may be possible for the inhibitory effects of aspirin and TYA (*3, 15*) on the platelet release reaction, because sodium arachidonate induces a substantial release of platelet-bound [^{14}C]serotonin from platelets resuspended (*1*) in a saline medium. This release can be as high as 80 percent with 25 μg of arachidonate (*6*).

Whether the enzymatic pathways involved in the formation of LASS and PG's from arachidonate are identical has yet to be established (the microsomal preparations used in this study must have contained several enzymes). If they are identical, then LASS is probably one of the chain of intermediates of prostaglandin production, such as the recently characterized cyclic endoperoxide (*20*). To support this assumption, we have found that LASS behaves similarly to the endoperoxide (*6*). For instance, it is inactivated by stannous chloride and is extractable as an acid lipid. Also, it can be isolated on silica gel when subjected to thin-layer chromatography in a solvent system consisting of toluene and dioxan (1 : 1); its mobility is midway between that of PGE_2 (which remains near the origin) and arachidonate (which travels near the solvent front).

The above hypothesis thus explains the relation which apparently exists between inhibition of platelet prostaglandin production (*1, 2, 15*), inhibition of platelet aggregation, and prolongation of bleeding time (*3, 21*) observed for drugs such as indomethacin, aspirin, acetaminophen, and sodium salicylate because platelet aggregation is a primary event in hemostasis. Also, some bleeding disorders might involve defects in the ability of platelets to produce LASS or to respond to it.

Finally, irreversible aggregation of platelets is thought to be a terminal pathway in the many processes involved in the etiology of arterial thromboembolism. Furthermore, studies in laboratory animals (*22*) imply that aspirin could be clinically useful in the prophylactic treatment of arterial thrombosis. It is therefore feasible that production of LASS is a causal event in thrombotic episodes. If this is so, there is now a biochemical basis for the development of prophylactic therapy.

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18. The concentration (1 μg/ml) of PGE_2 used routinely to demonstrate its potentiating effect upon aggregation is some 10 to 100 times greater than those detected after PG production by platelets, induced by thrombin (*1, 2*) or by collagen and epinephrine [J. B. Smith, C. Ingerman, J. J. Kocsis, M. J. Silver, *J. Clin. Invest.* **52**, 965 (1973); also (*15*)]. However, in several experiments we have observed significant potentiating effects on LASS-induced aggregation with concentrations of PGE_2 or between 1 to 100 ng/ml. The effects with small concentrations of the PG are most noticeable on rate of aggregation and size of the aggregates. In addition, during platelet prostaglandin production (which is extremely rapid), local concentrations of PGE_2 inside and closely adjacent to the platelets may be considerably higher than in the plasma. Also, PGE_2 content of inflamed tissue can reach 100 ng/ml (*19*) and, after infarction, the injured tissue could produce these quantities of prostaglandins.
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Acetylenic Analog of Arachidonate That Acts Like Aspirin on Platelets

Abstract. *Development of irreversible platelet aggregation and the accompanying release of platelet-bound serotonin and production of prostaglandins is suppressed by 5,8,11,14-eicosatetraynoic acid (TYA). These findings may be explained by an ability of TYA to inhibit the enzymatic conversion of arachidonate to a newly recognized factor, labile aggregation-stimulating substance, which induces platelet aggregation, and to prostaglandins E_2 and $F_{2\alpha}$.*

Like aspirin (*1*) 5,8,11,14-eicosatetraynoic acid [TYA, Ro 3-1428 (Roche)] inhibits production of prostaglandins (PG's) at many sites and potently inhibits conversion of arachidonate to PG's E_2 and $F_{2\alpha}$ in broken cell preparations (*2, 3*). Also, as has been shown (*4*), it acts like aspirin in suppressing the enzymatic generation of a labile aggregation-stimulating substance (LASS) from arachidonate. However, aspirin inhibits other enzymes, such as platelet phosphodiesterase (*5*). Similar properties have not been reported for TYA, and, because of its structure (it is an acetylenic analog of arachidonic acid), one might expect it to compete directly with the substrate in arachidonate-utilizing enzyme systems. We have used TYA to

