Differential Contributions of Major Lipid Components of Atheroma to Outcome of Cerebral Atheroembolism

A Study in an Animal Model

D. L. H. Rail M.B., F.R.A.C.P., T. J. Steiner, M.B., Ph.D., and F. Clifford Rose, F.R.C.P.

SUMMARY Cerebral atheroembolism, in which mainly lipid emboli are released from rupturing atheromatous plaques in vessels supplying the brain, is a term used for the release into the blood stream of mainly lipid emboli following rupture of advanced atheromatous plaques in vessels supplying the brain. Recognition of its clinical importance hinges upon the argument that it is common. The precise prevalence is difficult to determine, partly because it may occur without neurological sequelae and remain unrecognized during life, and partly because difficulties in histological technique may prevent cerebral atheroemboli being found during postmortem examination. In one autopsy study of unselected patients, atheromatous lesions were found in one or more of the major arteries to the brain in all patients over 50 years of age, and over two-thirds harbored advanced lesions. In patients with abdominal aortic atherosclerosis, distal atheroemboli were found postmortem in 12% of those with advanced disease. Although minor variations in plaque constitution may be expected, there is no reason to suppose that the process of atheroembolism is subject to major regional differences once severe atheromatous disease is established locally.

Cerebral atheroembolism can be clinically silent, but a number of published patient reports (e.g., Refs. 2, 4, 10, 11) demonstrate that cerebral ischemia, with or without infarction, may result. The nature of atheroemboli, and their behavior in the cerebral circulation, need to be better defined before their effects on the brain can be understood.

Chemical analysis of atheroemboli after release is seldom feasible, but the pultaceous contents of advanced atheromatous plaques from which they might arise have been described. By dry weight, up to 60-70% of this material is lipid. Although to some extent varying from one plaque to another and from one region to another within the same plaque, the main lipid constituents have been determined. Cholesterol esters (principally of oleic and linoleic acids) and cholesterol itself predominate (about 50% and 30% by weight respectively), with phospholipids (15%) and triglycerides (5%) present in smaller amounts. In early lesions (fatty streaks), cholesterol esters are laid down preferentially. Being in a liquid crystalline state (although fluid, maintaining a symmetrical molecular order), these are birefringent droplets. As the plaque develops, triglycerides accumulate and, together with a relative increase in amount of the more polyunsaturated cholesterol esters, reduce the melting point of the mixture, which forms oily, isotropic droplets. Cholesterol is poorly soluble in this mixture, saturating the esters at a concentration of about 8%. Progressive accumulation beyond this results in precipitation as cholesterol monohydrate crystals.

Phospholipid molecules have hydrophilic and hydrophobic groups. In a mainly aqueous medium, such as is found in the center of a plaque, they form liposomes. These structures are well characterized, consisting of bilayers of phospholipid molecules oriented with hydrophilic poles at each surface, organized concentrically into multilamellar spheres; layers of water alternate with, and separate, the lipid layers. In the presence of cholesterol and its esters, liposomes have the capacity to incorporate, into the lipid bilayers, molecules of the former up to about 33% weight for weight of phospholipid. The esters are very poorly taken up (2% weight for weight only), but, instead, may be more subject to the emulsifying effect of phospholipids, another property consequent upon the possession of hydrophilic and hydrophobic molecular poles.

It has been suggested that the size and number of cholesterol crystals are the central determinants of...
the effects of atheroemboli because, acting as solid supporting skeletons and resisting lysis and removal, they maintain the integrity of emboli for long periods. We found that cholesterol crystals, dispersed into the cerebral circulation of rats or rabbits via the internal carotid artery, caused little hemodynamic disturbance, though aggregates of these crystals, if stable, were less innocuous.5 Pure crystals, however, were usually disaggregated readily in the bloodstream, suggesting that physical interactions within the embolus between cholesterol and the other lipids, determining over-all physical state,18 might be a more relevant factor than the crystals themselves to causation of infarction.

