Effects of Freezing on Major Arteries

BY IRVING S. COOPER, M.D., PH.D.,* KHairyY SAMRA, M.C., M.Ch.,† AND KRSTYNA WISNIEWSKA, M.D.*

Thirty experiments were performed in dogs to assess the effects of freezing on carotid and femoral arteries. These vessels withstood freezing and thawing without rupture. Blood within the vessels did not clot and was lysed during thawing.

Histological changes of vessel walls consisted of early degeneration followed by a reparative process. Occasional secondary thrombosis occurred close to bifurcation of ligated branches and was related to extent and frequency of freezing and thawing.

ADDITIONAL KEY WORDS
cryogenic approach
cryosurgery cold effects
freezing and thawing
experimental cryosurgery

Introduction

For the past several years laboratory and clinical investigation has been under way in our department dealing with the effects of extreme cold on blood vessels, on clotting mechanisms, and on preexistent intravascular thrombi. In our clinic, as well as in others, the potential usefulness of varying degrees of extreme cold as a therapeutic modality for various types of vascular disease is being evaluated.1-5 These studies have also helped to establish the exact risk of hitting a vessel with the cold cautery while, for example, freezing a nearby tumor.

In future reports we will elaborate our clinical investigations with regard to possible treatment of vascular anomalies and aneurysms. The purpose of this report, however, is to summarize our laboratory studies of the effects of extreme cold applied to the walls of major arteries.

For more than a century, there have been reports indicating the potential usefulness of cooling or freezing biological tissues to produce controlled physiological inhibition or anatomical destruction.4-7 Until recently, however, the lack of adequate instrumentation has prevented the realization of the potential value of extreme cold as a surgical tool. During the past 20 years there have been classic advances in cryobiology as exemplified by the work of Luyet and Gehenio,8 Rey,9 Smith,10 Parkes11 and others.12-15 The investigations of these workers form a reservoir of basic information applicable to medical and surgical research. Also, cryogenic engineering16 has now reached the point in which the application of cryogenics to surgery will be limited only by the imagination of the surgeons and their interest in investigating this new approach. A combination of efforts on the part of biologists, cryogenic engineers and surgeons offers the possibility of productive investigation and therapy not only in the central nervous system17-22 but in many other areas of human disease as well.23-26

Methods

Thirty experiments were performed upon the carotid and femoral arteries of 16 mongrel dogs weighing from 35 to 54 lb. With the use of sodium pentobarbital anesthesia, and sterile operative technique, the arteries were exposed by incision of overlying tissues. A segment 1 to 2 in. long was isolated from the operative field by the use of specially constructed plastic foam sacs,
Carotid artery (dog) frozen solid by liquid nitrogen bath. The arterial segment was passed through a specially designed plastic foam sac which was kept filled with liquid nitrogen for one minute. The photograph shows the appearance of the artery frozen solid immediately after the liquid nitrogen had completely boiled off.

each individually tailored at the time of surgery to permit passage of the chosen vessel through the sac which was otherwise watertight on all but its most superior aspect (fig. 1). Freezing was achieved either by filling the sac with liquid nitrogen (−196°C) poured from a container at a rate to maintain complete filling for one minute, or by applying the cryogenic probe (2.2 mm) directly to the vessel wall with good contact and achieving a tip temperature of −180°C for ten minutes. To study the effect of freezing on aneurysms, artificial pouches were produced by placing ligatures distally across the external carotid artery or a branch from the femoral artery. The cryogenic probe was used in freezing these artificial aneurysms (stumps) (fig. 2). The process of freezing and thawing was repeated in some instances up to nine times.

Angiography was done routinely before, during, and after freezing. At varying intervals, ranging from several minutes up to one year after freezing, the arteries were excised and individually embedded in paraffin for histological examination. Serial sections, ten microns in thickness, were made. Every fifteenth section was mounted for microscopic study. Most specimens were cut transversely. Longitudinal sections were made in a few instances. The sections were stained with hematoxylin and eosin and, when desired, with Verhoeff's stain for elastic tissue, Van Gieson stain for collagen fibers, or Kossa stain for calcium. Normal control sections were taken proximal and distal to the frozen segment.

