Early and Late Effects of Coronary Artery Occlusion on Canine Purkinje Fibers

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ABSTRACT

The electrophysiological characteristics of subendocardial Purkinje and myocardial cells were studied during acute (within 30 minutes) and chronic (after 10 days) phases of myocardial infarction. Endocardial Purkinje and myocardial electrograms were recorded in vivo with bipolar electrodes before and after occlusion of the anterior descending coronary artery. Also, intracellular and extracellular potentials were recorded in vitro from the endocardial surface of infarcted regions of hearts excised during the acute and chronic phases. In the acute phase, Purkinje and myocardial potentials within the ischemic zone deteriorated in vivo, but they were not markedly delayed. Intracellular recordings in vitro showed partial depolarization of both Purkinje and myocardial cells. In the chronic phase, extracellular and intracellular Purkinje potentials recorded in vivo and in vitro from the infarcted zone usually did not differ from normal. No myocardial potentials were recorded from the endocardial surface of the chronic infarcts. Thus, subendocardial Purkinje and myocardial cells are affected early in infarction, but many Purkinje cells survive and return to normal.

KEY WORDS

chronic infarcts myocardial cells transmembrane potentials

acute ischemia automaticity conduction

In the first 30 minutes after occlusion of the anterior descending coronary artery in the dog, ectopic ventricular beats frequently occur and culminate in ventricular fibrillation (1). Existing evidence suggests that an important mechanism underlying this ectopic activity is reentry (2-7) resulting from slow conduction in ischemic myocardial cells, especially those in the subepicardial layers. The state of Purkinje fibers in this early stage of ischemia has not been studied; their role in the early arrhythmia is unknown. On the day following coronary artery occlusion, Purkinje fibers within the infarcted region manifest distinctive alterations of their electrophysiological properties: diminished resting potentials, prolonged action potentials, and enhanced automaticity (8-10). These altered Purkinje fibers are probably instrumental in the genesis of the ectopic ventricular beats occurring at this later stage. The eventual fate of the Purkinje fibers within the borders of a chronic infarction has not been determined. The purpose of the present study was to assess the electrophysiological properties of fibers during the early stages of ischemia when the initial burst of ventricular ectopic activity occurs and during the stage of chronic infarction when there is no longer any arrhythmia.

Methods

The methodology employed in this study was similar to that described in detail in a previous investigation of Purkinje fibers in 1-day-old myocardial infarctions (8). Recordings were made from the intact dog heart and from excised cardiac tissues. Eleven mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv), and a left thoracotomy was performed. After the heart had been exposed, stainless steel bipolar plunge wire electrodes (diameter 0.003 inches) were hooked into endocardial and subepicardial sites within and outside the region of distribution of the left anterior descending coronary artery. The location of the wire tips was verified when the heart was excised and the left ventricle opened. The vagosympathetic trunks were isolated in the neck and stimulated at intervals with pulses 0.05 msec long and 2-10 v in magnitude at 20 Hz for 1-2 minutes to produce transient periods of atrial arrest. After control recordings had been made (EEP amplifier, DB8 recorder, Electronics for Medicine), the left anterior descending coronary artery was abruptly tied 1-2 cm from the bifurcation of the left main coronary artery. Recordings were then repeated at frequent intervals. After 20-30 minutes of occlusion, the hearts were rapidly excised and the free wall of the left ventricle was bisected. Dissected preparations contained most of the endocardial surface...
of the left ventricle including the lower septum, the papillary muscles, the apex, and the lower free walls. The preparations were superfused with Tyrode's solution of the following millimolar composition: Na⁺ 151.1, K⁺ 4.05, Ca²⁺ 1.35, Mg²⁺ 0.5, Cl⁻ 131.25, HCO₃⁻ 24.0, HPO₄²⁻ 1.8, and dextrose 5.5.

