Cholesterol Crystal Embolization
In Rat Brain: A Model for Atheroembolic Cerebral Infarction

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SUMMARY Acute and delayed effects of embolizing cerebral surface vessels with cholesterol crystals were studied by direct observation in anesthetized rats and rabbits, using an open-skull technique, and by histological examination of brains at intervals of one day and one week following embolization. The number and size spectrum of crystals, which were infused into the ipsilateral internal carotid artery, were believed to approximate those released by a rupturing large atheromatous plaque in man, but the other lipid materials contained in such plaques were intentionally excluded. It was found that cholesterol crystals had only limited ability to impede blood flow in the 20-80μ diameter arteries in view. They were also inert within the lumen, causing no vessel wall reaction even after a week; nor was any evidence seen of a thrombogenic effect. Local caliber changes in the containing artery were reproducibly seen, with dilatation of the arterial segment proximal to the embolus and narrowing of the segment in front. These changes appeared to represent an active response of the vessel wall, rather than a passive response to alterations in intraluminal pressure. The difficulty in subsequently locating cholesterol emboli histologically was confirmed. Possible therapeutic implications for atheroembolic cerebral infarction in man were discussed.

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EMBOLIZATION from atheromatous plaques (atheroembolism) has been increasingly recognized over the past 40 years, and the association shown between atheroemboli and infarction of many major organs has indicated its clinical importance.

During development of an atheromatous plaque, saturation of the phospholipid and cholesterol ester phases is followed by precipitation of cholesterol as cholesterol monohydrate crystals, which eventually account for approximately one-third, by weight, of the gruel plaque. Histological evidence in infarcted tissue of crystalline material, usually intravascular or intramural "clefts" left as a result of dissolution during processing, is consequently regarded as pathognomonic of atheroembolism, unless the infarct is very recent, other lipid components of atheroemboli are seldom seen.

Ulcercated plaques found at postmortem examination in the aortic arch and carotid sinus and syphon, and birefringent emboli seen in the retinae of arteriopathies, both indicate that cerebral atheroembolism is a common event. Paradoxically, however, clefts are infrequently noted in routine sections of cerebral infarcts, leaving uncertain the relationship between cerebral atheroembolism and infarction.

The explanation of this discrepancy may be that many cerebral atheroemboli do not cause clinically significant infarction; although the subject of few adequate studies, cerebral infarcts due to atheroemboli are said to be small. Alternatively, even should the associated infarcts be of sufficient extent to draw attention, the atheroemboli may not be recognized because of the technical difficulties involved. Histological examination requires multiple thin sections and special fixing and staining techniques may be necessary. Furthermore, there is little tendency for material to become available for postmortem examination during the acute stage of atheroembolic cerebral infarction since death does not result. Should it be obtained later, the crystals meanwhile might have dispersed into the distal circulation as minute particles, even more easily missed, or disappeared altogether. If so, the part played by atheroemboli in cerebral infarction may be substantially more important than realized.

The clinical importance of this problem is considerable. After more than 15 years of extensive use, anticoagulants are still of uncertain prophylactic value in cerebral infarction; and among the antiplatelet agents, only aspirin has any proven benefit.

Atheroembolism is not affected by either agent and the embolus, once lodged, is not influenced by the lytic processes that act on fibrin. Its role in cerebral infarction, therefore, needs to be defined precisely in any new attempt to improve prevention and treatment of this condition.

Previous experimental investigations of atheroembolism are difficult to interpret because the embolic material may have been antigenic or the crystals were of variable composition. However, cerebral infarction was a reproducible result, with thrombosis of importance in its genesis during the first few hours after embolization. Since it was suggested that the central determinant of the effects of the atheroembolus was its crystalline component, the present study set out to examine, in an animal model, the events resulting from embolization of cholesterol crystals into cerebral vessels. Investigation of the role in cerebral infarction of other lipid components of atheroemboli can proceed from this model.