Results

PATHOLOGICAL OBSERVATIONS

Gross Appearance
Details concerning the vessels frozen, number and duration of freezings, time elapsed between freezing and pathological evaluation, and the occurrence of thrombosis are presented in table 1. The direct application of liquid nitrogen to the vessel wall for one minute was enough to freeze it solid. When using the cryogenic probe, three to five minutes of cooling were needed to achieve the same result. It was possible to ascertain visually when the artery was totally frozen. It became white and all pulsation ceased distally. Angiography done during freezing showed no filling of the vessel. Within three minutes after turning off the flow of liquid nitrogen the vessel regained normal pulsation as blood flowed through the lumen (fig. 3). Remarkably little evidence of injury was evident on inspection, even after repeating the process of freezing and thawing (fig. 4). Some adventitial hemorrhages were noted occasionally, but there was scarcely any perceptible vascular dilation.

Several days after freezing, the artery looked normal, apart from mild perivascular adhesions which normally accompany arterial mobilization. Long-term observations showed that the vessel wall became thicker and adherent to the surrounding tissues. No evidence of aneurysm formation was seen. Vascular function as a blood conduit was essentially undisturbed. Rupture of the vessel wall never occurred.
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FIGURE 3
Angiogram of the femoral artery (a) before, (b) during, and (c) three minutes after freezing.

Microscopic Appearance
The tissue reaction in arteries injured by freezing showed a constantly changing pattern which was dependent, to some extent, on the degree of freezing. For example, a vessel subjected to freezing and thawing nine times showed more changes than that frozen only once. It was possible to divide these changes into two distinct processes. A process of limited duration terminating in some disturbance of the normal structure of the vessel was defined arbitrarily as degenerative. The other one was of prolonged duration and tended to reproduce the normal structure of the arterial

FIGURE 4
Right carotid angiogram showing good filling of the aneurysm (arrow) (a) before freezing, (b) after freezing three times, (c) after freezing six times, (d) after freezing nine times.
TABLE I
Summary of Experiments

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Right carotid</th>
<th>Right carotid pseudoaneurysem (stump)</th>
<th>Right femoral artery</th>
<th>Right femoral pseudoaneurysem (stump)</th>
<th>Right femoral vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Frozen solid one minute</td>
<td>Frozen solid one minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Frozen solid, thawed and refrozen six times</td>
<td>Frozen solid one minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Frozen solid one minute</td>
<td>Control stump not frozen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Frozen solid and thawed six times</td>
<td>Frozen solid and thawed six times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Frozen solid and thawed six times</td>
<td>Frozen solid and thawed six times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Frozen solid and thawed six times</td>
<td>Frozen solid and thawed six times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Frozen solid and thawed six times</td>
<td>Frozen solid and thawed four times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Frozen solid and thawed six times</td>
<td>Frozen solid and thawed four times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Frozen solid for three minutes</td>
<td>Frozen solid for three minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Frozen solid for 30 seconds</td>
<td>Frozen solid for one minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Frozen solid for one minute</td>
<td>Frozen solid for one minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Frozen and thawed</td>
<td>Frozen solid for one minute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Frozen solid for three minutes</td>
<td>Frozen solid for three minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>Frozen solid for one minute</td>
<td>Frozen solid for one minute</td>
<td></td>
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<tr>
<td>64</td>
<td>Frozen solid for five minutes</td>
<td>Frozen solid for five minutes</td>
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<td></td>
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<tr>
<td>65</td>
<td>Frozen solid for three minutes</td>
<td>Frozen solid for three minutes</td>
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</tbody>
</table>

wall and was defined as reparative. The degenerative process occurred during the first three to four weeks to be followed by the reparative process, although evidence of regeneration appeared as early as the second week after the freezing.

Shortly after freezing, within a day or two, the vessel wall looked pale as compared to normal vessel walls. The endothelial cells were swollen. The smooth muscle fibers in the media became swollen and refractory to stains and their nuclei became irregular and pyknotic. The adventitia showed swollen collagen fibers, areas of fresh necrosis, hemorrhage, and congested nutrient vessels (fig. 5). The internal elastic membrane showed no significant changes apart from occasional discontinuity. On the other hand, the external elastic membrane demonstrated early fragmentation and gradual disintegration. By the end of the
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<table>
<thead>
<tr>
<th>Left femoral artery pseudoaneurysm (stump)</th>
<th>Time elapsed after freezing</th>
<th>Pathological evaluation of thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>One week</td>
<td>No evidence of thrombosis in artery or stump.</td>
<td></td>
</tr>
<tr>
<td>One hour</td>
<td>No evidence of thrombosis in artery or stump.</td>
<td></td>
</tr>
</tbody>
</table>

Frozen solid for one minute

Three months

Animal not yet sacrificed. No thrombosis in artery on arteriography. Control and frozen stump not yet pathologically evaluated.

