trated in patches at separate nodal regions so as to allow for distinct zones of inward current, thereby causing slowed saltation (*5*) and conduction block. Alternatively, the distribution of the channels can be relatively continuous, allowing for spatially continuous inward current (*6, 7*) and continuous conduction along the demyelinated region.

Our results suggest morphological correlates for both predicted patterns of organization of the axolemma in peripheral demyelinated axons. Figure 1, A and B, presents the anatomical correlate of the type of axonal membrane that might conduct with slowed saltation or exhibit conduction blocking. When an action potential reaches the demyelinated zone from the myelinated portion of the fiber, conduction may be blocked because of reduced excitability of the demyelinated membrane (*1, 2*) or impedance mismatch at the junction of the myelinated and demyelinated regions (*3, 4*). Saltation may continue, although slowed because of changes in passive properties of the internodal membrane, namely an increase in internodal capacitance and a decrease in internodal transverse resistance (*5*).

The electron micrograph in Fig. 1C presents a possible morphological correlate of continuous conduction. The spatial distribution of FeFCN stain that we have described suggests a high density of Na^+ channels along the demyelinated (former paranodal or internodal) region. This pattern of staining offers one morphological correlate for the physiological observation of spatially continuous inward current associated with continuous conduction along the demyelinated axon. In this case the demyelinated axolemma, which presumably contained a low density (*2*) of Na^+ channels prior to demyelination, reorganized by redistributing channels or by acquiring new ones.

These data concerning reorganization of the axon membrane after demyelination are of interest not only in terms of understanding the pathophysiology of demyelinated fibers, but also with respect to the mechanisms that mediate subsequent recovery of conduction. Future studies incorporating freeze-fracture or pharmacological methods should provide further information about this phenomenon.

ROBERT E. FOSTER
CHRISTOPHER C. WHALEN
STEPHEN G. WAXMAN
Department of Neurology, Stanford University School of Medicine, Veterans Administration Medical Center, Palo Alto, California 94304

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8. Using ^3H-labeled saxitoxin, Ritchie and Rogart (*2*) showed that in the normally myelinated axon, Na^+ channels are concentrated in nodal axolemma and are present at a very low density in the internodal axolemma. Likewise, a high density of external-face intramembranous particles (*9*) and a specific pattern of FeFCN staining (*10, 11*) are found at the node, indicating specialization of the nodal membrane.
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13. The tissue was fixed for 3 hours in cold 5 percent glutaraldehyde buffered with 0.2*M* sodium cacodylate (*p*H 7.4; 360 mOsm), washed three times in fresh 0.2*M* cacodylate buffer, postfixed for 1½ hours in 2 percent OsO_4, washed in distilled water three times at 5 minutes per wash, and stained by the FeFCN technique. For this the tissue sample was placed in 0.01*M* ferric chloride, washed three times in distilled water (5 minutes per wash), placed in 1 percent potassium ferrocyanide, and washed two more times in distilled water. The sample was then dehydrated and embedded in Epon-Araldite (*11*). Thick (3 μm) serial sections were examined by light microscopy, and selected thick sections were reembedded. Ultrathin sections were cut from these, stained on a grid with aqueous uranyl acetate and Reynold's lead citrate, and examined with a JEOL 100 CX electron microscope.
14. In normal mammalian peripheral nerve, the FeFCN technique stains the cytoplasmic surface of the axon membrane at nodes of Ranvier but not at internodal regions of the same fibers. Absence of internodal membrane staining was shown not to be due to lack of ability of the stain to reach these areas (*10, 11*). Specific staining of initial segment membrane rather than soma or dendritic membrane is consistent with the hypothesis that this stain is specific to regions densely supplied with Na^+ channels [S. G. Waxman and D. C. Quick, in *Physiology and Pathobiology of Axons*, S. G. Waxman, Ed. (Raven, New York, 1978), pp. 125–130]. In the electrocyte axons of *Sternarchus albifrons*, in which there are both excitable and inexcitable nodes [M. V. L. Bennett, in *Fish Physiology*, W. S. Hoar and D. J. Randall, Eds. (Academic Press, New York, 1971), pp. 374–491; S. G. Waxman, G. D. Pappas, M. V. L. Bennett, *J. Cell Biol.* **53**, 210 (1972)], the excitable nodes stain densely with FeFCN while inexcitable nodes along the same fiber do not (*10*). It should be emphasized that absence of staining with FeFCN does not necessarily imply membrane inexcitability, since C fibers are not stained with this technique (*11*). Sodium channel density for C fibers, estimated from measurements of the binding of ^3H-labeled saxitoxin, is approximately 110 per square micrometer [J. M. Ritchie, R. B. Rogart, G. R. Strichartz, *J. Physiol. (London)* **261**, 477 (1976)]. Axolemma staining with FeFCN thus appears to reflect quantitative differences in membrane structure.
15. This staining is not due to preferential accessibility of the demyelinated membrane to the extracellular milieu, since C fibers do not stain even when directly exposed to the extracellular space (*11*), and since the inexcitable type II nodes in *S. albifrons* (*14*) also do not stain.
16. Supported in part by NIH grant NS-15320, National Multiple Sclerosis Society grant RG-1231, and by the Medical Research Service of the Veterans Administration. We thank S. Cameron and M. Smith for technical assistance.