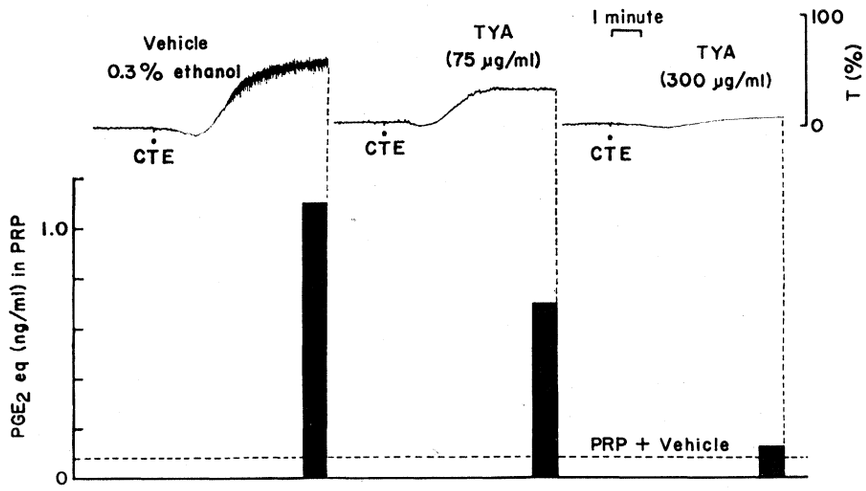

Fig. 1. Inhibition by ammonium tetraynoate of aggregation and platelet prostaglandin production induced by a saline extract of human connective tissue. Aggregation is indicated by an increased transmission of light through stirred (1000 rev/min) platelet-rich plasma (PRP) at 37°C. Difference in light transmission (T) between PRP and platelet-free plasma is taken as 100 percent. Ethanolic saline vehicle (final concentration of 0.3 percent ethanol) or ammonium tetraynoate (75 and 300 μg/ml) was first incubated with the PRP for 5 minutes prior to the addition of a diluted extract (*8*) of connective tissue (*CTE*) (50 μl per 2 ml of PRP). Platelet-rich plasma was collected from several aggregation tubes (broken lines), pooled to a volume of 8 ml, and examined for PG content (*15*). Basal levels of PG (44 pg per milliliter of PGE_2 equivalents per milliliter of PRP) were estimated by extracting 12 ml of PRP (concentrated into 1 ml for assay), which had been stirred for 10 minutes with the ethanol saline vehicle only. [Donor: D.K. (male); platelet count: 368,000 cell/mm^3.] The ability of TYA to greatly reduce collagen-stimulated production of platelet PG's was also demonstrated in two other experiments, in one of which TYA was used as the free acid (*6*).

Table 1. Effects of TYA on release of platelet-bound serotonin. Release of [^{14}C]serotonin (Radiochemical Centre, Amersham, U.K.), bound in the platelets was assessed (*13*). Aggregating agents used were connective tissue extract undiluted or 1/5 dilution (25 μl per each milliliter of platelet-rich plasma); epinephrine (5 μ*M*), adenosine diphosphate (ADP) (4 μ*M*), and thrombin (0.25 unit/ml) (*18*). The ammonium tetraynoate was used at a final concentration of 200 or 300 μg/ml, and the free acid at 225 μg/ml [in 0.15 ml of platelet-free plasma (PFP)]. Only the first wave of aggregation (induced by ADP or epinephrine) remained in the presence of TYA; T, light transmission.

Aggregating agent	Vehicle or TYA	Aggregation (% T)	[^{14}C]Serotonin release (%)	Percent reduction	
				Aggregation	Serotonin release
		D.K. (male)			
Connective tissue (undiluted)	Vehicle	70.1	68.9	27.8	41.1
	TYA	50.6	40.6		
		R.O. (female)			
Connective tissue	Vehicle	66.7	35.2	36.3	80.0
	TYA	42.5	7.0		
Epinephrine	Vehicle	69.4	52.7	60.4	100.0
	TYA	27.5	0		
ADP	Vehicle	55.6	16.8	55.0	100.0
	TYA	25.0	0		
		M.W. (female)			
Connective tissue	Vehicle	10.5	6.4	100.0	40.6
	TYA	0	3.8		
Epinephrine	Vehicle	84.2	50.9	75.5	100.0
	TYA	20.6	0		
		B.M. (male)			
Connective tissue (undiluted) (10 μl)	Vehicle (PFP)	62.7	32.8	96.2	80.4
	TYA (free acid)	2.4	6.4		
Connective tissue	Vehicle	23.3	20.8	91.4	34.6
	TYA	2.0	13.6		
		C.P. (female)			
Thrombin	Vehicle	59.3	24.8	36.6	66.4
	TYA	37.6	9.8		