It was usually possible to make extracellular recordings within 10 minutes and intracellular recordings within 15 minutes of excision. Recordings were made using techniques and instrumentation that have been described previously (8). Sites outside the distribution of the occluded vessel (normal zone) were monitored for comparison with recordings from sites within the ischemic zone. In the excised specimens it was not possible to distinguish the ischemic and normal zones by inspection. However, from dye injections of the anterior descending coronary artery in ten dogs and from observation of many 1-day-old infarctions, which are clearly visible, the zone of ischemia could be estimated. Despite individual variation, the endocardial surface of the base of the anterior papillary muscle, the apex, and the anterior and inferior portion of the septum were consistently within the ischemic zone. The base of the posterior papillary muscle and the posterior septum and free wall were considered to reliably represent nonischemic normal areas.

In another group of eight dogs, the anterior descending coronary artery was tied under aseptic conditions and the chest was closed. Another thoracotomy was performed after 10 days to 3 months, and endocardial and epicardial recordings were made from normal and infarcted zones. The hearts with chronic infarctions were excised and studied in vitro in a manner similar to that employed with the dogs with acute ischemia. In these specimens, the infarcted zone was clearly distinguishable from the endocardial aspect; it was seen as a white apical scar with overlying yellowish Purkinje strands.

**Results**

**EARLY ISCHEMIA**

*In Vivo Findings.*—Endocardial recordings made in vivo presented separate deflections attributable to activation of Purkinje (P) and ordinary myocardial (M) cells in the vicinity of the electrodes. The P deflections preceded the M deflections; at sites on the upper septum, they preceded the QRS complex. They were of shorter duration (< 5 msec) and usually of smaller amplitude (< 2 mv) than the M deflections (10-30 msec, 2-10 mv). The M deflections often had a more rapid component, lasting 5-15 msec, and a slower component lasting throughout the QRS complex or into the S-T segment. The M deflections of Figure 1 show prominent slower components. The slow components tended to diminish with time after electrode insertion, sometimes disappearing entirely leaving only a rapid component, as in the epicardial recording of Figure 1. The slow component was considered to represent an injury potential. There was no essential difference in the behavior of rapid M components that were relatively pure and those that were associated with slow components. Consequently, the slow components were ignored when
the rapid components were analyzed. At all sites from which P potentials were recorded, the tips of the electrodes were observed to be in close proximity to grossly visible pale Purkinje strands when the heart was inspected after excision. In a few instances, the two wires were not hooked together into the endocardium, but usually both tips were snug against the endocardium within 2 mm of one another. Within minutes after the occlusion, the amplitude of P deflections at 16 of 21 sites in the ischemic zone had diminished; however, delays in activation of P deflections were slight with the maximum observed delay being 10 msec. In a few instances the P deflection disappeared because of attenuation or delay sufficient to displace it into the M deflection; the delay of activation could not be measured accurately under this condition. Release of the occlusion during the initial 30 minutes was followed by recovery of the timing and the amplitude of the P deflections. The sequence of deterioration after occlusion and the recovery after release could be repeated in the same dog, but partial resistance developed after several trials. Figure 1 illustrates records from one of several occlusions and releases in one dog. The P deflections recorded from both endocardial sites supplied by the anterior descending coronary artery deteriorated within 15 seconds after occlusion and recovered completely about 2 minutes after release. Frequently, as in this experiment, the P potentials appeared to be affected more severely than the M potentials in the very early period (first 10 minutes) after occlusion. However, endocardial M potentials in the ischemic zone invariably diminished in amplitude within the first few hours after occlusion (8). M deflections, like P deflections, were only slightly delayed (20 msec maximum), although they were diminished in amplitude. The severity and the rapidity of evolution of the changes were not uniform within the ischemic zone. Electrograms from some sites within the ischemic zone were little affected, although those from other sites showed definite changes.

In this early period after occlusion, the ventricular escape time and ventricular escape pacemakers during vagally induced atrial arrest did not differ from control, a finding previously reported by Scherlag et al. (2). During ventricular escape rhythms, P potentials recorded from the ischemic zone always followed P potentials recorded from more proximal sites in the left bundle branch, indicating escape pacemakers in the proximal bundle branches.

**In Vitro Findings.**—After excision and isolation of the heart, the earliest extracellular recordings from the ischemic zone showed diminutive slow deflections of uncertain cellular origin. In contrast, recordings from the normal zone contained clearly distinguishable separate P and M deflections with amplitudes and durations comparable to those recorded from uninfarcted preparations. P deflections were 1–3 msec in duration and M deflections were 6–10 msec in duration. The contrast between recordings from the normal and the ischemic zone is illustrated in Figure 2B.