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Aspirin: An Unexpected Side Effect on Prostacyclin Synthesis in Cultured Vascular Smooth Muscle Cells

Abstract. *Monolayer cultures of rat aorta smooth muscle cells synthesized the anti-aggregatory substance prostacyclin via the cyclooxygenase pathway from ^{14}C-labeled arachidonic acid. The product was identified both by bioassay and by mass spectrometry. Labeled cells produced prostacyclin only when exposed to the initiator thrombin: treatment with therapeutic concentrations of aspirin (0.2 millimolar) for 30 minutes completely destroyed the cells' ability to synthesize prostacyclin. Prostacyclin synthesis from exogenous arachidonic acid recovered fully within 1 to 2 hours by a cycloheximide-sensitive process. Thrombin responsiveness, which was permanently impaired in confluent nondividing cultures, recovered substantially and within 24 hours only when cells were stimulated to divide by subculturing. These results indicate that resting vascular cells can rapidly synthesize new cyclooxygenase, but that aspirin destroys additional components of the prostacyclin system which can only be replaced during cell division.*

Thromboxane (TXA_2) and prostacyclin (PGI_2) are synthesized from arachidonic acid by way of a common endoperoxide precursor (*1, 2*). When synthesized in platelets, TXA_2 stimulates aggregation and is a vasoconstrictor (*3*); in contrast, PGI_2 synthesized in blood vessel walls is a potent inhibitor of aggregation and acts as a vasodilator (*4*).

A common property underlying the action of a number of antiplatelet drugs such as aspirin is their ability to inhibit the cyclooxygenase enzyme that converts arachidonic acid to the cyclic endoperoxide intermediate and thus to prevent the synthesis of the proaggregatory substance TXA_2 in platelets (*5*). A number of these drugs are being tested both

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in clinical trials and in animal studies for their ability to inhibit atherogenesis and myocardial infarction (*5-8*).

Aspirin permanently inactivates cyclooxygenase (*9*). Platelets, which have essentially no protein synthesizing capacity, are therefore inactivated for the remainder of their 10-day circulating lifetime when they are exposed to aspirin. Because prostacyclin synthesis requires a similar cyclooxygenase, the effects of aspirin on prostacyclin synthesis by the vascular wall is a matter of some concern.