While it can be assumed that plaque contents do not alter their chemical composition with embolization, the same cannot be expected of their physical state after release into a turbulent, aqueous bloodstream. For this reason, theoretical extrapolation from plaque to embolus has limited scope and the effects in emboled lipid mixtures of these complex factors and relationships need to be studied directly. In the same way as with cholesterol, therefore, the behavior of other important lipids has been observed in the cerebral vessels of the rat and rabbit. We have also looked at interactions between these lipids, both in these animal models and in vitro, with the primary aim of relating findings to their possible effects in man and, in particular, to their potential ability to cause cerebral infarction.

Methods

a) Animals

Young adult albino rats (350–450 g) were used in experimental procedures of 2 types (see below); New Zealand White rabbits were used in a few non-recovery experiments. These methods have been fully described previously1,8 and outline details only are given here.

1) Open-skull Experiments

Rats were anesthetized with ethyl carbamate (Urethane, BDH). Blood pressure and body temperature were maintained. In each animal, a Portex cannula was introduced through a distal opening in the left external carotid artery so that its tip lay at the carotid bifurcation with the proximal stump of the external carotid totally occluded and flow from common to internal carotid unimpeded. Substances infused through such a cannula necessarily entered the internal carotid bloodstream. A dorsal craniotomy was carried out on the left side to expose an area approximately 10 × 5 mm of the dorsal cerebral surface which, after dural resection, was preserved under a pool of liquid paraffin at 37°C. The arteries from the middle cerebral trunk, with the largest about 80 μ in diameter, were viewed through a Zeiss Op-Mi 6 operating microscope at magnifications of up to 40 ×. The 3-way beamsplitting facility permitted simultaneous color video-monitoring, recorded on tape when appropriate, and

still photography with a Nikon F2 camera body and motor drive to take frames singly or repetitively at up to 2.5 per second. Enhanced lighting and filters were available for fluorescein angiography, which demonstrated arterial, capillary and venous phases of perfusion and readily revealed areas of hemodynamic disturbance or extravasation.

In rabbits, a larger craniotomy opening could be made but all procedures were otherwise essentially similar.

In a few rats, the iris circulation was directly viewed through the microscope. The prominent vessels, supplied by the pterygopalatine branch of the internal carotid artery, were arterial arcades of much smaller caliber, about 20–30 μ.

Materials were embolized (see below) by infusion through the intracarotid cannula with either the cerebral or iris vessels under direct observation to control the quantity given. It was possible to monitor emboli arriving in the vessels and their progress distally, alterations in vessel caliber, changes in blood flow whether amounting to stasis or not, prolongation of circulation time, and areas of blood-brain barrier breakdown.

These experiments were terminal.

2) Recovery Experiments

These animals, anesthetized with pentobarbital sodium (Sagatal, May and Baker) intraperitoneally, were cannulated in the same way and then embolized immediately with predetermined quantities of material. The cannula was then withdrawn, the external carotid stump ligated, and the skin closed before recovery. Survivors were examined regularly for signs of neurological deficit and sacrificed between one and 7 days later. Brains were removed from all animals upon death (and, in some cases, parts of the lungs) and examined macroscopically and histologically.

b) Histology

Material for histological examination was fixed in 10% formal saline and then sectioned frozen (at 15 μ) or after paraffin embedding (5 to 15 μ). Frozen sections were examined microscopically either directly or through partially or fully crossed polarizing filters, or after staining for lipid with Sudan IV. Paraffin sections were stained with hematoxylin and eosin, Martius scarlet blue (for fibrin) or Luxol fast blue (for myelin).

c) Embolic Materials

Material of 2 types was used: mixed lipids extracted from human atheromatous plaques, and pure preparations of individual lipids representative of those most important in the plaque, singly or in combinations.

