ture as "glial" by morphological criteria must be considered as quite tentative.

I submit that Wahn *et al.* have possibly demonstrated that certain cyclic AMP derivatives influence some of the cells in their explant cultures to extend long thin processes, but the "neuronal" nature of these cells has yet to be established.

MARC A. DICHTER
Beth Israel Hospital,
Boston, Massachusetts 02215

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The identifications of simple motor neurons, melanophores, and nerve bundles [(*1*), figure 1, c, e, and g] are unambiguous. The identifications of glial cells and complex neurons [(*1*), figure 1, d and f] are more tentative. We have additional evidence which could not be included in a short communication owing to space limitations. These include a comparison of cultured cyclic AMP–induced cells with cultured neural fold or neural tube cells. The morphologies seen in the former are identical to those seen in the latter. In addition we have made ultrastructural identification. Setting aside the tentative identification of glial cells and complex neurons, the formation of simple neurons, melanophores, and nerve bundles clearly establishes that neural differentiation took place.

HARVEY L. WAHN
LAURACE E. LIGHTBODY
T. T. TCHEN, JOHN D. TAYLOR
Departments of Chemistry and *Biology,*
Wayne State University,
Detroit, Michigan 48202

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Fatty Acids, Platelets, and Microcirculatory Obstruction

Furlow and Bass (*1*) observed that sodium arachidonate injection into the carotid artery of rats produced unilateral cerebrovascular occlusion due to obstruction of the hemispheric microcirculation by platelet aggregates. In a similar study, Silver *et al.* (*2*) found that arachidonate infusion into the ear vein of rabbits produced sudden death due to occlusion of the lung microcirculation by platelet aggregates. We are concerned that these observations may be misinterpreted by those unfamiliar with the biological effects of fatty acid solutions. For example, a reasonable inference is that arachidonate or the prostaglandins E_2 or $F_{2\alpha}$, which are synthesized from arachidonate and known to cause platelet aggregation (*3*), may be involved in the pathogenesis of some types of microvascular occlusive disease. Because of the way in which the fatty acid was administered in these studies (*1, 2*), however, we question whether the responses observed have any pathophysiological significance.

In the stroke study, 50 μl of sodium arachidonate was injected rapidly in concentrations ranging from 0.33 to 33 m*M* (*1*). When a fatty acid salt is infused in this manner, it enters the plasma unesterified fatty acid pool, known commonly as plasma free fatty acid. Normally, 99 percent or more of these free fatty acids exists as a physical complex with plasma albumin (*4*). In the concentrations usually present in the plasma, 0.2 m*M* to about 1.7 m*M* (*5*), free fatty acids are bound very tightly by albumin, so that the concentration of unbound fatty acid anions is in the range of 0.01 to 10 μM (*6*). Fatty acids are not toxic when complexed with albumin; in fact, they are excellent substrates for a variety of cells and tissues (*7*). By contrast, fatty acids that are not firmly bound to albumin exhibit detergent actions, denaturing proteins and damaging cells and organelles (*8*). Therefore, when fatty acids are said to produce toxic effects in biological systems, special care must be taken to be certain that these effects are not due to the nonspecific detergent actions of a soap solution. The key point is that the fatty acid must be combined with albumin or another carrier protein *prior* to contact with cells or tissues. Suitable methods have been developed for the injection of fatty acid solutions into animals (*9*). We have reservations about the interpretation of the studies of Furlow and Bass (*1*) and Silver *et al.* (*2*) because these precautions were not taken when arachidonate was injected.

