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American Society for Pharmacology and
Experimental Therapeutics
American Association of Pathologists
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The American Association of Immunologists

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Clinical Immunology Society
Society for Mucosal Immunology
American Association of Veterinary
Immunologists
International Society for Bioelectricity

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EFFECTS OF SC-49992 IN INHIBITION OF PLATELET AGGREGATION
N.S. Nicholson, B.B. Tait, S.G. Panzer-Knodle, A.K. Salyers, L.W. King, L.P. Feigen, Searle, Skokie, IL 60077
 Platelet aggregation is important in rethrombosis of coronary arteries following thrombolysis and angioplasty. SC-49992 (SC), a mimetic of the peptide binding sequence of fibrinogen (fgn) prevents platelet aggregation by blocking the platelet glycoprotein IIb/IIIa receptor. In vitro, SC inhibited fgn binding to human platelets (IC_{50} : 6×10^{-7} M). SC inhibited ADP-induced aggregation in human platelet rich plasma (PRP) with an IC_{50} of 2.4×10^{-6} M and in dog PRP with an IC_{50} of 2.1×10^{-6} M. In washed human platelets with thrombin as the agonist, the IC_{50} was 6.0×10^{-7} M. In rats, SC blocked collagen induced thrombocytopenia with an ED_{50} of 0.053 mg/kg. Platelet responsiveness in the rat recovered with a half life of about 12 min. In dogs, I.V. bolus administration inhibited ex vivo platelet aggregation (ED_{50} : 0.6 mg/kg). During 2 hour infusions SC inhibited collagen induced ex vivo platelet aggregation (ED_{50} : 7.5 μ g/kg/min). Full recovery occurred 30 min after termination of a 10 μ g/kg/min infusion.

3081

ADENOSINE DIPHOSPHATE-INDUCED PLATELET AGGREGATION AND HYPERCHOLESTEROLEMIA. D.J. Saxon, Morehead State University, Morehead, KY 40351.

Platelet aggregation may contribute to the development of atherosclerosis and thrombi. The purpose of the study was to determine if hypercholesterolemia influences ADP-induced platelet aggregation. Blood from rats administered a 10 week diet enriched with 5% cholesterol + 1% sodium taurocholate were compared to samples from rats on a normal diet. Total cholesterol, LDL, HDL and triglycerides were significantly elevated ($p < 0.05$) by the cholesterol diet. Platelet counts were not significantly different ($p < 0.05$). Aggregation was measured in citrated- whole blood with Tyrode buffer (1:1), pH 7.4, using impedance aggregometry. Platelets from the hypercholesterolemic animals had a slightly higher relative frequency (89%) of aggregation induced by 0.5 μ M of ADP compared to the control group (82%). The hypercholesterolemic platelets had a significantly greater ($p < 0.05$) maximum aggregation response (maximum impedance increase) to 0.5 μ M ADP, and also aggregated in response to 0.5 μ M ADP at a significantly ($p < 0.05$) faster rate (impedance change per second). Hypercholesterolemia may alter the basic platelet reaction involving ADP-induced aggregation. (Supported by MSU Grant 1990-91)

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INCREASED PLATELET SENSITIVITY TO EPINEPHRINE-INDUCED AGGREGATION DURING CHRONIC STRESS. H. Tracy, W. Patterson, D. McGlassen, J. Marshall, and J. Patterson (SPON: R. Burton), USAF School of Aerospace Medicine, Brooks AFB, Tx, and Wilford-Hall USAF Medical Center, Lackland AFB, Tx.

Epinephrine-induced platelet aggregation was measured in a group of candidates at the USAF Officer Training School (OTS) to investigate whether a chronic stress situation changed the sensitivity of platelets to epinephrine-induced aggregation as a possible link in stress-induced atherosclerosis. Subjective responses of the test group indicated that the first two to three weeks of the twelve week course were highly stressful, and that the candidates adapted to the situation after the third week. Our results showed a 150% increase in the number of candidates whose platelets aggregated with epinephrine during the second week of OTS, compared to the number of candidates exhibiting epinephrine-induced aggregation prior to beginning the course. Twenty-three percent of the candidates exhibited baseline epinephrine-induced aggregation, which increased to 58% at the second week sample. The number of candidates who exhibited epinephrine-induced platelet aggregation from the fourth through the twelfth week dropped to an average of only 38%. One candidate exhibited no epinephrine-induced aggregation until the eighth week, which coincided with his dismissal from OTS due to poor performance. These data indicate that platelet sensitivity to epinephrine-induced aggregation increases under stress, which may reveal a potential link in stress-induced atherosclerosis.