test the concept (*4*) that activity of an arachidonate-consuming enzyme system is essential for the irreversible or second-phase type of aggregation of human platelets, which is associated with the release of platelet constituents. The TYA was used routinely as the more soluble ammonium salt (*3*) but similar results were obtained with the free acid (*6*). Aggregation of human platelets in citrated platelet-rich plasma (PRP) was assessed by means of standard turbidometric techniques (*6–8*). The difference in light transmission between PRP and platelet-free plasma was taken as 100 percent on the aggregation scale.

Platelet aggregation and serotonin release induced by various agents can be inhibited by aspirin; we have examined the problem of whether similar effects can be produced by TYA.

Aggregation induced by collagen is thought to be purely of the second-phase type and can be suppressed by aspirin (*8–13*). Using a saline extract of connective tissue as the source of collagen (*8*), we have shown that ammonium tetraynoate at 75 to 300 μg/ml could produce a concentration-dependent reduction in aggregation and in platelet prostaglandin production (Fig. 1). These effects of TYA were shown in eight experiments (six donors of both sexes), and in four of these experiments TYA was used as the free acid (*6*). In one such experiment in which PG's were assayed, it was shown that the free acid of TYA at 150 μg/ml reduced appearance of PGE_2-like activity from a concentration in the PRP of 3.3 to 1.0 ng/ml with reduction in aggregation response of 54.3 percent.

Epinephrine-induced aggregation has two distinct phases, and only the second phase is suppressed by aspirin-type drugs (*8, 10, 13*). It was shown that ammonium tetraynoate (100 to 300 μg/ml) could produce a similarly marked reduction or abolition of second-phase epinephrine aggregation (five experiments, five donors). In one of these experiments, the appearance of PG which occurs during the second phase (*14*) was also examined (*15*). During the first phase of aggregation, amounts of PGE_2-like activity extracted from the PRP were not distinguishable from basal levels (44 pg/ml); during the second phase, significant amounts (0.33 ng/ml) appeared, but not when second-phase aggregation had been

abolished by ammonium tetraynoate (300 μg/ml). In a similar experiment in which TYA (225 μg/ml) was used as the free acid, appearance of PG (0.94 ng of PGE_2 equivalents per milliliter of PRP) was also reduced to basal levels, in a manner that corresponded to the complete suppression of second wave aggregation (which was observed).

Concentrations of adenosine diphosphate (ADP), between 2 and 4 μ*M* (the exact amount varies between experiments), can induce distinctive first and second phases of aggregation similar to those observed with epinephrine; again, only the second phases are inhibited by aspirin-type drugs (*8, 10, 11, 13*). Like aspirin, ammonium tetraynoate (100 to 300 μg/ml) could abolish the second phase of aggregation (six experiments, six donors). In three experiments, similar effects were observed with TYA in the form of the free acid (150 to 300 μg/ml).

Aggregation induced by low concentrations of thrombin can also be reduced by aspirin (*10–12*). In two experiments (two donors), it was shown that TYA, either as the ammonium salt (200 μg/ml) or free acid (150 μg/ml), could inhibit aggregation induced by thrombin (0.25 to 0.3 unit per milliliter) by 37 and 42 percent, respectively.

We then studied the effects of TYA on the platelet release reaction. The release of platelet constituents (including serotonin) that occurs during second-phase aggregation is suppressed by aspirin. It can be seen (Table 1) that, like aspirin (*11–13*), TYA inhibited the release of platelet-bound serotonin induced by connective tissue, ADP, epinephrine, and thrombin.

