration of norepinephrine in the bath was increased to \(10^{-6}\) for measurement of maximal contraction, as described above.

RESULTS

Strength of Contraction. Table 1 lists the
TABLE 2.—Isometric Relaxation of Aortas of Hypertensive and Normotensive Rats on Exposure to Nitrite, 10-4 M (as NaNO2)

<table>
<thead>
<tr>
<th>Group Description</th>
<th>Hypertensive rats</th>
<th>Normotensive rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood pressure</td>
<td>Relaxation</td>
</tr>
<tr>
<td></td>
<td>(mm Hg)</td>
<td>(Gm.)</td>
</tr>
<tr>
<td>1. Partial constriction of one renal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>artery, operation 2½ months before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>obtaining aorta</td>
<td>158</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>154</td>
<td>0.12</td>
</tr>
<tr>
<td>2. Constriction of one renal artery,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>removal of opposite kidney</td>
<td>202</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>204</td>
<td>0.22</td>
</tr>
<tr>
<td>3. &quot;Post-desoxycorticosterone&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>188</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>204</td>
<td>0.05</td>
</tr>
<tr>
<td>4. Desoxycorticosterone plus renal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ischemia</td>
<td>170</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>0.12</td>
</tr>
<tr>
<td>5. Unoperated rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>188</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>148</td>
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<td></td>
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<tr>
<td></td>
<td>144</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>0.06</td>
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</table>

The contraction of aortas of hypertensive rats, when exposed to norepinephrine, was slightly weaker than that of the normotensive group (0.33 Gm., compared with 0.43 Gm. for the control group). This difference can be accounted for entirely by 10 aortas from the hypertensive group which displayed a weaker contraction than any of the control aortas. If these 10 results are excluded, the aortas of the remaining 19 hypertensive rats developed contractions identical in strength to those of the control group.

Interestingly, the 19 hypertensive rats whose aortas showed entirely normal contractile responses include all the rats from 4 of our 6 experimental groups with hypertension. The 10 "hyporesponsive" aortas include 4 of the 6 aortas from rats having hypertension as a result of constriction of one renal artery 4 months previously and 6 of the 7 aortas from rats which received desoxycorticosterone plus constriction of one renal artery.
these 10 instances contractions occurred in a normal fashion at concentrations of norepinephrine of $10^{-11}$ or $10^{-9}$; however, increasing the concentration of norepinephrine to $10^{-7}$ produced either no increase or else only a very small increase in tension.

With the data available at present no good explanation can be offered for the frequent "hyporesponsiveness" among these two groups of rats. The finding of normal responses in the aortas of rats with hypertension of either longer or shorter duration as a result of constriction of one renal artery suggests that the acuteness of the hypertension is not a likely explanation for the isolated occurrence of hyporesponsiveness. Further study of larger groups of rats with hypertension of various types will be required before the significance of this observation can be assessed.

**Spontaneous Tone.** Table 2 shows the amount of relaxation which occurred when the aortas of various rats were exposed to sodium nitrite. (The number of observations in this table is larger than that in table 1 because a few spirals were used in other experiments, and were not exposed to norepinephrine following their initial exposure to nitrite.)

Thirty-two aortas from hypertensive rats lost an average of 0.140 Gm. of tension when exposed to nitrite. The relaxation of 32 control aortas was virtually identical, with a mean of 0.142 Gm.

Considering each subgroup individually, the amount of relaxation was significantly different only when rats with hypertension as a result of constriction of one renal artery 4 months earlier were compared with operated controls ($p < .01$). In this instance, aortas of hypertensive rats lost an average of 0.186 Gm. of tension when nitrite was added to the bath, compared to a decrement in tension of 0.132 Gm. for aortas of 15 unoperated control rats. This difference remains an isolated, unexplained finding. We do not know whether the use of nitrite in these experiments provides a quantitatively accurate reflection of "spontaneous tone." If it does, it is indeed possible that in certain stages of certain forms of hypertension spontaneous tone is increased. On the same assumption, however, we could conclude that in the majority of instances the aortas of hypertensive rats have normal tone.