1) Lipid Extract

Suitable human aortae were obtained from the post-mortem room. After removal of all coagulated debris from the intimal surface, pultaceous material was
scooped from the centers of advanced plaques. The lipid component was extracted from weighed quantities into chloroform and methanol, using the method described by Folch, Lees and Sloane Stanley, and stored at −20°C under nitrogen until required.

2) Individual Lipids

Cholesterol esters (cholesterol oleate and linoleate), cholesterol (monohydrate crystals), phospholipid (phosphatidylcholine) and triglyceride (triolein) were obtained in a high degree of purity (usually 99%, Sigma London Chemical Co. or Lipid Products). Principal impurities in these preparations, other sterols and their esters of fatty acids of varying degrees of unsaturation, were unlikely to be of any significance in the proportions present. The lipids were dissolved in known concentration in chloroform and stored under nitrogen at −20°C until needed.

d) Preparation of Emboli

Measured quantities (usually 10 mg) of the required lipid materials (extract or one or more of the pure preparations) were added to 2 ml of saline-for-injection and separated from the chloroform or chloroform-methanol mixture in a rotary-evaporator at 37°C. When complete, this process left the suspended particles in saline, which were maintained at 37°C until embolized.

A variety of mixtures of the pure lipids were prepared either to be embolized or for assessment of the physical nature of the precipitated particles. These are summarized in the table. The particulate matter in the extract, as well as in these lipid mixtures, was examined by naked eye and then microscopically, both directly and through polarizing filters, and photographed. Agitation of the coverslip established how readily particles could be disaggregated. The appearance of individual particles was noted. Particle sizes were measured by micrometer and approximate numbers determined in a modified Neubuer counting chamber.

Results

The lipid extract had the naked eye appearance of a greasy, watery liquid in which tiny suspended particles could be seen. It consisted of a suspension in saline of non-coalescent isotropic droplets, ranging downwards in size from 5 μm to less than 1 μm in diameter, and crystal aggregates in a concentration of the order of 10⁶/ml; the latter, variable in both size and shape, ranged up to 400 μm greatest dimension (fig. 1A), but only 1 or 2% were greater than 100 μm and 90% were smaller than 30 μm. Perturbation of the coverslip, which partially broke up the aggregates, yielded single crystals up to 100 μm in length, though most were under 20 μm, together with further discrete droplets of oil and amorphous lipid material.

In the open-skull animals, the extract passed through the larger epicerebral arteries (50–80 μm diameter) and no disturbance of flow was apparent at this level. In the 30–40 μ side branches, some emboli lodged but discernment of flow disturbances in these vessels was hindered by their course into the cerebral substance. Fluorescein angiography (fig. 2) revealed no delay in the arterial phase of circulation but, subsequently, there was progressive extravasation of the albumen-bound dye deeply in the cortex (fig. 2D), from much smaller vessels. In the 20–30 μ vessels of the iris, although emboli were not seen, stasis was rapidly produced in some vessels and often occupied whole segments or evidence quadrants of the iris.

Animals recovering after infusion of 1–3 mg of this material showed no effect, or only transient limb paresis of less than 24 hours duration. Larger doses, up to 10 mg, produced contralateral hemiparesis in a high percentage of animals and postmortem examination revealed discrete ipsilateral infarcts involving cortex and white matter, often extending deeply as far as the ventricle (fig. 3). Sudan IV-stained frozen sections demonstrated lipid material in vessels of 20–30 μ diameter; occasional intravascular crystals were also seen through polarizing filters, but in neither case was there evidence of associated cellular reaction in the vessel wall to the emboli, or evidence of intraluminal thrombosis.

The appearance and behavior of the pure lipids and mixtures were dependent upon which lipids were included and on their relative proportions.