The final point of similarity between TYA and aspirin was the interaction of TYA with arachidonate (*16*) on platelet aggregation (Fig. 2). When sodium arachidonate (final concentration in the PRP of 25 μg/ml) was present in the PRP, the suppression of ADP-induced second wave aggregation induced by TYA could be prevented. A similar interaction with dihomo-γ-linolenic acid (the precursor for PGE_1 biosynthesis) was not observed (one or more experiments with PRP from three donors in each case).

The ability of many drugs to prevent the development of irreversible platelet aggregation parallels their ability to inhibit platelet prostaglandin production. Furthermore, both aspirin-type drugs and TYA inhibit the generation of LASS from arachidonate (*4*), thus raising the question of whether TYA could act like aspirin on platelets, especially because of its close chemical similarity to arachidonate.

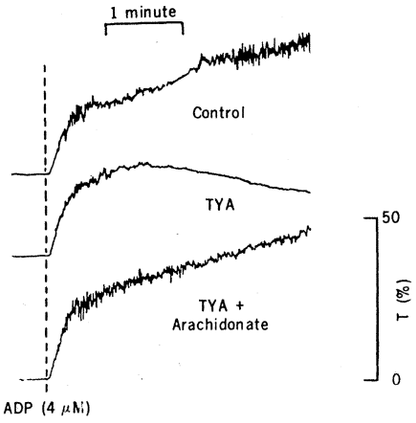

Fig. 2. Prevention by arachidonate of the antiaggregating effects of ammonium tetraynoate. For the control (upper record), 20 μl of the 10 percent ethanol saline vehicle (for TYA) was added to PRP (1 ml) containing 5 μl of vehicle for the arachidonate (*4*). Three minutes later, ADP (4 μ*M*) was added (broken line) and two wave aggregation developed. To show the effects of TYA (second record), 20 μl of ammonium tetraynoate (final concentration of 200 μg/ml in the PRP) was present during the preliminary incubation; TYA prevented the second wave of aggregation from developing. The bottom record shows the effects of TYA + arachidonate. In this case, sodium arachidonate (final concentration of 25 μg/ml) was added prior to the TYA, and the inhibitory effects of TYA were prevented; T, light transmission.

We found that TYA did act like aspirin on platelets, and our findings can only be explained adequately if an arachidonate-utilizing system plays an essential role in irreversible aggregation and the platelet release reaction. The concept outlined by Willis (*4*) is thus supported, although it has yet to be determined whether TYA has antihemostatic or antithrombotic properties in experimental animals or man (*17*).

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6. The effects of TYA or its vehicle were compared simultaneously with both channels of a dual aggregation module (Payton Associates Inc.). The basic findings with ammonium tetraynoate were repeated with the free acid of TYA. Solid TYA was dissolved in ethanol (to 10 mg/ml), and 0.3 ml of this solution was evaporated to dryness at 25°C under a stream of nitrogen. The TYA residue was dissolved in 2 ml of autologous platelet-free plasma (PFP) at 37°C. For control purposes, a similar PFP sample was prepared with 0.3 ml of ethanol. These PFP preparations (0.1 to 0.2 ml) were added slowly (in 20-μl portions) to PRP in the aggregometer tube. Stock solutions (10 mg/ml) of ammonium tetraynoate were prepared in warm ethanolic saline [10 percent (by volume) ethanol in 0.9 percent sodium chloride solution].
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15. Samples (6 to 12 ml) of frozen PRP were thawed in saturated sodium chloride solution containing hydrochloric acid (final *p*H 2.5 to 2.8). Prostaglandins were extracted (twice) into three volumes of ethyl acetate; centrifugation and freezing (— 60°C) were used to separate the phases. The combined ethyl acetate fractions from each sample were collected through phase-separating paper (Whatman 1PS) and a small column (0.25) of granular sodium sulfate. This extract was diluted (1 : 4) with petroleum ether (b.p. 30° to 60°C) and dried over sodium sulfate (10 g), that was subsequently rinsed with 1 ml of ethanol and 25 ml of a mixture of ethyl acetate and petroleum ether (1 : 4). The total extract was then passed through a column (3 g) of activated silicic acid (Unisil: Clarkson), and the effluent (containing neutral lipids) was discarded. After the columns were rinsed again with 15 ml of the ethyl acetate–petroleum ether mixture (the effluent being discarded), combined PGE and PGF fractions were eluted with 30 ml of methanol–ethyl acetate (3 : 17). This was evaporated to dryness under nitrogen, and the extract was dissolved in 1 to 2 ml of Tyrode's solution for bioassay on isolated superfused rat stomach strip [A. L. Willis, *J. Pharm. Pharmacol.* **21**, 126 (1969)]. The biological activity assayed was due predominantly to PGE_2, because two to four times more PGF_2 is produced than $PGF_{2\alpha}$ (by thin-layer chromatography), and the rat stomach strip is up to five times more sensitive to PGE_2 than to $PGF_{2\alpha}$. Each value obtained was corrected for recovery (~ 60 percent) of tritiated PGE_1.
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17. Injected intraperitoneally in rats, TYA has antipyretic and anti-inflammatory properties similar to those of aspirin, but the doses required (100 to 300 mg/kg) are much higher than suggested by its inhibitory effects on PG synthesis in vitro [A. L. Willis, in *Symposium on Analgesics*, A. Van Poznak, Ed.