The resting potentials and the amplitudes and upstroke velocities of action potentials recorded in the ischemic zone were reduced compared with those recorded in the normal zone. The possibility that these changes were due to poor impalements was considered to be remote for various reasons. The recordings that were analyzed did not manifest other evidence of poor impalements such as instability, motion artifact, or gradual rather than abrupt change from the zero potential level to the resting potential level. Recordings with those characteristics sometimes were encountered, but they were discarded. Because the ischemic zone was less contractile than the normal zone, it was actually easier to obtain stable impalement in this zone. In addition, there were abnormalities in the extracellular recordings from the ischemic zone both in vivo and in vitro which corresponded to the changes in the intracellular recordings. Finally, it seems unlikely that microinjury from poor impalement would produce the observed changes in the time course of repolarization. The duration of

![FIGURE 2](http://circres.ahajournals.org/)

**FIGURE 2**

Potentials recorded from the endocardial surface of a superfused specimen including normal (NZ), border (BZ), and ischemic (IZ) zones of a heart excised 20 minutes after occlusion of the left anterior descending coronary artery. A: Recordings made 10 minutes after excision of the heart. B: Recordings made 90 minutes after excision. Purkinje (P) and myocardial (M) deflections can be distinguished. Note that activation time for the ischemic zone sites is reduced about one-half in B compared with that in A. The preparation was driven, and the stimulus artifacts appear in most of the traces of A and B.
action potentials of P and M cells in the ischemic zone was shorter than that of cells in the normal zone. Diastolic depolarization of P fibers in the ischemic zone was minimum. Representative examples of recordings from P fibers and M fibers are illustrated in Figure 3A and Figure 4. In time, transmembrane potentials of P and M cells in the ischemic zone recovered toward normal (Figs. 3 and 4). Figure 5 shows normal transmembrane potentials from fully recovered P and M cells in the ischemic zone after 1.5 hours of superfusion. The rate of recovery was variable. In about one-third of the preparations (4 of 11), there was little difference between the ischemic and the normal zone at the time of the earliest recordings made 10–15 minutes after excision. At the other extreme, in some preparations after several hours cells from the ischemic zone had not returned to normal. There was heterogeneity with respect to the degree of depression and the rate of recovery of cells at different sites within the ischemic zone. Minimally affected cells were often found. The mean values of the resting potentials and the amplitudes and durations of the action potentials of Purkinje and myocardial cells in the ischemic and the normal zone are shown in Table 1. None of the preparations showed a proclivity for spontaneous rapid firing. The mean spontaneous firing rate of the infarcted preparations (8 ± 3 beats/min) did not differ from the mean rate of similarly dissected uninfarcted preparations (6 ± 3 beats/min). Mean conduction velocity (linear propagation) in the Purkinje network was 0.73 ± 0.15 m/sec in the

FIGURE 3
Intracellular potentials of Purkinje fibers in a preparation from a heart excised 20 minutes after occlusion of the left anterior descending coronary artery. A: Potentials recorded from a cell within the ischemic zone (IZ) 15 minutes after excision of the heart. B: Potentials of the same cell recorded 20 minutes later and compared with those of a cell within the normal zone (NZ).

FIGURE 4
Intracellular potentials of myocardial (M) and Purkinje fibers (PF) in ischemic zones (IZ) and normal zones (NZ) of a preparation from a heart excised 20 minutes after occlusion of the left anterior descending coronary artery. The recordings from the ischemic zone were initially made 13 minutes after excision. An M action potential was recorded from the ischemic zone 28 minutes after excision and is shown on the far right.