3080

THE INHIBITORY EFFECT OF TETRAHYDROAMINOACRIDINE ON HUMAN PLATELET FUNCTIONS. S.-Y. LIU and D. M. SYLVESTER (SPON: R. BULL), Washington State University, Pullman, WA 99163

Oral administration of tetrahydroaminoacridine (THA) has been reported to significantly improve of the symptoms of patients with Alzheimer's disease (A.D.). Although THA was initially used to treat A.D. because of its anticholinesterase activity, the exact mechanism responsible for the clinical efficacy of THA is not clear. A.D. also produces cellular changes in tissues other than the CNS, including blood platelets. Specifically, membrane abnormalities of intracellular organelles of platelets correlates well with patient prognosis in A.D. In addition, platelet functional defects may contribute to the progression of A.D. In this study, we determined the effect of THA on several different actions of human platelets. We found that THA inhibited human platelet aggregation induced by various agonists in a dose dependent manner. THA inhibited the release of platelet dense granules during activation and also, THA dispersed platelets that had been activated by platelet agonists. Antiplatelet actions of THA are dependent upon inhibition of intracellular ionized calcium mobilization.

3082

Prevention of reocclusion after thrombolysis in a canine model of coronary occlusion.

L.P. Feigen, L.W. King, J.C. Campion, N.S. Nicholson, and S.G. Panzer-Knodle, Searle, Skokie, IL 60077.

SC-49992 (8 Guanidino-Octanoyl- ASP-PHE) (SC) is a peptidomimetic of the fibrinogen binding sequence RGDF which blocks the platelet GPIIb/IIIa fibrinogen receptor. In anesthetized, open chest dogs, with thrombin-induced occlusion of the LAD, effects of heparin (5000U), aspirin (10 mg/kg) and SC (10-80 μ g/kg/min iv) were evaluated on lysis and reocclusion following repeated iv injections of tPA. Vessel patency was determined with an electromagnetic flowmeter. Heparin alone (5000 U) had a significant, but limited, effect reducing time to lysis from 45 ± 10 min to 21 ± 3 min. Reocclusion time was not changed. All subsequent studies were done in the presence of heparin, and heparin data were taken to be control. Aspirin (10 mg/kg) had no effect on time to lysis but prolonged the time to reocclusion from 3.4 ± 0.6 to 29 ± 10 min. SC completely prevented reocclusion at doses $> 30 \mu$ g/kg/min. At a dose of 80 μ g/kg/min, it also significantly shortened the time to lysis from 21 ± 3 to 7.4 ± 1.4 min. When platelet aggregation was inhibited $> 50\%$ in dog platelets ex-vivo (30 μ g/kg/min in this model), template bleeding times exceeded 30 min. SC has a short pharmacodynamic half life (≈ 10 min), suggesting that if bleeding were to become a problem, platelet function would be restored rapidly after administration of the compound were terminated.

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CORRELATION OF VERY RAPID PLATELET ADHESION TO COLLAGEN WITH PLATELET DENSITY AND SIZE. Renata Polanowska-Grabowska, Sanghamitra Reha, and Adrian R.L. Gear, Dept. Biochemistry, Univ. of Virginia, Charlottesville, VA 22908.

We have previously shown that adhesion of human platelets to immobilized collagen is extremely rapid, with initial rates approaching 300% of single particles adhering per second. Here, we have investigated adhesion efficiency as a function of platelet density. Platelet subpopulations were separated by Percoll density-gradient centrifugation into three fractions; light ($1.040 < \rho < 1.065$ g/ml), intermediate ($1.065 < \rho < 1.070$ g/ml) and heavy ($1.070 < \rho < 1.080$ g/ml), which constituted 20, 50 and 30% of the total platelet population, having mean volumes of 5.45, 6.27 and 7.25 fl, respectively. Using a continuous-flow, micro-affinity column, we found that the densest (largest) platelets adhered to collagen 4 and 1.5 times faster than the light and intermediate populations. They were also less sensitive to inhibition by PGI_2 . In contrast, there was no difference in ADP or collagen-induced aggregation between light and heavy fractions, indicating that heavy platelets were not preferentially involved in collagen-induced aggregation. These results suggest that normal circulating platelets are distinctly heterogenous in their ability to adhere to collagen under arterial-flow conditions. The greater efficiency of heavy platelets may be related to an increased content or different types of collagen receptors compared to the lighter platelets. It is not a reflection of varying platelet number, since adhesion efficiency is not affected by particle count. (Supported by NIH grant HL-27014 and the Carman Trust).