That fatty acid soap solutions cause platelet aggregation actually is not a new finding. The initial observation was that fatty acid infusion or massive mobilization produced thrombosis (*10*). Subsequent in vitro studies established that fatty acids can activate the plasma clotting system (*11*) and cause platelet aggregation (*12*). When human platelets are incubated with fatty acids that are properly complexed with plasma albumin, the platelets take up, oxidize, and esterify large quantities of fatty acid (*13*). Incubation with albumin-bound saturated fatty acids such as palmitate and stearate made the platelets more sensitive to adenosine diphosphate–induced aggregation, but only when the molar ratio of fatty acid to albumin was greater than 2 (*14*). This is due presumably to fatty acid binding to the platelet membrane (*13*) and perhaps destabilizing the lipid bilayer structure (*15*). In no case, however, did any of the fatty acids themselves cause the platelets to aggregate (*14*). Even at molar ratios of 6, the unsaturated fatty acids oleate and linoleate did not enhance adenosine diphosphate–induced platelet aggregation when they were added as a complex with albumin.

Arachidonate was not tested in our experiments because it comprises only a very small fraction of the plasma free fatty acids (*7, 16*). Even if the plasma free fatty acid concentration is elevated greatly, such as after exercise or injection of heparin (*17*), the total arachidonate concentration will be less than 0.1 m*M*, and most of it will be bound to albumin. The dose of arachidonate employed to produce microvascular occlusion in the rabbit was 1.4 mg/kg (*2*). Of the six fatty acids tested, only arachidonate caused this effect. In interpreting these observations, one must remember that arachidonate, because of its four double bonds, is much more water-soluble than the sodium salts of other long-chain fatty acids. Therefore, sodium arachidonate in high concentrations may have a greater detergent action because, as compared with other long-chain fatty anions, much more of it remains in solution after entering the plasma.

In conclusion, the recent studies with sodium arachidonate provide a potentially useful method for producing microcirculatory obstruction in experimental animals (*1, 2*). We believe, however, that neither of these studies provides any conclusive evidence that arachidonate or prostaglandins are involved in the pathogenesis of platelet aggregation or microcirculatory obstruction. A more likely interpretation is that arachidonate produced a nonspecific detergent effect because it was injected into the blood as a soap solution. This is not to say that elevations in arachidonate or other fatty acids are completely innocuous. Our point is that these studies are not valid tests of the pathophysiological question because of the manner in which arachidonate was administered to the animals.

ARTHUR A. SPECTOR
JOHN C. HOAK
Departments of Biochemistry and
Medicine, University of Iowa,
Iowa City 52242

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Spector and Hoak have raised cogent questions concerning our report (*1*) on arachidonate-induced stroke. Firm data (*2*) would suggest that sodium arachidonate exerts a nonspecific detergent action in our model in the rat. However, we feel that a substantial body of evidence can be mustered to support the theory that arachidonate also exerts a unique thrombogenic action in vivo. We have discussed the complex mechanisms of action of this fatty acid, including its detergent properties, in a report submitted elsewhere for publication. In the meantime, we offer the following remarks.

Our model for producing stroke is just that, namely, a model, which may or may not reflect natural pathophysiologic processes leading to stroke in man. "Properly complexing" arachidonate with albumin in this model would assuredly diminish or eliminate the pharmacologic effects of the fatty acid, as is the case with protein-bound drugs in general (*3*). Although the proposed experiment with albumin complexation should be performed, its absence does not impeach the observation that arachidonate and other drugs (*4*) can induce cerebral microvascular occlusion in vivo.

Evidence that arachidonate acts by a biochemical reaction rather than by physical means as a detergent comes from various sources. Many fatty acids closely related to arachidonate do not produce platelet aggregation under identical experimental conditions in vitro and in vivo (*5*). Indeed, certain fatty acids, in particular the acetylenic analog of arachidonate, inhibit the action of arachidonate (*6*), a phenomenon inconsistent with any anticipated detergent effects that would presumably be additive or synergistic. Furthermore, the aspirinlike drugs and some antioxidants block the actions of arachidonate in vivo (*7*), most likely by enzymatic inhibition (*8*) rather than by an undefined antidetergent action.