FIGURE 5
Intracellular potentials recorded from fully recovered Purkinje (IZ-P) and myocardial (IZ-M) cells within the infarcted zone of the preparation of a heart excised 20 minutes after occlusion of the left anterior descending coronary artery. These recordings were made after 1.5 hours of superfusion in vitro. P and M potentials recorded in vivo and soon after isolation in vitro had been abnormal.
TABLE 1

<table>
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<tr>
<th>Time (min)</th>
<th>Zone</th>
<th>RP (mv)</th>
<th>AP amplitude (mv)</th>
<th>AP duration (msec)</th>
<th>RP (mv)</th>
<th>AP amplitude (mv)</th>
<th>AP duration (msec)</th>
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</thead>
<tbody>
<tr>
<td>0-60</td>
<td>IZ</td>
<td>62 ± 12*</td>
<td>71 ± 12*</td>
<td>265 ± 53*</td>
<td>71 ± 12*</td>
<td>89 ± 16*</td>
<td>229 ± 44*</td>
</tr>
<tr>
<td>0-60</td>
<td>NZ</td>
<td>82 ± 9</td>
<td>107 ± 7</td>
<td>330 ± 29</td>
<td>83 ± 7</td>
<td>105 ± 10</td>
<td>281 ± 24</td>
</tr>
<tr>
<td>60-120</td>
<td>IZ</td>
<td>69 ± 9†</td>
<td>85 ± 17†</td>
<td>213 ± 35†</td>
<td>74 ± 4‡</td>
<td>87 ± 15‡</td>
<td>213 ± 30‡</td>
</tr>
<tr>
<td>60-120</td>
<td>NZ</td>
<td>83 ± 7</td>
<td>105 ± 10</td>
<td>281 ± 24</td>
<td>83 ± 7</td>
<td>105 ± 10</td>
<td>281 ± 24</td>
</tr>
</tbody>
</table>

All values are means ± SD. Time refers to the time interval after excision during which recordings were made. IZ = ischemic zone, NZ = normal zone, RP = resting potential, and AP = action potential.

* Values differ from control during the same time interval with a P value < 0.001.
† Values differ from control with a P value < 0.01.
‡ Values differ from control with a P value < 0.05.

Infarcted preparation; this value was significantly (P < 0.05) less than normal (1.18 ± 0.22 m/sec).

CHRONIC INFARCTS

In Vivo Findings.—Dogs with chronic infarction did not have arrhythmias. Automaticity of ventricular escape pacemakers was not enhanced (Fig. 6B). Recordings from the endocardial surface of the scar often disclosed P potentials of normal amplitude (up to 2 mv) and duration (< 5 msec), but there were no deflections which could be attributed to local M activation in any of the preparations. The P potential was associated with a small slow deflection (< 1 mv) lasting throughout the QRS complex. Because this deflection occupied the entire duration of the QRS complex and usually lacked a sharp slope, it was attributed to activation of distant myocardium (extrinsic deflection). Similar deflections have been recorded from Ivalon sponges sutured into the left ventricular wall (11). Representative recordings from the endocardial surface of the infarct in the intact dog are shown in Figure 6A. The small slow deflection about 100 msec before the P spike is of uncertain origin; it

![Image of Figure 6A and 6B](http://circres.ahajournals.org/)

**FIGURE 6**

Purkinje potentials and automaticity in a 2-month-old myocardial infarct. A: ECG leads 1 and 2 and a bipolar recording from the endocardial surface of the infarct. B: Vagal stimulation (VS) was applied to unmask enhanced Purkinje automaticity. There was no escape pacemaker for 10 seconds. The vertical bar on the third trace of A represents 1 mv. Time lines are at intervals of 1 second in B.

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might have been generated by Purkinje repolarization. Note that during vagal stimulation, automaticity was not enhanced.

**In Vitro Findings.**—In the excised specimens the chronic infarcts appeared as hard white scars, but pale yellowish Purkinje strands were visible on the endocardial surface of the scar. Immediately after isolation, electrograms recorded from the infarcted zone consisted of sharp deflections suggesting P origin (1–3 msec in duration). Slower deflections typical of M origin (6–10 msec) were never recorded in the infarcted zone. As in the specimen from the early infarctions, the electrograms from the normal zone contained both P and M deflections. A representative set of electrograms is illustrated in Figure 7. In most preparations, intracellular resting and action potentials recorded from P cells in the infarcted zone did not differ from those recorded nearby in the normal zone at any time after isolation. A comparison of the intracellular potentials in the infarcted and normal zones is shown in Figure 8. In two preparations (infarcts 10 and 14 days old), occasional cells with slightly decreased resting potentials or abnormal action potentials were found. A record from a cell with an abnormal action potential is shown in Figure 9 in comparison with records from a Purkinje cell and a myocardial cell in the normal zone. Enhanced automaticity was not noted in chronic infarctions. Transmembrane action potentials characteristic of M cells were not recorded from the endocardial surface of the infarcted zone. There was no significant difference between the P cells of the normal zone and the infarcted zone with respect to mean values of the resting potential (84 ± 8 and 81 ± 6 mv, respectively) and the amplitude (110 ± 10 and 108 ± 9 mv) and duration (320 ± 28 and 302 ± 31 msec) of the action potentials.