Frozen solid and thawed three times

One month

No thrombosis in carotid artery. Partial thrombosis at bifurcation of a branch of femoral artery included in frozen area.

Three weeks

No thrombosis.

Seven weeks

No thrombosis.

Frozen solid and thawed three times

Two weeks

No thrombosis.

Three weeks

No thrombosis in either artery.

Frozen solid for one minute

Four days

No thrombosis in either vessel.

Two weeks

No thrombosis.

One year

No thrombosis.

One day

No thrombosis.

Frozen solid for three minutes

One month

Thrombosis in right carotid pseudoaneurysm but not in any of the other frozen arteries or in vein.

One month

No thrombosis in artery or vein.

Three weeks

No thrombosis in artery or vein. Thrombosis in femoral pseudoaneurysm.

Nine months

No thrombosis in artery or vein. Thrombosis in pseudoaneurysm.

first week, the degeneration of the smooth muscle cells became conspicuous. Degeneration of the elastic lamellae was apparent after degeneration of smooth muscle had proceeded to an advanced stage. The elastic fibers lost their normal wavy contour and became linear (fig. 6). Two vessels showed calcification of the media two weeks and six months after freezing (fig. 7). An inflammatory response was present in many lesions for as long as three weeks. This took the form of scattered polymorphonuclear leukocytes and monocytes in the adventitia, mostly around nutrient vessels (fig. 8). In three cases, where the freezing process was severe, polymorphonuclear leukocytes were also seen in the subintima.

Minimal focal proliferation of the intima was found in almost all lesions at the beginning of two weeks. First, a thin strip of tissue
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FIGURE 5

Cross section of the carotid artery two days after freezing. The vessel wall is swollen and refractory to stain. Hematoxylin and eosin (×40).

appeared along one side of the lumen. In some vessels, this gradually proliferated around the lumen and advanced centrally toward the axis of the vessel (fig. 9). Evidence of proliferation of smooth muscle fibers appeared within the media at the end of two to three weeks in the form of large, pale cells containing pink-staining cytoplasm and vesicular nuclei. These

FIGURE 6

A. Control: Normal vessel wall showing the arrangement of the internal and external elastic membranes. B. Cross section of the femoral artery, one week after freezing. The internal elastic membrane is intact, whereas the external membrane shows fragmentation and disintegration. The elastic fibers in the media have lost their wavy contour and become linear. Verhoeff’s stain (×40).
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Figure 7
Cross section of the carotid artery two weeks after freezing, showing calcification of the media limited to one side of the vessel wall. Hematoxylin and eosin (×16).

Immature cells gradually matured and attained the spindle-like form of smooth muscle cells. In between muscle cells, some degree of fibrosis was present. Proliferation of elastic tissue was seen in lesions four to five weeks old. Then scattered elastic fibrils appeared within the media. These fibrils gradually increased in number but still the external elastic membrane remained prominently thinner than normal (fig. 10). Also proliferation of collagen fibers took place as early as three weeks and gradually became thicker, especially the adventitia.

The histological changes in the vessel walls three, six, nine, and 12 months after freezing were similar to those observed after five weeks, except that the reparative process was more advanced. The vessel layers were well preserved and the internal elastic membranes well formed.

Except for minimal focal intima proliferation, more advanced hyaline changes (and, because of that, less cellularity), some fibrotic changes, slight increase of elastic fibers in the muscle, and decrease in thickness of external elastic fibers, the vessels looked normal. No thrombosis was found in any of these vessels. In small ligated branches of the femoral artery, well-organized thrombosis was found nine months after freezing. Close to the bifurcation, slight to medium intima proliferation was observed.