Methods

Plaque Constituents

To determine the quantity and sizes of particles in the crystalline component, human atheromatous...
Plaques in the aortic arch — common carotid artery system were examined postmortem. The pultaceous material, scraped from a number of lesions of varying sizes, weighed less than 15 mg (dry weight) in most cases. Samples examined by polarized light microscopy showed birefringent particles in large numbers, with the characteristic elongated appearance of cholesterol crystals. Measurements in these circumstances could be only approximate, though adequate for this investigation. In their longest dimension, particles varied from less than 10μ to greater than 150μ, but most were under 20μ.

Animals

Experiments of 2 types (recovery and non-recovery) were carried out in adult rats of various strains, and in a few rabbits. Non-recovery experiments were performed in one group of animals, anesthetized with ethyl carbamate (Urethane; BDH), to observe through a craniotomy the immediate effects of embolizing crystals into the cerebral surface vessels. The other group, anesthetized with pentobarbitone sodium (Sagatal: May and Baker), were intended to survive the embolization to be sacrificed a day or a week later.

Body temperature was maintained in non-recovery animals by overhead lighting and blood pressure monitored via a femoral arterial cannula. A cannula in the other femoral artery permitted arterial blood sampling for measurement of respiratory gas levels, and a femoral venous line was used for injections of norepinephrine (Levophed: Winthrop). Tracheostomy was performed in some cases so that blood gases could be manipulated by artificial ventilation or rebreathing.

In all animals, the left carotid bifurcation and proximal part of the external carotid artery were exposed, the vagus nerve and sympathetic trunk being preserved. In a few of the non-recovery group, the procedure was carried out bilaterally. Branches arising from the external carotid artery were ligated before it was cannulated toward the heart (Portex intravenous cannula, 1.02 mm outside diameter); a Zeiss operating microscope was used so that the tip of the cannula could be positioned exactly at the carotid bifurcation, without obstructing flow into the internal carotid artery. In some cases the pterygopalatine branch of the internal carotid artery was also ligated to reduce mis-routing during embolization. In non-recovery animals, the cannula was secured in position; however, in the survival group, embolization (see below) was carried out at this stage, the cannula then removed and the external carotid artery ligated proximally and distally.

Non-recovery animals after cannulation were positioned prone in a standard headholder over a heated perspex table. The dorsal surface of the skull was exposed and a left craniotomy performed with a low-speed dental drill, used intermittently and with saline irrigation to facilitate dissipation of generated heat. The craniotomy was limited medially by the superior sagittal sinus, posteriorly by the transverse sinus, laterally by the upper margin of the origin of the temporalis muscle and anteriorly, in front of the coronal suture, by a transverse line at the point where the frontal bone tapers. A pool was fashioned, using a glass ring (1 cm deep, 2.5 cm diameter) with a flanged lower edge securely tied beneath the reflected skin flaps, and filled with liquid paraffin at 37°C. The dura was then removed from the entire extent of the craniotomy, with care taken not to expose the cerebral surface at any time to mechanical contact or to air. Hemostasis, if required, was achieved at the cut dural edges with strands of saline-moist cotton wool so that free blood was not allowed to settle in contact with the cortex. The vascular field, in which the diameter of the largest arteries was about 80μ, was continuously observed through the Op-Mi 6 microscope with a 100 mm objective lens, giving a maximum magnification of approximately × 40 with its zoom facility. The 3-way beam-split permitted simultaneous color video monitoring, and recording on tape when appropriate, as well as still photography.

The external carotid artery cannula enabled injections to be made into the main arterial stream at the carotid bifurcation, the injected material necessarily entering the internal carotid artery. Small bolus injections of saline, visible firstly in the cerebral arteries and then in the draining veins, were used to demonstrate patency and continuity of the system. Routinely carried out at an early stage, this invariably confirmed that the territory of the ipsilateral internal carotid artery included the central part of the exposed cortex. Bolus injections of saline through the contralateral internal carotid artery demonstrated that this vessel contributed very significantly to blood flow in the anterior part of the exposed field, but much less to the central part. The more posterior part of the field received no significant supply from the contralateral carotid artery and only a proportion from the ipsilateral carotid artery.) Subsequent angiography of this sort, standardized in terms of bolus volume and injection rate by use of an infusion pump (Harvard Apparatus Co), showed areas where arterial flow was retarded or obstructed.