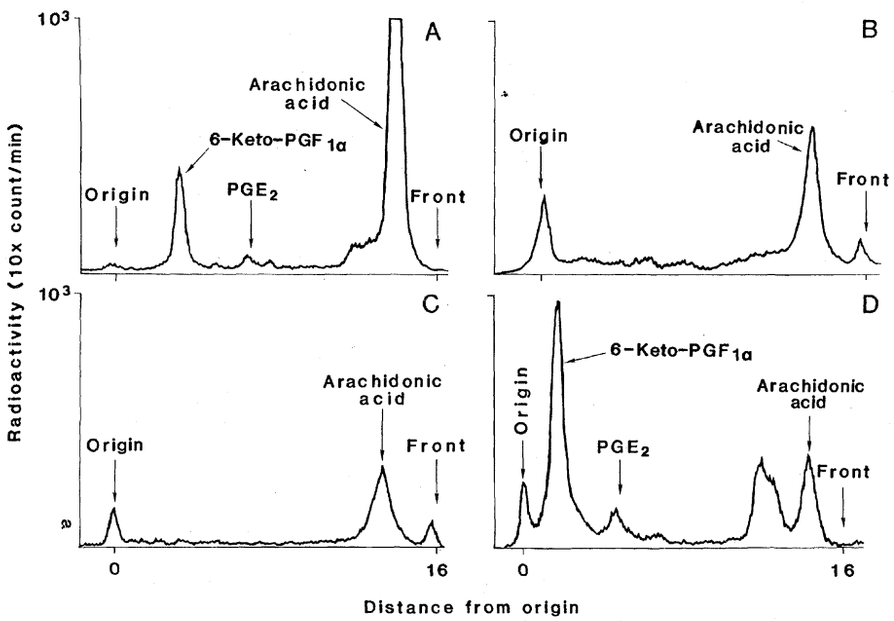

Fig. 1. Thin-layer radiochromatograms of products from arachidonic acid metabolism in rat aorta smooth muscle cells from rat aorta. (A) Profile of the metabolic products from exogenous arachidonic acid. Confluent cultures of cells in 25-cm² flasks were superfused with [^{14}C]arachidonic acid (0.75 μCi, 3.8 μg) in 1 ml of medium (NCTC-135 buffered with 15 m*M* Hepes, *p*H 7.4). After 5 minutes the medium was removed, acidified, and extracted with ethyl acetate. Extracts were evaporated to dryness under nitrogen and applied to heat-activated silica gel G thin-layer plates. The plates were incubated in a water-saturated atmosphere for 30 minutes before development in the organic phase of ethyl acetate, isooctane, acetic acid, and water (11:50:20:100, by volume). The metabolic profile was obtained by measuring the radioactivity in the chromatograms with a radioactivity scanner. To examine metabolites from endogenous arachidonic acid, we labeled confluent cultures of cells with [^{14}C]arachidonic acid (2.5 μCi) in 2 ml of medium supplemented with 10 percent delipidized fetal bovine serum. After 18 hours the medium was removed, and the washed cultures were incubated for an additional 24 hours in the same medium without radioactive substrate. The labeled cultures were washed with medium and then incubated with either medium alone or medium containing stimulating agent for 5 minutes at 37°C with gentle rocking. The medium was removed and subjected to analysis by thin-layer chromatography as described above. (B) Medium from cells labeled with [^{14}C]arachidonic acid and maintained in culture for 48 hours. (C) Labeled cells incubated with medium alone. (D) Labeled cells incubated with 0.5 unit of thrombin.

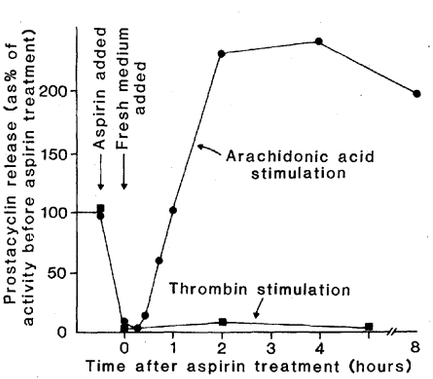

Fig. 2. Prostacyclin release by rat aorta smooth muscle cells treated with aspirin. (Top curve) Confluent monolayers of cells were treated with aspirin (0.2 m*M*) for 30 minutes. The aspirin was removed, the cells were washed, and fresh growth medium was added. Ability to synthesize PGI_2 from exogenous [^{14}C]arachidonic acid was measured in duplicate cultures at intervals after removal of aspirin. Activity recovered to 100 percent of baseline within 1 hour and reached a maximum of 250 percent of baseline levels within 2 to 5 hours. Recovery was dependent on protein synthesis since no activity was observed in cultures to which cycloheximide (20 μ*M*) was added (data not shown). (Bottom curve) Confluent cultures of rat aorta smooth muscle cells were labeled with [^{14}C]arachidonic acid (4 μg, 2.5 μCi) as described in the text and treated with aspirin (0.2 m*M*) for 30 minutes. The aspirin was removed, the cells were washed, and fresh growth medium was added. The ability of the cells to release PGI_2 in response to stimulation by thrombin (0.5 unit) was measured at 0, 2, and 5 hours after aspirin removal.