**Discussion**

The electrophysiological properties of isolated Purkinje fibers are somewhat insensitive to oxygen deprivation. Trautwein et al. (12) have demon-
strated that the properties of Purkinje fibers bathed in solutions equilibrated with 95% N₂-5% CO₂ show no change for hours and then deteriorate only if the fibers are driven at higher rates. Resistance to hypoxia has also been demonstrated in intact animals; conduction within the His-Purkinje system is well maintained for hours when the coronary arteries are perfused with hypoxic blood (13). This low requirement for oxygen could be a reflection of the paucity of myofibrils in Purkinje fibers (14) and their relatively weak contractile activity, since contractile activity requires much more energy than does electrical activity (15, 16). In addition, anaerobic metabolic pathways could be more important in Purkinje fibers than they are in myocardial cells (17, 18).

In light of this background of data from previous studies, the rapid effects produced by coronary artery occlusion on the electrical activity of Purkinje fibers in the intact working heart was unexpected. It was anticipated that oxygenated left ventricular chamber blood should provide adequate sustenance for at least several hours, since Purkinje fibers can be sustained in a relatively normal state by superfusion in vitro even with hypoxic solutions. To the contrary, the deterioration in vivo of Purkinje potentials was often more rapid than that of myocardial potentials recorded from the endocardial surface (Fig. 1).

Since the recordings were made with plunge wire electrodes hooked into the endocardium, it is conceivable that the tips of the wires could have dislodged after occlusion because of the outward bulging of the ischemic portion of the ventricular wall during systole. If so, diminution of Purkinje potentials would be an experimental artifact. For several reasons this explanation is unlikely. In some experiments, the wires were tugged on after the occlusion to ensure that their tips remained hooked into the endocardium; this procedure did not change the character of the recordings. Also, both tips of the wires were always snug against the endocardium at the time of inspection of the endocardial surface after excision of the heart. Finally, the intra- and extracellular recordings from the excised specimens showed corresponding changes in the electrical activity of Purkinje fibers.

Changes in the transmembrane potentials of Purkinje fibers in the early period (20-30 minutes) after occlusion are not similar to changes observed on the day after occlusion during the later period of arrhythmias. In the early period, the action potentials of Purkinje fibers are shortened and diastolic depolarization is minimum, whereas, on the day after occlusion, action potentials are strikingly prolonged and diastolic depolarization is enhanced (8-10). During both periods, the amplitudes of resting and action potentials are reduced. Also, recovery toward normal during superfusion occurs during both periods. It has been postulated that the changes of the later period are produced not primarily by hypoxia but by substances released from damaged myocardial cells (8). A similar hypothesis can be applied to the changes of the earlier period, but the nature of the offending factors must differ at different times after occlusion. During this early period, potassium released from myocardial cells into the extracellular space is a plausible candidate for the induction of some of the observed changes. The amplitude of the resting potential, the amplitude and duration of the action potentials, and the rate of diastolic depolarization are all inversely related to the extracellular potassium concentration (19). Moreover, it is clear that there is a very early transfer of potassium from the intracellular to the extracellular space after coronary occlusion (20-23). However, the magnitude of the rise in extracellular potassium concentration in the vicinity of subendocardial Purkinje fibers has not been accurately quantified. The finding that recovery sometimes requires several hours of superfusion in vitro militates against the hypothesis that elevated extracellular potassium is solely responsible for the changes. The rapidly diffusing potassium ion would be expected to equilibrate rapidly between the tissue extracellular space and the potassium of the superfusate; hence, rapid recovery of Purkinje fibers should occur after isolation and during superfusion. On the other hand, the slow recovery could be the result of a continuing source of potassium moving from deeper layers to the tissue outward toward the endocardial surface.