Embolization

A dilute suspension of cholesterol crystals (BDH) in saline was prepared in the following way: 5 mg of crystals were vigorously agitated in 5 ml of N-saline in a closed 50 ml glass container; the suspension was allowed to stand for 30 min, so that the larger particles settled, before 3 ml were drawn from the middle depths through a 19-gauge needle into a 5 ml hypodermic syringe. Collected in this way, the suspension contained 2.5-3 × 10⁶ evenly dispersed crystalline particles per ml ranging downwards in size from 100μ greatest dimension, but 80% to 90% were smaller than 20μ. In recovery animals, between 0.5 and 3 ml of the suspension was injected through the carotid cannula at a steady rate over about 30 sec. In craniotomy experiments infusions were much smaller, being discontinued as soon as a crystal appeared in the field, although further infusions were sometimes needed until one or more crystals lodged within the arteries in view.
Histology

Brains from all animals intended to survive (recovery experiments) were examined following spontaneous death or sacrifice. After removal, they were fixed in formol saline and cut at 15μ on a freezing microtome or at 5μ after paraffin embedding. Frozen sections were stained for cholesterol with modified Schultz stain, or with toluidine blue, and viewed under a microscope, either directly or through partially or fully crossed polarizing filters; paraffin sections were stained with hematoxylin and eosin and examined for clefts.

Results

In general, the effect of cholesterol crystal emboli depended upon the size and number of crystals entering the field. The great majority of those seen passed rapidly through the cerebral surface arteries to disappear deep into the cortex, though none was observed returning in the veins. In those cases where smaller crystals (up to 20μ) lodged within arteries in view, they tended to do so at bifurcations, or at origins of sidebranches. They produced no visible disturbances of blood flow and usually moved on after a few seconds.

Larger crystals (40–80μ) showed the expected tendency to lodge more proximally in arteries, again particularly at divisions of the main trunk (fig 1). These emboli occasionally caused obvious hemodynamic disturbances. A much more constant finding was the occurrence of associated changes in the caliber of the containing vessel. These were invariably of similar nature: dilatation to 2 or 3 times its previous diameter of the segment of artery immediately proximal to the embolus, with narrowing of the segment in front (fig 1 B). Usually these changes were very localized, the affected segment in each case

![Figure 1. Single cholesterol crystal (indicated by arrow) embolized via internal carotid artery into cerebral surface artery. Scale in mm. (Photographs taken from color videomonitor.) A: Normal vascular field, before embolization. The artery coursing diagonally from bottom left to top right is of uniform caliber throughout. B: Cholesterol crystal temporarily lodged at origin of sidebranch. Although no significant increase in resistance to flow is apparent in the main arterial trunk, there is localized narrowing of this vessel just distal to the embolus, and also of the sidebranch. Immediately proximal to the embolus, the main artery is slightly dilated. C and D: Embolus now progressing at steady rate of about 1 mm/10 sec. The caliber changes noted in B in the main arterial trunk keep pace with the crystal, although there is persistence of the proximal dilatation because of the relative rapidity of crystal movement. (See text.)](http://stroke.ahajournals.org/fig/1.jpg)
being only 2 to 3 vessel diameters in length, but the extent of the proximal dilated segment depended to some degree on the rate of passage of the crystal. They took place rapidly so that the crystal, if moving sufficiently slowly along the vessel for its progress to be followed, was pursued continuously by a wave of dilatation in the segment behind and preceded by a wave of constriction (fig 1 C,D). In each part of the artery, as the crystal passed through, narrowing gave way to dilatation, with a return to normal caliber within a minute if the crystal moved on. If the crystal lodged permanently, the caliber changes tended to persist indefinitely, or at least for as long as the field remained viable.