We have therefore studied the effects of aspirin in a cultured vascular smooth muscle cell line from rat aorta which produces prostacyclin in vitro (*10*), and investigated the ability of the cells to recover from aspirin treatment under a variety of experimental conditions.

Cultures were routinely grown in NCTC-135 medium supplemented with 15 m*M* Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], 10 percent fetal bovine serum, penicillin (50 unit/ml), and streptomycin (50 μg/ml). The experiments reported here were carried out with cells in the 20th to 25th passage. Confluent cultures in 25-cm² flasks produced a number of metabolic products when perfused briefly with [^{14}C]-arachidonic acid (Fig. 1A). The major metabolite was 6-keto-$PGF_{1\alpha}$, the stable breakdown product of prostacyclin. This compound was identified by mass spectrometry after conversion to the tetra-trimethylsilyl hydroxime methyl ester. The mass spectrum of the cell-derived material was identical to that of the authentic compound and showed characteristic major ions at mass-to-charge ratios of 687, 672, 598, 507, 436, 418, and 245. The production of biologically active prostacyclin by the cell monolayers was also confirmed by measuring its inhibitory effects on platelet aggregation in a standardized system (*10*).

To examine conversion of endogenous arachidonic acid to PGI_2, we labeled the cell phospholipids with radioactive arachidonic acid by incubating confluent cultures in 25-cm² flasks with 2.5 μCi of [1-^{14}C]arachidonic acid in NCTC-135 medium containing 15 m*M* Hepes and 10 percent serum protein from which the lipid had been removed (*11*). After 12 to 18 hours, the labeling medium was removed, and the monolayers were cultured in the same medium without the radioactive substrate for an additional 24 to 48 hours. Medium removed from the labeled monolayers after 48 hours of culture contained free arachidonic acid, but no release of prostacyclin into the culture medium was detected in these unstimulated cultures (Fig. 1B). Brief treatment of the labeled cells with 0.5 unit of thrombin for 5 minutes, however, resulted in the release of a large quantity of prostacyclin, as well as prostaglandin E_2 (PGE_2), arachidonic acid, and unidentified products into the medium (Fig. 1D). Controls, treated with buffer alone, produced no detectable PGI_2, although some arachidonic acid was released into the medium (Fig. 1C, lower left). These results demonstrate that vascular smooth muscle cells in culture do not constantly

produce and release prostacyclin but require some specific stimulating agent, such as thrombin. Similar observations have been made for vascular endothelial cells of human origin (*12*).

Cultures were exposed to aspirin at a concentration of 0.2 m*M* for 30 minutes. The aspirin was then removed, the cell monolayer washed, and the ability of these cells to synthesize prostacyclin from exogenously supplied [^{14}C]arachidonic acid was measured at intervals. After aspirin treatment the synthesis of 6-keto-PGF$_{1\alpha}$ from exogenous arachidonic acid was completely eliminated, but within 1 hour after removal of aspirin the levels of prostacyclin synthetic activity approached those found in untreated cells, and by 2 to 3 hours had increased to 2.5 times the concentrations in cells before aspirin treatment (Fig. 2). This recovery was completely blocked in cultures to which the protein synthesizing inhibitor cycloheximide (20 μM) was added.

The ability of aspirin-treated labeled cells to release prostacyclin in response to the initiator thrombin was measured in similar experiments (Fig. 2). In contrast to the rapid and full recovery of the cyclooxygenase as evidenced by prostacyclin synthesis from [^{14}C]arachidonic acid, the cells were completely unresponsive to thrombin even 5 hours after removal of aspirin in confluent nondividing cell cultures.

In longer-term experiments, thrombin responsiveness was still 75 percent impaired even 4 days after aspirin treatment. However, when parallel cultures were stimulated to divide by trypsinization and subculturing in fresh medium, substantial recovery occurred within 24 hours (Fig. 3).