Complete thrombosis was found in three artificial aneurysms (stumps) (fig. 11) one week, three weeks, and nine months after freezing. No thrombosis was noted in the three other artificial aneurysms (stumps). One other case showed partial thrombosis (fig. 12) at the bifurcation of a branch of the femoral artery included in the frozen area. This happened one month after freezing. All these cases were frozen and thawed for at least six times. On histological examination, the vessel wall showed marked changes particularly in the intima, and discontinuity of the internal elastic membrane was a constant finding.
Discussion

The walls of major arteries have a peculiar ability to withstand the effects of extreme cold, relative to other tissues. Freezing and thawing a major artery in the dog within the time-temperature parameters described thus far has not produced rupture of the vessel within a 12-month period of observation. Moreover, blood which is frozen solid within the artery does not clot, but rather is lysed when the vessel thaws and is carried off as circulation is resumed. When these vessels are excised and examined histologically, they show unequivocal microscopic changes. These changes are especially conspicuous in the media and intima and consist of a process of degeneration followed by a reparative process tending to reproduce the normal structure of the arterial wall. Inflammation is mild or negligible, and inflammatory cells are mainly seen in the adventitia. Although devitalized by freezing, the collagenous and elastic tissue of the wall permits function of the structure as a blood conduit until repair occurs. Repair requires approximately five to six weeks, and at the end of this time a relatively normal blood vessel is present. Taylor et al. studied the effects of freezing on the abdominal aorta of juvenile rabbits using CO₂ as a freezing agent and came to the same conclusion. He found more constant changes in the media with calcification simulating Monckberg degeneration. We found such calcification in two cases only.

The fact that major blood vessels are remarkably resistant to freezing indicates a potential clinical advantage with regard to tumor therapy. Apparently one need not fear injury to an artery the size of those used in these experiments in the region of a tumor to be treated by cryotherapy. For example, cryosurgical techniques might be considered for carotid body tumors which are notoriously difficult to excise safely because they arise from the adventitia of the carotid artery and the

FIGURE 8

Inflammatory response around nutrient vessels in the adventitia, two weeks after freezing. Hematoxylin and eosin (×40).
blood supply to the brain may be imperiled by excision. Knowledge about the effect of freezing on smaller arteries will be obtained from future experiments.

Whether freezing an artery causes secondary thrombosis or not is still a point of dispute; Taylor et al., Gage et al., and Kindt and Youmans deny the presence of thrombosis. Walder, in studying the effect of freezing on cortical vessels of cats, found that some vessels indeed showed thrombosis, while others showed incipient thrombotic changes. Furthermore, he explored the possibilities of thrombosing vascular anomalies of the brain with encouraging results. Our own study showed thrombosis in four vessels, all of which were frozen at least six times. Three of these were artificial aneurysms (stumps). Another experiment will be needed to determine the incidence of thrombosis in such artificial aneurysms (stumps) not subjected to freezing. Vessels frozen once or three times failed to show thrombosis. It is our impression that the incidence of thrombosis is directly related to the degree of freezing and subsequent damage to the vessel wall.

We hope to continue our experiments in order to more clearly delineate the time-temperature parameters necessary to produce thrombosis. We also hope to carry the same study on cerebral vessels. This may lead perhaps to new approaches in the surgical therapy of aneurysms and vascular malformations.

Summary
Thirty experiments were performed in mongrel dogs to study the effects of freezing upon the carotid and the femoral arteries. The cryogenic technique is described and the results are demonstrated by angiography as well as histological study.
Major arteries seem to withstand freezing and thawing without subsequent rupture. Also, blood, when frozen solid in vivo, does not clot but is lysed once the vessel thaws.

Histological examination of a vessel wall subjected to freezing reveals definite changes. These changes consist of a process of degeneration soon followed by a reparative process. Secondary thrombosis is occasionally seen and is related, to a great extent, to the degree of freezing and subsequent damage to the vessel wall. When thrombosis occurred in the artificial aneurysms (stumps), the thrombosis extended back into the parent artery.

Acknowledgment
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References
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Figure 11

Complete thrombosis of "artificial aneurysm" two weeks after freezing. Verhoeff's stain (X50).

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A. Angiography of a femoral artery 33 days after freezing showing that the vessel was still patent. B. Femoral artery of the same case showing incomplete thrombosis. Verhoeff's stain (X55).

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