INTRODUCTION

ADENOSINE DIPHOSPHATE (ADP) IS AN AGONIST FOR PLATELET AGGREGATION. ERYTHROCYTES AND ENDOTHELIAL CELLS MAY BE THE SOURCES OF ADP AS AN AGONIST FOR PLATELET AGGREGATION. PLATELET ACTIVATION BY A VARIETY OF AGONISTS INDUCES ADP RELEASE FROM THE DENSE GRANULES OF PLATELETS AS A PART OF A POSITIVE FEEDBACK LOOP FOR IRREVERSIBLE AGGREGATION. ADP AT CONCENTRATIONS $\leq < 0.5 \mu\text{M}$ PRODUCES REVERSIBLE PLATELET AGGREGATION. WITH ADP CONCENTRATIONS OF $2 - 5 \mu\text{M}$ IRREVERSIBLE AGGREGATION OCCURS WITH SECRETION OF PLATELET CONSTITUENTS FROM α GRANULES (ADHESIVE PROTEINS, PLATELET-DERIVED GROWTH FACTOR, TRANSFORMING GROWTH FACTOR- β , PLATELET FACTOR, THROMBOGLOBULIN) AND DENSE GRANULES (ADP, ATP, Ca^{++} , SEROTONIN).

REVERSIBLE PLATELET AGGREGATION MAY BE RELATED TO THE PHYSIOLOGICAL FUNCTION OF REPAIRING ENDOTHELIUM AND SECRETION OF GROWTH FACTORS FROM α GRANULES. THE IRREVERSIBLE RESPONSE IS INVOLVED IN THE HEMOSTATIC FUNCTION OF PLATELETS.

HYPERCHOLESTEROLEMIA AND OXIDIZED LOW DENSITY LIPOPROTEIN (LDL) ARE REPORTED TO HAVE A PROMINENT ROLE IN INJURY TO MANY CELL TYPES AND TO OCCUPY A SIGNIFICANT ROLE IN ATHEROSCLEROSIS.

PLATELETS IN WHOLE BLOOD ARE CERTAINLY EXPOSED TO A MULTITUDE OF MOLECULES, INCLUDING OXIDIZED LDL AND AGONISTS FOR AGGREGATION LIKE ADP FROM ERYTHROCYTES AND ENDOTHELIAL CELLS. IMPEDANCE AGGREGOMETRY PERMITS THE UTILIZATION OF WHOLE BLOOD TO MEASURE THIS PLATELET FUNCTION.

THEREFORE THE OBJECTIVE OF THIS STUDY WAS TO DETERMINE IF HYPERCHOLESTEROLEMIA ALTERS THE RESPONSE OF PLATELETS TO ADP-INDUCED AGGREGATION AS MEASURED IN WHOLE BLOOD BY IMPEDANCE AGGREGOMETRY.

METHODS

DIETARY GROUPS OF MALE SPRAGUE-DAWLEY RATS (275-299 GRAMS):

1. NORMAL DIET: PURINA 5001 LABORATORY RODENT CHOW FOR 10 WEEKS
2. HIGH CHOLESTEROL DIET: RODENT CHOW ENRICHED WITH 5% CHOLESTEROL + 1% SODIUM TAUROCHOLATE (BIOSERV) FOR 10 WEEKS

BLOOD SAMPLES:

TAIL ARTERIAL BLOOD + 3.2% SODIUM CITRATE (9:1 RATIO)

PLATELET COUNTS:

PLATELET COUNTS OF PLATELET-RICH PLASMA WERE DETERMINED WITH A COULTER Z_f COUNTER

DETERMINATION OF PLASMA LIPID LEVELS:

- 1. TOTAL CHOLESTEROL: MODIFICATION OF ALLAIN *ET AL* PROCEDURE
(1974) SIGMA DIAGNOSTICS PROCEDURE
NO. 352**

- 2. TRIGLYCERIDES: MODIFICATION OF MCGOWAN *ET AL*
PROCEDURE (1983) SIGMA
DIAGNOSTICS PROCEDURE NO. 339**

- 3. HDL-CHOLESTEROL: WARNER *ET AL* PROCEDURE (1982)
SIGMA DIAGNOSTICS PROCEDURE
NO. 352-3**

- 4. LDL-CHOLESTEROL: CALCULATION BASED ON FRIEDEWALD
ET AL (1972)**

MEASUREMENT OF ADP-INDUCED PLATELET AGGREGATION IN WHOLE BLOOD:

- 1. BLOOD SAMPLES WERE PREPARED IN A 1:1 RATIO (0.5 ML + 0.5 ML) OF CITRATED BLOOD AND TYRODE SOLUTION CONTAINING ALBUMIN, pH 7.4.**
- 2. 0.5 MICROMOLAR CONCENTRATION OF ADP PER FINAL VOLUME IN EACH AGGREGATION CUVETTE WAS USED.**
- 3. MEASUREMENT OF PLATELET AGGREGATION WAS PERFORMED IN A CHRONO-LOG MODEL 530 WHOLE BLOOD AGGREGOMETER.**

ANALYSIS OF DATA:

POOLED t-TESTS WERE USED TO COMPARE LIPID LEVELS, PLATELET COUNTS, MAXIMAL IMPEDANCE RESPONSES, AND RATE OF IMPEDANCE RESPONSES BETWEEN THE CONTROL AND EXPERIMENTAL GROUPS.