Aspirin is known to irreversibly inhibit the cyclooxygenase by acetylating the enzyme (*9*). It must be presumed that the rapid recovery of prostacyclin synthesis from arachidonic acid in aspirin-treated cells represents synthesis of a new enzyme. This conclusion is confirmed by the observations that recovery of the cyclooxygenase is inhibited by cycloheximide. This rapid replacement of the cyclooxygenase indicates that synthesis and turnover of the enzyme are probably a continuous process even in nondividing cells. The failure of aspirin-treated cells to produce prostacyclin in response to thrombin, despite full recovery of the cyclooxygenase, indicates therefore that aspirin must also inactivate additional components of the prostacyclin-releasing system that are not continuously replaced in resting cells.

Thrombin normally functions as a pro-

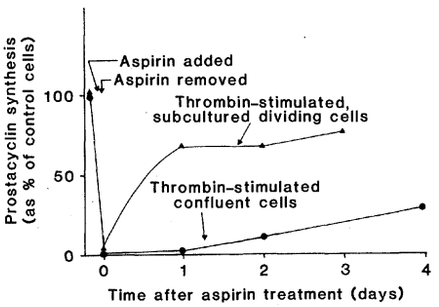

Fig. 3. Confluent cell cultures were labeled with [^{14}C]arachidonic acid and exposed to aspirin (0.2 m*M*) for 30 minutes as described in Fig. 1. The aspirin was removed and the cultures divided into two groups. One group was treated with fresh growth medium only. Cells in the second group were stimulated to divide by trypsinization and subculturing at a 1:2 ratio. Prostacyclin synthesis in response to added thrombin (0.5 unit) remained substantially impaired during the entire 4-day experimental period for confluent nondividing cells (bottom curve), whereas activity rapidly recovered in dividing cells (top curve).

aggregatory substance. It is believed that the ability of thrombin to induce synthesis of the anti-aggregatory substance prostacyclin may be an important control feature of thrombin-induced hemostasis.

The prolonged inactivation of this system in confluent cultures suggests that recovery of the vasculature after aspirin treatment may require more than mere replacement of the cyclooxygenase component of the prostacyclin synthetase system. Full recovery of the system in a functional sense may require replacement of cellular components that are regenerated only during cell division.

JOHN WHITING
KALMAN SALATA
J. MARTYN BAILEY
Department of Biochemistry, George Washington University, School of Medicine and Health Sciences, Washington, D.C., 20037

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Kin Selection: Its Components

Abstract. *Change in gene frequency under kin selection is the sum of two components, namely, $\overline{\Delta q_I}$, a change in gene frequency caused by individual selection, and Δq_G, a change caused by group selection. For the evolution of altruistic traits by kin selection, $\overline{\Delta q_I}$ is always negative—that is, individual selection operates against altruism—and Δq_G is always positive, so that selection between groups favors altruism. Hamilton's rule specifies the conditions under which $\Delta q_G > |\overline{\Delta q_I}|$—that is, the conditions necessary for intergroup selection to override individual selection.*

Kin selection (*1*, *2*) is the evolutionary process that occurs when individuals within a population interact with one another in a nonrandom way with respect to kinship, and these interactions affect fitness. This process is believed to have exerted a significant influence on the evolution of social behaviors—that is, on the ways in which individuals interact (*3*).

Maynard Smith (*1*) originally defined kin selection in contrast to, and as an alternative for, group selection. The key distinction between the two evolutionary processes was the presence or absence of discontinuities in the population breeding structure. Kin selection did "not require any discontinuities in population breeding structure" (*1*), whereas the existence of partially isolated breeding groups was "an essential condition for group selection" (*1*). Smith later proposed (*2*) that the term "group selection" be restricted to those cases in which the group was the unit of selection; that is, to those cases in which changes in gene frequency are brought about by the differential extinction and proliferation of groups (*4*). However, similarities between kin and group selection are pronounced. Kin selection depends upon the structuring of the population into kin groups, whether the groups exist as actual physical entities or exist as a result of the facultative expression of social behaviors (*5*). This feature of kin selection has led several authors (*3*, *6*) to consider that kin selection is a form of group se-

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