In chronic infarction, most of the surviving subendocardial Purkinje fibers are electrophysiologically normal. From our data, it is not possible to assess quantitatively the survival rate. It is possible that the damage which occurs is sublethal and that all subendocardial Purkinje fibers survive. On the other hand, many fibers, particularly those in deeper layers, may not survive. The observation that the surviving fibers are for the most part normal fits with the absence of arrhythmias in the dogs at this stage and with the normal ventricular automaticity. Within the borders of the chronic infarctions, the duration of Purkinje action potentials was normal despite the absence of myocardial cells and Purkinje-myocardial coupling. This finding indicates that the shorter action potentials of
the subendocardial myocardial cells coupling with the Purkinje system have relatively little influence on the duration of the action potentials of the superficial Purkinje cells. Furthermore, this finding suggests that the disappearance of myocardial cells in 1-day-old infarctions does not account for the prolongation of the action potentials of Purkinje cells at that time (8, 9). The occasional abnormal cells in the chronic infarcts could represent permanently altered cells or slowly recovering cells. Observation of these occasional abnormal cells only in the younger infarcts (10 and 14 days) suggests the latter. It is possible that slowly recovering or persistently altered Purkinje fibers could play a role in late arrhythmias after myocardial infarction.

Myocardial cells were affected in the early period in a manner similar to Purkinje fibers. Resting and action potentials were diminished, and action potentials were shortened in duration. At this time (20–30 minutes after occlusion), the damage was not necessarily lethal, since recovery was observed in vitro. In the case of myocardial cells, it is likely that lack of oxygen was a major damaging factor, since working myocardium has a higher requirement for oxygen. However, it is possible that other factors engendered by hypoxia were also deleterious. Increased extracellular potassium concentration, for example, could contribute to the electrophysiological changes in the myocardial cells. In 1-day-old infarctions (8) and in chronic infarctions, no subendocardial myocardial cells survived within the infarcted zone. Strictly interpreted, the failure to record myocardial potentials indicates only that the recording electrodes at the endocardial surface were too far from electrically active myocardial cells. This situation could result from the loss of myocardial cells in the subendocardial layers or the overgrowth of subendocardial fibrous tissue. Since the lesion is caused by ischemia and the myocardial potentials disappear after 24 hours, it appears reasonable to assume that there was an actual loss of subendocardial myocardial cells. Previous histological studies have shown that the extent of the infarction is greatest in the subendocardial region (11).

There is no clear indication that any of the electrophysiological changes observed in vivo or in vitro in Purkinje or myocardial cells are important in the genesis of the arrhythmias of the early phase. Despite the partial depolarization of the cells, there was only a slight or moderate conduction delay in activation of either the Purkinje cells or the subendocardial myocardial cells. Subendocardial myocardial cells differ from subepicardial myocardial cells in this respect. The latter show marked conduction delays in the early phase (4). The finding that conduction delay is greatest in the subepicardial layers of the ischemic zone has been reported previously (24). The lesser delay of the subendocardial layers probably relates to the close linkage of these cells to the Purkinje network, which is not affected severely or lethally by the occlusion. Also, the severity of the changes in subendocardial cells was spotty, so that conduction pathways utilizing minimally affected cells could mitigate the conduction delays. The greater delay of the subepicardial layers probably relates to the traversal of numerous layers of severely ischemic intramural myocardial cells. The lack of marked conduction delays in activation of subendocardial cells probably accounts for the failure to elicit repetitive firing from the endocardial surface in vitro. In contrast, in 1-day-old infarcts (8, 10), marked and heterogeneous conduction delays result from excitation during the prolonged phase 3 of the Purkinje action potentials. As a consequence, repetitive firing can be easily elicited. Finally, disorders of automaticity cannot account for ectopic beats in this early phase. Automaticity appears to be depressed rather than enhanced. We have reported elsewhere that ectopic firing of the early phase probably originates largely from markedly delayed conduction to the subepicardial myocardial cells, which eventuates in reentry (4).

References


Circulation Research, Vol. 35, September 1974
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Circ Res. 1974;35:391-399
doi: 10.1161/01.RES.35.3.391

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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