If the systemic Pco sub 2 was raised by re-breathing, dilatation of all visualized parts of the arterial tree became evident. This response was markedly delayed in arterial segments distal to contained emboli, where constriction was maintained for many seconds after a maximal response was noted elsewhere, although dilatation of comparable degree occurred finally.

Raising the mean arterial pressure (e.g. from 80 to 130 mm Hg) with intravenous injections of norepinephrine also abolished the caliber changes. In these circumstances, the dilated proximal section rapidly constricted, reaching the same diameter as other parts of the artery which had also constricted to some extent. Associated with this change was an increase in caliber, to a similar diameter, of the previously narrowed distal segment, giving the artery a uniform size throughout, constricted slightly and with the contained crystal bulging the vessel walls.

Total obstruction of blood flow by a single crystal was never seen, but splitting of the stream and local turbulence were common. Cyanosis in the distal segment was a rare event. If 2 or more crystals entered the same vessel and lodged at different points, the hemodynamic disturbances were more profound; on occasion, the degree of blood flow retardation was sufficient to fractionate flow, with the slow progress of individual red cells clearly discernible. In these circumstances, total stasis and cyanosis distally were common events. Not infrequently, there would be a reversal of flow in the main trunk beyond the most distal embolus, with "run-off" into the side branches.

The presence of more than one crystal also complicated the caliber changes. Beyond the most distal embolus, the direction of flow to some extent affected the picture but, nevertheless, marked narrowing was usual. Narrowing of the segments between crystals was almost invariable and sometimes very marked; although total collapse was not seen, complete stasis in these segments was not uncommon. Behind the most proximal embolus, dilatation might occur but, once there was a multiplicity of larger-sized crystals in the field, it was always uncertain whether other crystals were lodged unseen even more proximally.

Occasionally, proximal emboli progressed to join a more distal one. Such aggregations were more likely than single crystals to cause severe obstruction of blood flow, with total stasis and collapse of the distal segment sometimes seen in smaller arteries. Thrombosis was never in evidence in association with these crystals. If flow could be reconstituted after a period of stasis lasting many minutes, the blood had clearly remained fluid in the segments affected. Occasional small white bodies were dislodged from the surface of crystals to travel on in the bloodstream, but were never seen on the vessel wall.

Individual crystals lodging permanently in the field remained intact throughout several hours of viewing and showed no tendency to break up. In some survival animals re-anesthetized after one week, a few crystals were seen in the cerebral surface vessels; they were lodged intraluminally without evidence of associated thrombus or platelet clumps, or vessel reaction to them. More commonly, however, in these animals, whether after one day or one week, no evidence of the prior embolization was detected.

Animals that were embolized and allowed to recover fell into 2 "dose-dependent" groups, with a narrow margin between. A larger quantity (2-3 ml) of embolic material caused death of the animal, usually within 12 to 24 hours. Examination of the brain showed marked or gross swelling of the embolized hemisphere, with brainstem softening and herniation through the foramen magnum. Nevertheless, few crystals were demonstrated histologically, and those seen were always intraluminal with no evidence of associated damage to the vessel wall. There were, however, definite early cellular changes of infarction, with the appearance of irregularly shaped pyknotic nuclei in comparison with the smooth spherical nuclei seen in the contralateral hemisphere. After embolization with a lesser quantity of material (1-1.5 ml), survival with no obvious neurological disability (other than ipsilateral enophthalmos and anopia) was usual. Mild contralateral forepaw paresis was seen in one animal but in this, and most others, no crystals were seen in the cerebral surface vessels and no brain damage was apparent histologically.

**Discussion**

Both in the amounts used and in size distribution, the crystals in these experiments were similar to those seen in large atheromatous plaques in the human. They differed most significantly in that plaque crystals are not dispersed when released at the time of rupture and may be of different chemical compositions (cholesterol esters or cholesterol-phospholipid mixtures), at least in part. This heterogeneity of crystal type may explain the differing estimates given for the time crystals remain in the sites to which they have embolized. In this study, they were seen after one week, but others have been seen at one month following embolization. Since clefs in postmortem sections are the diagnostic feature of atheroembolic infarction, this is an important consideration.