Phospholipid (phosphatidylcholine) suspended in

<table>
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<th>TABLE Lipid Mixtures (Identified in Text by Nos. 1-18) Prepared for Embolization and/or Examination of Physical State</th>
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E in column 2 indicates preparations that were embolized. CE: cholesterol oleate (equal proportions of oleate and linoleate); Ch: cholesterol TG: triolein; PL: phosphatidylcholine. Figures in these columns indicate percentage of each component by weight. *In preparation 4, the proportion of cholesterol was increased by adding dispersed crystals in suspension (6–6 X 10⁶ in 2 ml of saline) after rotary evaporation of the cholesterol/phospholipid mixture. (See Steiner et al. for details of preparation of crystals.)
saline formed into liposomes in general smaller than 1 μ in overall diameter. About $2 \times 10^5$/ml were counted in the pure suspension (preparation 1 in the table). In preparations 2 to 4, increasing numbers of free crystals were apparent microscopically, with little tendency to aggregate and forming particles in the size range 5-100 μ greatest dimension. Embolization experiments with these mixtures were uneventful. Liposomes alone passed rapidly through the circulation without hindrance. Cholesterol crystal behavior was unaltered by the presence of liposomes and any aggregates reaching the epicerebral vessels could be seen breaking up readily if they lodged, with the individual crystals passing out of sight distally.

Cholesterol esters (1:1 mixture of oleate and linoleate) at 37°C were in a mixed state of crystals and liquid crystals. In the presence of triolein at a concentration of 4% (preparation 5), the microscopic appearances were of isotropic droplets, in size ranging from less than 1 μ to about 5 μ and in number about $0.5 \times 10^6$/ml, and some birefringent aggregates up to 30 μ in size. Increasing the proportion of triolein reduced the number of crystals further so that little birefringence was seen in preparation 6; droplets more readily coalesced and a few were up to 10 μ in size. Adding phospholipid (preparation 7) inhibited coalescence of droplets which were consequently smaller (less than 5 μ) and more numerous (about $1.5 \times 10^5$/ml).

These mixtures had little effect in acute animal preparations, with only brief stasis seen in smaller vessels such as those of the iris, sometimes in association with observed passage of small crystals. Of those animals injected with large quantities (10 mg) before withdrawal of anesthesia, most died within 6-12 hours, but material staining positively for lipid, and hemorrhagic interstitial and alveolar exudate, were prominent in the lungs. Those that did survive such treatment, and those injected with smaller quantities, recovered without neurological deficit; their brains showed no neuronal changes, though intra-arterial lipid in small amounts and occasional crystals could be demonstrated in vessels of the order of 20 μ in diameter.
After this preparatory examination of the major lipid components, cholesterol and cholesterol esters, the 2 were mixed, revealing a practical difficulty. As the proportion of cholesterol was increased (preparations 8 to 12), transition from the crystal/liquid crystal state of the esters to the solid crystalline form of cholesterol itself occurred through a series of sticky gum-like materials of increasing viscosity, many of which (particularly 9, 10 and 11) could not be handled because they stuck avidly to their containing flask. Microscopically, they were birefringent hydrophobic aggregates (fig. 1B) with little tendency to disperse unless one or other component was very greatly in excess to dictate behavior (preparations 8 and 12).

Preparations 9 to 11 could not usefully be emulsified. Repeated attempts with the 1:1 mixture of esters and cholesterol (preparation 10) were unsuccessful because little of the material escaped the walls of the delivery apparatus. Mixtures in a 9:1 ratio (preparations 8 and 12), bearing little relationship to the proportions found in atheromatous material, were emulsified only in a few recovery animals. Neither mixture was associated with neurological deficit or histological change.

Practical problems with ester/cholesterol mixtures were eased with the addition of triolein or of phosphatidylcholine in a concentration of 12% (preparation 15: see table). The ester/cholesterol glutinous aggregate has been dispersed into microscopic particles which, however, retain their birefringence, starkly demonstrated by polarizing filters crossed almost fully. The mixture of esters and cholesterol, in a 5:3 ratio, with 5% of triglyceride and 15% of phospholipid (preparation 16: see table) approximates to the composition of the lipid extract, and the appearances are broadly similar (cf. A). Triolein impairs the phospholipid disaggregating effect demonstrated in C by conversion mainly of cholesterol esters to oil, so that birefringence is less prominent and there are oily droplets. As in A, the central aggregate, selected for demonstration, is at the upper extreme of the size spectrum; the smaller surrounding clusters are more typical, but many others are below the limits of resolution at this magnification. E: with triolein in greater excess than in D (preparation 17: see table), aggregates, though of similar size spectrum, appear less compact because of the more prominent and less viscous oily component. Removal of the polarizing filters allows the quantities of free isotropic droplets to be seen.