**Threshold Studies.** No gross difference in threshold for norepinephrine between aortas of hypertensive and normotensive rats was observed during the course of our studies. Meticulous exploration of the effect of very low concentrations of norepinephrine in 12 hypertensive rats revealed a threshold which ranged from $2 \times 10^{-18}$ to $2 \times 10^{-11}$. This represents essentially complete overlap with the thresholds determined in 6 "operated normotensive" controls, which ranged from $2 \times 10^{-13}$ to $1 \times 10^{-10}$.

**DISCUSSION**

In addition to the criticism previously directed at the interpretation of in vivo experiments seeming to demonstrate hyper-responsiveness, one other highly significant factor should also be considered. It is recognized that the lumens of arteries and arterioles of persons with hypertension have a decreased "resting" diameter. If waterlogging or muscular hypertrophy of arteries were not present in hypertension this would have to be due to a contracted ring of arteriolar smooth
muscle. We believe that a given amount of additional constriction of an already constricted vessel will cause a disproportionately great increase in resistance to flow. The following example will demonstrate this point.

If one considers a small arteriole, having a lumen 10 \( \mu \) in diameter and a wall 5 \( \mu \) thick (fig. 1), what happens to vascular resistance as the circular smooth muscle undergoes successive increments of contraction? Let us assume that the outer contracting fibers in such a vessel form a circle whose initial diameter is 20 \( \mu \). The cross-sectional area of the ring of arterial wall contained between these outermost constricting fibers and the innermost constricting fiber would then be \((10^2 - 5^2) \pi \mu^2 = 75 \pi \mu^2\). This area can be assumed to remain constant as the muscle fibers contract and the lumen becomes smaller. The area of the lumen of the vessel may then be calculated as the difference between the area of the large circle (bounded by the outer contracting muscle fibers) and the cross-sectional area of this ring of arterial tissue. It would be 25 \( \pi \mu^2\). Arteriolar resistance, according to Poiseuille's law, will be proportional to the reciprocal of the fourth power of the radius of the lumen.

Let us now assume a 2 per cent shortening of the circumferential ring of arteriolar muscle fibers. The diameter of the circle formed by these fibers is thus reduced from 20 \( \mu \) to 19.6 \( \mu \) (fig. 1). The area of the lumen would then be reduced from 25 \( \pi \mu^2 \) to \([(19.8)^2 - 75] \pi \mu^2 = 21.04 \pi \mu^2\). Let us suppose another 2 per cent shortening of the circular smooth muscle, so that the outer diameter is reduced to 19.208 \( \mu \), and continue, repeating our calculations of the area of the lumen and the resulting resistance for this and further 2 per cent increments of shortening. If the initial outer muscular circumference of the arteriole is 20 \( \pi \), the supposed successive shortening of the muscular circumference would be represented by \(20\pi \times (.98)^1, 20\pi \times (.98)^2, 20\pi \times (.98)^3, 20\pi \times (.98)^4, \ldots\)

Figure 2 presents the results of these calculations graphically. With progressive 2 per cent increments in shortening of circular smooth muscle, each actually slightly less than the preceding one in absolute terms, there is a decrease in the cross-sectional area of the lumen, which occurs as a geometric progression. The calculated resistance, however, rises exponentially, so that each additional 2 per cent shortening produces an increasingly greater per cent rise in resistance to flow. Hence, with arterioles already markedly narrowed (as in hypertension) a truly minute additional shortening of the circular smooth muscle could result in an extremely large increment of resistance. From our calculations, in an arteriole of these approximate dimensions in a hypertensive patient, in which but a 4 per cent shortening of circular muscle had already occurred, the cross-sectional area of the lumen would be reduced to about 70 per cent of its normal value. The increment of resistance to flow produced by an additional 2 per cent shortening of the circular muscle fibers would be 50 per cent greater than the increment of resistance produced by an equivalent shortening of muscle fibers of a normal, uncontracted arteriole.

Thus, arteriolar narrowing in hypertension, as a result of muscular shortening in itself, could result in a significant shift on the curve (fig. 2) which relates increments of muscle shortening to increments of resistance. This would occur even if "waterlogging" and muscular hypertrophy were excluded or ignored.