Older literature emphasized the thrombogenic efficacy of long-chained, *saturated* fatty acids (*9*). Many reports even minimized the procoagulant activity of the long-chained, *unsaturated* fatty acids (*10*). This trend has been bolstered by the fact that arachidonate constitutes only 2.39 ± 0.22 percent (mean ± S.E.M.) of the nonesterified fatty acid pool in the bloodstream (*11*). The greatest quantity of arachidonate, however, is present in esterified form within tissue lipids (*12*). For instance, arachidonate is the single most plentiful fatty acid in the phospholipid fraction of platelet membranes, where it comprises nearly 20 percent of the total fatty acid content (*13*). Thus, very high local concentrations of free arachidonate could conceivably be generated by hydrolysis, as might occur during tissue injury (*14*). Released arachidonate can be converted within seconds into the labile compounds, prostaglandins G_2 and H_2, which trigger platelet aggregation in nanogram quantities (*15*), presumably far below concentrations necessary for major detergent action. Clearly, carotid infusion of prostaglandins G_2 and H_2 instead of their arachidonate precursor could largely obviate any undesired detergent effect in a stroke model.

In conclusion, we concur with the suggestion of Spector and Hoak that arachidonate exerts a nonspecific detergent action in achieving microvascular occlusion in our model of stroke (*1*). However, we believe it would be premature to imply that the detergent property of arachidonate explains all of the actions of this fatty acid in vivo. We also recognize that our report (*1*) contains indirect experimental evidence implicating prostaglandin pathways in the pathogenesis of the stroke syndrome. Hence, inferences as to pathophysiology must be regarded as provisional pending more definitive experiments.

THOMAS W. FURLOW, JR.
Department of Neurology, National Naval Medical Center, Bethesda, Maryland 20014

NORMAN H. BASS
Department of Neurology, University of Virginia Medical Center, Charlottesville 22901

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Above, Spector and Hoak conclude that our "studies with sodium arachidonate provide a potentially useful method for producing microcirculatory obstruction in experimental animals"; we agree. Indeed, our animal model system (*1*) is currently in use in several laboratories for testing potential antithrombotic agents. However, they further state that these studies do not provide any conclusive evidence that ara-

chidonate or prostaglandins are involved in the pathogenesis of platelet aggregation or microcirculatory obstruction. These studies were not designed to provide such evidence. They were designed to test the hypothesis that arachidonic acid, the essential fatty acid precursor of platelet prostaglandins, might induce platelet aggregation in vivo which would culminate in the sudden death of the animal by obstruction of the pulmonary microcirculation with platelet aggregates. The hypothesis was tested and found to be correct.

Spector and Hoak suggest that arachidonate or prostaglandins may have nothing to do with platelet aggregation but that, more likely, arachidonate "produced a nonspecific detergent effect." Since this may be denigrating important findings of several investigators, as well as those from our laboratory, we shall briefly review the evidence that the conversion of arachidonic acid into prostaglandins is involved in platelet aggregation and thus in hemostasis and thrombosis.

Human platelets contain considerable amounts of arachidonic acid esterified in phospholipids (*2*). During the aggregation of platelets (*3*) and during blood clotting (*4*) platelet prostaglandin synthesis occurs. This prostaglandin synthesis involves the transformation of arachidonic acid into endoperoxide intermediates designated as PGG_2 and PGH_2 (*5*). These substances are more potent inducers of platelet aggregation than arachidonic acid or even adenosine diphosphate (*6*). However, they are rapidly converted to PGE_2, $PGF_{2\alpha}$, and other nonprostanoate compounds (*7*), and there are no reports that any of these end products cause the aggregation of human platelets. Direct evidence that the arachidonic acid in platelet phospholipids is utilized during platelet aggregation is found in the work of Bills and Silver (*8*). They labeled the phospholipids of intact platelets with [^{14}C]arachidonic acid, and showed that in response to thrombin this labeled arachidonic acid is released and transformed into radioactive products of prostaglandin synthetase and arachidonate lipoxygenase. Further support that prostaglandins are involved in platelet aggregation stems from the observations that aspirin inhibits prostaglandin synthesis and aggregation in vitro and in vivo (*9*). Aspirin also prolongs the bleeding time (*10*), causes serious bleeding problems in some patients (*11*), and is being considered as an antithrombotic agent (*12*).