As the proportion of cholesterol was increased (preparations 13 and 14) produced greater numbers of isotropic droplets at the expense of the birefringent particle clumps. Particularly in 14, aggregates appeared more fluid, maintaining their integrity less well, so that mechanical agitation achieved more effective dispersal through the aqueous suspension. Addition of phospholipid to esters and cholesterol, on the other hand (preparation 15), produced ready and rapid dispersal of the birefringent aggregates into a suspension of smaller particles in much larger numbers (fig. 1C); many were smaller than 1 μ, and, although the largest ranged up to 150 μ, 90% were under 10 μ greatest dimension and only 1-2% over 40 μ. In mixtures of all 4 types of lipid, esters, cholesterol, phospholipid and triglyceride (preparations 16 to 18), the major interactions were to some extent predictable from the demonstrated properties of the simpler mixtures and the following account is a generalization. In the presence of triolein, phospholipid disaggregated the particles less effectively, which were somewhat larger in preparations 16 and 17 than in 15 (5% over 40 μ and 10% over 20 μ, though with similar upper and lower limits). Although particles appeared microscopically as crystal aggregates, birefringence was less prominent than in preparation 15; as in 13 and 14, which also included triolein, there were many isotropic droplets both within the aggregates and free. Aggregates were more compact in preparation 16 (fig. 1D) than in 17, in which they appeared loosely bound (fig. 1E). Preparation 18 consisted mainly of isotropic droplets though they might contain tiny crystals and there were some small crystal aggregates. Particles were well dispersed in numbers of about 2 × 10⁶/ml and, though they were up to 50 μ in size, 90% were smaller than 10 μ greatest dimension.

With these mixtures, there was approximation to the lipid compositions of atheromatous gruel and plaque extract. Preparations 16 and 18 were emulsified. While preparation 18, like 8 with a similarly low proportion of cholesterol, was without demonstrable effect in the animal model, preparation 16 was readily associated with cerebral infarction. Dosages as low as 2–3 mg (cf. lipid extract) produced infarcts that were small (0.25–1 mm) but often multiple (fig 4),
FIGURE 2. Fluorescein angiogram of cerebral surface vessels viewed through left craniotomy in a rabbit. About 5 mg of lipid extract has been embolized by slow infusion into the ipsilateral internal carotid artery, but none has lodged in view. A: early arterial phase; B: capillary and early venous phase: no areas of ischemia or delayed perfusion are demonstrated; C: late venous phase: drainage is uniform, but small foci of perivascular fluorescence are appearing as the background fades; D: after clearance of the venous phase, extravasated fluorescein is brightly visible; its origins, widely dispersed within the fluorescein-perfused territory, are mainly deep in the cortex but those in clear focus are related to distal arterioles.

FIGURE 3. Coronal section of rat brain (left side) near mid-collicular level, stained with hematoxylin and eosin. Scale in mm. 10 mg of lipid extract infused into the ipsilateral carotid artery produced a discrete hemisphere infarct extending in each direction for several millimeters at the dorsal surface and deeply to the ventricular margin.