There was a major discrepancy in our animals between the number of crystals thought to have embolized and the number later found histologically. This can only partly be explained by the possibilities that many of the crystals were filtered out in the can-
nula, or in the proximal arteries, or that they embolized selectively through other, inappropriate arterial routes. Even if the smaller crystals passed through the capillary circulation, it appears likely that a more important factor was inadequate histological technique. Careful treatment of frozen sections, transferred directly into a water-based mountant, increased the histological yield of crystals but these difficulties, nevertheless, underline the greater problems of finding crystals at postmortem examination in large expanses of necrotic tissue with fragile, poorly supported vessels.

Although single crystals in the larger arteries caused little blood flow obstruction, their effect in the smaller vessels at a more distal level might well have been different. Even in a large artery, profound hemodynamic disturbance, commonly amounting to total stasis, was a feature when 2 or more emboli lodged at different points within it. This may relate to the radiological observation that the effects upon blood flow of 2 separate stenotic lesions in an artery summate unpredictably. The response of the survival animals to varying quantities of injected crystals suggests that once a critical number of vessels feeding the capillary bed are blocked, the sequence of clotting tendency resulting from stagnation may be accelerated by several possible components of the embolus and, once there, had sufficient time to act; i.e. the tissue thromboplastin may be present in the embolus, or by its phospholipid components. Identification of the more important of these factors may enable prevention of this thrombosis in its early stages by other means than "blanket" anticoagulant therapy, which has dangers in embolic infarction. The arterial caliber changes of dilatation behind the embolus and narrowing in front appeared similar to those seen with artificial emboli by Gurdjian, Webster, Martin and Thomas, who dismissed them as "mechanical." They could be expected if the vessel behaved as a passively distensible tube subject to changes in its intraluminal pressure, if the embolus caused sufficient obstruction to produce a marked pressure drop at its site of lodgement, and if the distal part of the artery did not fill retrogradely from collaterals to balance the pressure. The evidence does not indicate that these conditions applied. The changes were seen when the mean systemic arterial pressure was within autoregulatory limits, but this might have little bearing on the pressure within the artery in question once emboli had been introduced, with the possibility that an unseen, but obstructing, cluster of emboli lay proximally. Since saline-angiographic evidence repeatedly failed to show any slowing of flow, and resistance in the embolized artery could not have been reduced, there was no likelihood that pressure within that vessel was significantly lower than that in the surrounding field. From the same evidence, these crystals presented no appreciable resistance to flow that could result in a drop in pressure beyond them. If the vessel was capable of autoregulating, the changes seen could not have been passive unless the pre-embolus pressure exceeded the upper limit for autoregulation and the post-embolus pressure fell below the lower limit; alternatively, it would be necessary to postulate that the pressure, while within the limits of autoregulation, was greater beyond the embolus than proximal to it. Quite clearly, none of these circumstances could have existed. If the vessel was not capable of autoregulating, or if the threshold for response had been altered, perhaps because of a local vasomotor paresis in some way induced by the passage of the embolus, caliber changes similar to those described might have occurred, but the responses to raising the systemic blood pressure or $P_{CO_2}$ could not then be explained.

In conclusion, although the number and size of cholesterol crystals may be the central determinant of...
the effects of an atheroembolus, the cholesterol crystal is itself inert within the vessels examined. Its influence on local tissue perfusion may largely depend on its ability to support, at a focus within the arterial side of the circulation, other more harmful lipids that would otherwise pass through the capillaries. Treatment aimed at dispersing such aggregates may prove a useful therapeutic measure in atheroembolic cerebral infarction. Simple mechanical obstruction of arterial flow by disaggregated crystals alone seems to be considerably less important, although this is influenced by the response of the containing artery, which under.

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