The above represents a strong body of evidence implicating arachidonic acid and platelet prostaglandin synthesis in platelet aggregation and thrombosis. Free arachidonate is *normally* not available to the prostaglandin synthetase in platelets, and this is perhaps one reason why platelets do not aggregate in normal circulating blood or platelet-rich plasma (PRP). However, if arachidonic acid (either dispersed in plasma or as the sodium salt) is stirred with normal human PRP at 37°C, rapid, irreversible platelet aggregation occurs (*13*). Eleven other fatty acids, when tested in the same manner, do not induce such aggregation (*13*). In fact, several of them, including fatty acids with five or six double bonds, actually inhibit arachidonic acid–induced aggregation. If arachidonic acid–induced aggregation were indeed due to a nonspecific detergent effect, then the addition of sodium salts of other fatty acids should increase the detergent effect of sodium arachidonate and produce better aggregation or lysis of platelets rather than inhibition of aggregation. Further evidence that arachidonic acid, as used in our in vitro experiments, does not cause detergent-like lysis of platelets was obtained by showing that arachidonic acid–induced aggregation involves the selective release of serotonin from platelets (*13*). This was supported by electron micrographs which showed that platelets responded to arachidonic acid in a manner similar to their response to other aggregating agents (*14*).

In our in vivo studies (*1*) we injected sodium arachidonate into the ear veins of rabbits and found that at a dose of 1.4 mg/kg 15 rabbits died within 3 minutes of the injection. Death was preceded by acute respiratory symptoms, and platelet aggregates were found in the heart blood and in the vessels of the microcirculation of the lungs. To show that this was a specific effect of arachidonate and not a nonspecific detergent effect, five closely related fatty acids were injected into rabbits as the sodium salt and at doses four times the LD_{50} of arachidonate. (The LD_{50} is the dose lethal to 50 percent.) None of these animals showed any signs of toxicity. Electron micrographs of lung tissue clearly show that the platelets in aggregates found in the lung vessels of rabbits that have succumbed to arachidonic acid are not lysed (*15*). A further argument against the possible detergent effect of arachidonate is our finding that aspirin, an inhibitor of platelet prostaglandin synthesis (*9*), protects rabbits from a challenging lethal dose of arachidonate (*1*). We are not aware of any evidence that aspirin interferes with detergent effects.

Spector and Hoak have stated that fatty acids must be combined with albumin prior to contact with cells or tissues. We question whether this is so. Arachidonic acid binds to albumin as other free fatty acids do and, of course, *normally* circulates in the blood so bound. It is known that albumin reduces the amount of arachidonic acid that is converted into prostaglandins by platelets and inhibits arachidonic acid–induced platelet aggregation (*13, 16*). However, we suggest that in an *abnormal* or pathological situation in vivo arachidonic acid could suddenly be released at a specific locus and momentarily exceed the capacity of albumin to bind it. If one wishes to show an effect of free arachidonic acid which can only be shown in the absence of large amounts of albumin it is unreasonable to combine it with albumin prior to testing.

Arachidonic acid is a special fatty acid because it is a substrate for prostaglandin synthesis which may lead to platelet aggregation and because, as has recently been shown (*17*), the origin of arachidonic acid in plasma, as well as the regulation of its levels, is different from other free fatty acids.

In summary, it is conceivable that under pathological conditions the local release of large amounts of arachidonic acid could cause platelet aggregation leading to obstruction of the microcirculation of vital organs such as lungs, brain, heart, or kidneys, thus resulting in a disabling illness or death (*1*).

MELVIN J. SILVER
WILLIS S. HOCH, JAMES J. KOCSIS
CAROL M. INGERMAN, J. BRYAN SMITH
Cardeza Foundation and *Departments of Pharmacology* and *Pathology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107*

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