Discussion

Our findings demonstrate that each of the main lipid components of atheromatous gruel, cholesterol, cholesterol esters and phospholipids, is harmless when dispersed in relatively pure form into the cerebral circulation via the internal carotid artery. They form innocuous emboli: either droplets, or particles, sufficiently small to pass through the capillaries without significant hindrance, or crystals of such shape that, though sometimes large enough to lodge at arteriolar or even arterial level, they do not create hemodynamic disturbance.* There is no vessel wall reaction and no thrombogenic effect. Nonetheless, both the total lipid extracted from gruel plaques, and synthetic mixtures prepared to simulate its constitution, caused infarction when embolized, and discussion needs to
LIPIDS AND OUTCOME OF CEREBRAL ATHEROEMBOLISM

FIGURE 4. A: Coronal section (dorsal part, left side) of rat brain at the level of the hippocampus and medial thalamic nuclei. Hematoxylin and eosin stain. Scale 1 mm. Approximately 3 mg of a synthetic mixture of lipids (cholesterol, 30%; cholesterol oleate and linoleate, 25% each; phosphatidylcholine, 15%; triolein, 5% [preparation 16: table]), infused as in figure 3, produced numerous small infarcts throughout much of the cerebrum, some contralateral. This section shows hemisphere infarcts, particularly one dorsally near the mid line, one (arrowed) lateral to the nucleus caudatus putamen, and one dorsolateral to the lateral ventricle which involves the radiation of the corpus callosum; in addition, there is a relatively large hippocampal infarct, and other, smaller infarcts around the medial part of the medial thalamic nucleus have resulted in degeneration of the section. B: The lateral hemisphere infarct arrowed in A has been further enlarged about 10x.

center around what property is possessed by these but not by any of their individual components that confers upon them their noxious quality.

A large proportion of the cholesterol is in crystalline form in the advanced plaque. When pure, such crystals have little tendency to aggregate in the cerebral circulation and are readily dispersed if they do. In the presence of the other lipids, their behavior is demonstrably different and, undoubtedly, the key to this question is held in this interaction.

Much of this other lipid component exists in the plaque as oils. These, by definition, are liquids immiscible with water, and form discrete droplets suspended in the aqueous medium of the plaque gruel or of the blood. The size of the droplets depends on a balance between their tendency to coalesce, a function primarily of surface tension, and the mainly mechanical forces promoting dispersal, such as agitation resulting from turbulence in the blood stream. Size and number are therefore inversely related (see below).

Cholesterol crystals, being hydrophobic, attract around them a layer of the oil which would otherwise form free droplets. Coalescence of this oil results in aggregation of the crystals and formation of oil/crystal mixtures with viscosities dependent on their relative proportions (cf. preparations 8–12). The size of such aggregates, though obviously dependent on crystal size, is now ultimately determined by the same factors of surface tension in the oil in competition with the mechanical dispersing forces, viscosity playing a modifying role.

Cholesterol and cholesterol ester mixtures, in combinations in which neither is in substantial excess (preparations 9–11), in practice form thick, gum-like substances so sticky that very few particles in suspension could be created. Mixtures of 1:1 cholesterol oleate and cholesterol linoleate at 37°C are in the form of crystals and liquid crystals, the latter, to the extent of their liquid properties, behaving as an oil. Addition of increasing amounts of triglyceride, itself a low-viscosity oil, progressively lowers the melting point of the mixture into the ambient temperature range, with consequent liquefaction and loss of birefringence. A similar effect on cholesterol itself is much less important in view of its higher initial melting point (over 100°C in, for instance, a 3:5 mixture with cholesterol oleate). Because of overall reduction in viscosity, aggregates are more fluid in the presence of triglyceride, and relatively weakly bonded.

Phospholipids play a quite different role, equally important, in modifying particle size and aggregability. This may be most readily understood if the phospholipid molecule is regarded simply as an emulsifying agent, for this appears to be its major effect. Such agents, by reducing the surface tension of oil droplets in water, aid and maintain their dispersal (cf. preparation 7). The interaction of this effect with those of the other 3 components is depicted in figure 5, the whole concept being represented by a regular 4-faced pyramid, or tetrahedron. Removal of free cholesterol by incorporation within the liposome, thereby reducing the crystalline component, is probably not without additional effect but of less importance in view of the quantities involved.