(Cornell Univ. Press, New York, in press)]. Similarly, TYA may lack significant effects on platelet aggregation in vivo.
18. Agents used to induce platelet aggregation were adenosine diphosphate (Sigma), epinephrine (adrenaline chloride: Parke-Davis), bovine thrombin (Parke-Davis), and saline extract of human subcutaneous connective tissue (*8*).
19. Supported in part by National Heart and Lung Institute grant HE 14595. We thank J. Rogers (Roosevelt Hospital) for technical assistance.

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Spheroid Chromatin Units (*ν* Bodies)

Abstract. *Linear arrays of spherical chromatin particles (ν bodies) about 70 angstroms in diameter have been observed in preparations of isolated eukaryotic nuclei swollen in water, centrifuged onto carbon films, and positively or negatively stained. These bodies have been found in isolated rat thymus, rat liver, and chicken erythrocyte nuclei. Favorable views also reveal connecting strands about 15 angstroms wide between adjacent particles.*

The packaging of DNA within eukaryotic chromosomes continues to be a formidable structural problem. Packing ratios greater than 100/1 (DNA length/chromatid length) are not uncommon for metaphase chromosomes (*1*). The DNA concentrations within localized regions of interphase nuclei may approximate 200 mg/ml or more (*2*). Acutely aware of this problem, investigators have postulated multiple orders of coiling or folding of a fundamental nucleohistone molecule (*1, 3*). Several models have been derived from low-angle x-ray diffraction studies, including: four DNA molecules packed into a single nucleohistone fibril (*4*); a single DNA double helix and associated proteins folded into an irregular superhelix 80 to 120 Å in diameter and 45 Å in pitch (*5*); and a single DNA-protein fiber constrained into a superhelix 100 Å in diameter and 120 Å in pitch (*6*). Ultrastructural studies have also yielded a profusion of models. Spreading of chromosomes on a Langmuir trough frequently yields fibrils about 250 Å in diameter, although differences due to tissue type, presence of chelating agents, and method of dehydration and drying have been reported (*7*). Direct adsorption of sheared chromatin onto microscope grids has revealed a network of fibers approximately 100 Å wide with numerous side branches 80 to 200 Å in length (*5*). Spraying of chromatin onto a grid yields a network of fibers (*8*) and separated filaments (20 to 30 Å in diameter) containing numerous "nodular" elements about 150 Å in diameter (*9*). Thin sections of nuclei and chromosomes reveal fragments of threads frequently 100 to 200 Å wide (*3, 10, 11*). Bram and Ris (*5*) regard the 250-Å fiber as a folding (or doubling) of a superhelix, due to divalent metal ions, and interpret the thin-section data as artifacts of chelation by buffer ions. Lampert (*12*) views the 250-Å filament as a folding of the superhelix of Pardon and Wilkins (*6*), and explains the thin-section data in terms of shrinkage due to fixation. Despite this divergence of views, there is a consensus that multiple levels of coiling or folding are required to explain the observed variation in chromatin fiber widths.