We have deliberately ignored considera-
tion of the viscosity of blood in our calculations. The studies of Pappenheimer and Maes on the perfused hind limb of the dog showed without exception that vasoconstriction per se increases the apparent viscosity of the blood. And when a decreased lumen size in small arterioles increases the resistance to flow, about one third of the increase is accomplished by increasing the apparent viscosity of blood.

Therefore, there are two distinct factors which might cause an "apparent" hyperresponsiveness of hypertensive subjects to pressor agents in the absence of an actually greater shortening of circular smooth muscle fibers. Such apparent hyper-responsiveness would occur whether the parameter measured was cessation of flow in a capillary bed, or over-all changes in blood flow or vascular resistance. First, "waterlogging" and hypertrophy by increasing the wall thickness of arterioles could produce apparent hyper-responsiveness without actual hyper-responsiveness. Second, even without "waterlogging" or hypertrophy, our calculations show that a slight decrease in cross-sectional areas of the arteriolar lumen in the hypertensive, as a result of possible pre-existing smooth muscle contraction, could create a situation of apparent hyper-responsiveness without actual hyper-responsiveness.

**SUMMARY**

Using a technic involving inhibition of spontaneous arterial tone, the contractile responses of spirally cut strips of aorta from 4 different types of hypertensive rats were studied. In no case did aortas from hypertensive rats show hyper-responsiveness upon exposure to norepinephrine, when compared with the responses of normotensive control animals.

The aortas of one group of rats with renal hypertension of 1 to 3 months’ duration demonstrated increased spontaneous tone, as evidenced by increased relaxation on exposure to nitrite. Several aortas from this group, as well as those from rats with a different form of hypertension of even shorter duration, developed less than the normal amount of tension on exposure to norepinephrine. We have no satisfactory explanation for these isolated findings. With these exceptions, both spontaneous tone and strength of contraction as a result of norepinephrine were normal in our investigation of hypertension of varying types and durations. Likewise, the threshold of the aorta for norepinephrine was not affected by the presence or absence of hypertension.

The direct evidence obtained in these in vitro experiments on the aorta of rats cannot be transferred immediately to the behavior of arterioles of humans with essential hypertension. However, technics by which the latter has been studied do not support the conclusion that actual rather than apparent hyper-responsiveness is present. The arterioles of patients with hypertension are in a different "resting" state than those of normotensive persons. Studies which fail to take this difference into account may be grossly misleading.

**SUMMARIO IN INTERLINGUA**

Un technica resultante in le inhibition del spontane tono arterial esseva usate pro studiar le responsas de contractilitate in seciones spiral de 4 differente typos de rattos hypertensive. Esseva trovate nulle caso in que le aorta de un ratto hypertensive manifestava hyper-responsivitate a norepinephrina in comparison con le aorta de normotensive animales de controlo.

Le aortas de un gruppo de rattos con hyper-tension renal de 1 a 3 menses de duration demonstrava un augmentate tono spontanee, manifeste in augmentos de relaxation post exposition a nitrito. Plure aortas in iste gruppo e etiam le aortas de rattos con un differente forma de hypertension de un duration mesmo plus curte disveloppava grados subnormal de tension post exposition a norepinephrina. Nos non pote offerer un satisfacente explication de iste isolate constatazioni. A parte iste exceptiones, tanto le tono spontanee como etiam le fortia del contraction effectuate per norepinephrina esseva normal in nostre inves-tigation de hypertension de varie typos e du-
VASCULAR HYPER-RESPONSIVENESS

rationes. In plus, le limine aortic pro le efecto de norepinephrina non esseva afficite per le presentia o absentia de hypertension.

Le constatationes facite in iste experimentos in vitro con aortas de ratto non es directemente transferibile al comportamento de arteriolas de humanos con hypertension essential. Tamen, technicas disveloppate pro le studio de tal arteriolas human non ha producire resultatos que supporta le conclusion que ver plus tosto que apparente hyper-responsivitate es presente. Le arteriolas de pacientes con hypertension difere in lor stato de "reposo" ab le arteriolas de personas normotensive. Studios que negligence iste differenia pote devenir grossiermente illusori.

REFERENCES
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