What is the relevance of these factors to the out-
Interactions between cholesterol, cholesterol esters, triglyceride and phospholipid, summarized as a tetrahedral concept. With the 4 lipids each represented at one of the 4 vertices, the principal effect of each on the others can be depicted theoretically on the 6 axes. In practice this requires some oversimplification. The interactions specified are those most relevant within the context of an atheroembolus; i.e., each indication of the effect of one lipid upon another assumes the modifying presence of the other two and, if considered in isolation, which is not the intention, may approximate variably to reality.

The obvious disparity between the size of such particles embolized and the level of vessel obstruction is explained by poor cohesiveness due to the viscosity-lowering effect of triglyceride. Disaggregation in the bloodstream and molding of particles in the smaller vessels are both expected consequences of increased fluidity.

The influence of phospholipid on lipid emboli, therefore, may be largely protective against vessel obstruction and, to some extent, countered by that of triglyceride. However, in the absence of mechanical energy, emulsification depends on random molecular movement and is likely to be incomplete. Such conditions almost certainly obtain in the advanced atheromatous plaque, and may have important consequences. Whatever the overall composition, differing degrees of emulsification throughout the plaque will unpredictably affect the size, number and nature of particles. Since the total number released by a single rupturing plaque may be many millions, such could be the effect on the target tissue if, say, just 1% reach a critical size that the nature of the remaining 99% is, for practical purposes, immaterial. This is a very important concept.

These various considerations indicate a cause of the apparently disparate sequelae of cerebral atheroembolism. Critically dependent upon possibly minor constitutional changes among the lipids, effects may well vary substantially from one affected individual to another. In routine clinical practice, the spectrum may be concealed. At one end, it seems certain, effects are insignificant and, if seen at postmortem examination, such cases may be those with ulcerated plaques in the neck vessels but no history or signs of ischemic brain damage. Towards the other extreme are the reported cases of atheroembolic cerebral infarction with obvious atheromatous material in the cerebral blood vessels at autopsy (e.g., Ref. 10). Intermediate in the spectrum is the unknown number of patients in whom symptoms of transient cerebral ischemia result but are not reported, or the possibility of an association is not appreciated. In addition, a large number of patients are seen postmortem with small cerebral infarcts in whom evidence suggesting the immediate cause is either inadequately sought or shown to be absent. Even macroemboli can disappear within hours of lodging; it is therefore highly probable that microemboli initially associated with a non-fatal infarct will not be present after the delay of months or years that is likely before autopsy becomes possible.

Infarcts resulting from multiple microemboli contrast with infarction due to macroembolic vessel occlusion not only in pathogenesis and the nature of the embolic material. Occlusion of small, distal vessels, with the collateral circulation similarly affected, may cause more profound tissue ischemia than blockage of a large (proximal) artery, whether by a macroembolus or atherosclerotic lesion, or otherwise. Such distal obstruction was characteristic of embolization with the total-lipid extract: in open-skull animals, extravasation of albumen-bound fluorescein from small vessels signified damage at that level.
(figure 2D); in recovery experiments, evidence of lipid was seen histologically in 20–30 μm vessels.

Atheroemboli, having larger aggregated cholesterol crystals than those obtained by lipid extraction, are able to obstruct vessels of the order of 80–100 μm in diameter. If, in keeping with our findings, they also block those of 20–30 μm they may be unique among the various spontaneously arising emboli in threatening both major terminal anastomotic beds, pial and precapillary. Since atheroemboli often occur where blood flow is compromised already by the proximal atheromatous lesions from which they originate, their potential ability to curtail supply below the limits compatible with tissue viability is only too clear. The expected result is multiple small infarcts (fig. 4), patchily widespread in accordance with the vagaries of laminar flow. The neurological consequences, for these reasons alone, would be unpredictably variable, governed not only by this chance distribution but also on the basis of availability of neuronal reserves that may be silently depleted.

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