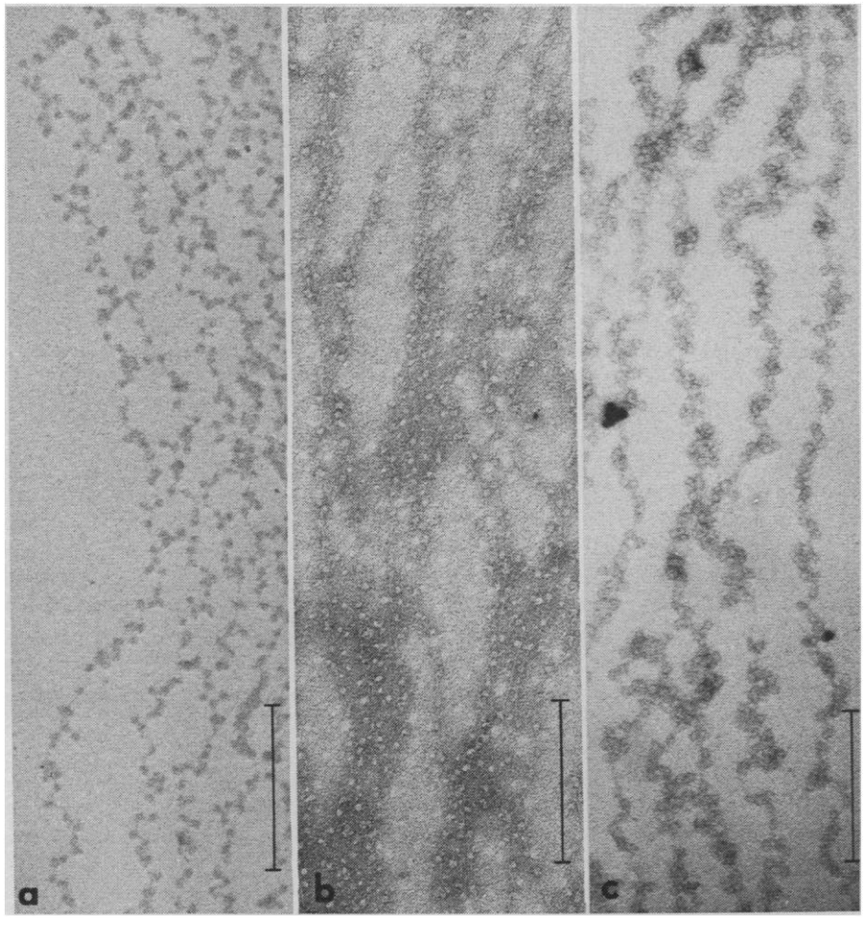

Fig. 1. Chromatin fibers spilling out of ruptured nuclei. The degree of fiber swelling and the proximity of individual *ν* bodies to each other varies within different regions of a single nucleus. Scale bars, 0.2 μm. (a) Rat thymus chromatin, positively stained with a mixture of 4 percent aqueous phosphotungstic acid and 95 percent ethanol (3 : 7), rinsed in 95 percent ethanol, and dried in air. (b) Rat thymus chromatin, negatively stained with 0.5 percent ammonium molybdate, adjusted to *p*H 7.4 to 8.0 with ammonium hydroxide. (c) Chicken erythrocyte chromatin, negatively stained as in (b). Clustering of *ν* bodies is most evident in (c), where groups of three or more are readily visualized. Connecting strands are most easily seen in (b).

We have attempted to visualize chromatin structure by methods different from those cited above. Interphase nuclei were isolated from fresh rat thymus (*13*), rat liver (*2*), and chicken erythrocytes (*2*), washed and centrifuged twice in CKM buffer (*14*) and once in 0.2*M* KCl, suspended in 0.2*M* KCl at a concentration of approximately 10^8 nuclei per milliliter, and diluted 200-fold into distilled H_2O. Nuclei were allowed to swell for 10 to 15 minutes, then made 1 percent in formalin (*p*H 6.8 to 7.0). Fixation proceeded for at least 30 minutes. All operations, up to this point, were at 0°