RESULTS

PLASMA LIPID LEVELS WERE SIGNIFICANTLY ALTERED BY THE HIGH CHOLESTEROL DIET. TOTAL CHOLESTEROL, LDL-CHOLESTEROL, AND TRIGLYCERIDES WERE SIGNIFICANTLY ELEVATED, WHILE HDL-CHOLESTEROL WAS SIGNIFICANTLY DECREASED (TABLE 1).

THERE WAS NO SIGNIFICANT DIFFERENCE IN PLATELET COUNTS OF RATS RECEIVING THE NORMAL DIET AND HIGH CHOLESTEROL DIET (TABLE 2).

PLATELETS FROM HYPERCHOLESTEROLEMIC RATS AGGREGATED IN RESPONSE TO 0.5 μ M ADP AT A SLIGHTLY HIGHER FREQUENCY THAN PLATELETS FROM NORMOCHOLESTEROLEMIC RATS (TABLE 3).

FIGURE 1 ILLUSTRATES TYPICAL ADP-INDUCED PLATELET AGGREGATION RESPONSES TO 0.5 μ M ADP FOR NORMOCHOLESTEROLEMIC PLATELETS AND HYPERCHOLESTEROLEMIC PLATELETS. THE AGGREGATION RESPONSES TO 0.5 μ M ADP BY PLATELETS FROM HYPERCHOLESTEROLEMIC RATS WERE SIGNIFICANTLY GREATER THAN PLATELET AGGREGATION RESPONSES FROM NORMOCHOLESTEROLEMIC RATS IN REGARD TO BOTH MAXIMUM CHANGE IN IMPEDANCE AND RATE OF CHANGE IN IMPEDANCE (TABLE 4).

TABLE 1: PLASMA LIPID LEVELS OF RATS FED FOR 10 WEEKS ON A NORMAL DIET OR A HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

Plasma Lipid	N Diet Rats		HC Diet Rats		t-test
	<u>n</u>	<u>mg%</u>	<u>n</u>	<u>mg%</u>	
TC	11	76.7 +/- 18.7	10	300.7 +/- 94.0	S2
LDL	11	5.6 +/- 4.9	8	230.9 +/- 98.3	S2
HDL	10	55.9 +/- 14.0	7	40.1 +/- 11.3	S1
TG	11	78.0 +/- 27.5	10	105.5 +/- 27.7	S1

mg% as mean, +/- standard deviation

n = number of animals

N = normal diet, HC = high cholesterol diet

TC = total cholesterol, LDL = LDL cholesterol

HDL = HDL cholesterol, TG = triglycerides

S1 = significant at $p < .025$, S2 = significant at $p < .005$

TABLE 2: PLATELET COUNTS OF RATS FED FOR 10 WEEKS ON A NORMAL DIET OR A HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

Diet	n	Platelet Counts in Millions Per mL of Whole Blood	t-test
N	11	105.6 +/- 5.4	NS
HC	11	102.0 +/- 6.3	

N = normal diet, HC = high cholesterol diet

n = number of animals

Platelet counts as mean +/- standard deviation

NS = not significant at $p < .005$

**TABLE 3: RELATIVE FREQUENCIES OF PLATELET AGGREGATION
FOLLOWING ADDITION OF 0.5 MICROMOLAR ADENOSINE
DIPHOSPHATE TO BLOOD FROM RATS FED FOR 10
WEEKS ON A NORMAL DIET OR A 5% CHOLESTEROL AND
1% SODIUM TAUROCHOLATE DIET**

Diet Group	n	Frequency of Aggregation	Percentage
N	11	9	81.8
HC	9	8	88.8

N = normal diet

HC = high cholesterol diet

n = number of animals

TABLE 4: ADENOSINE DIPHOSPHATE-INDUCED PLATELET AGGREGATION FOLLOWING ADDITION OF 0.5 MICROMOLAR ADP TO BLOOD FROM RATS FED FOR 10 WEEKS ON A NORMAL DIET OR A 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE DIET

Diet Group	n	Delta Ohms/Sec	Maximum Delta Ohms
N	9	0.077 +/- .034	8.00 +/- 5.28
HC	7	0.213 +/- .103	15.53 +/- 8.55
t-test		p < .001	p < .001

N = normal diet

HC = high cholesterol diet

Delta Ohms/Sec as mean +/- standard deviation

Maximum Delta Ohms as mean +/- standard deviation

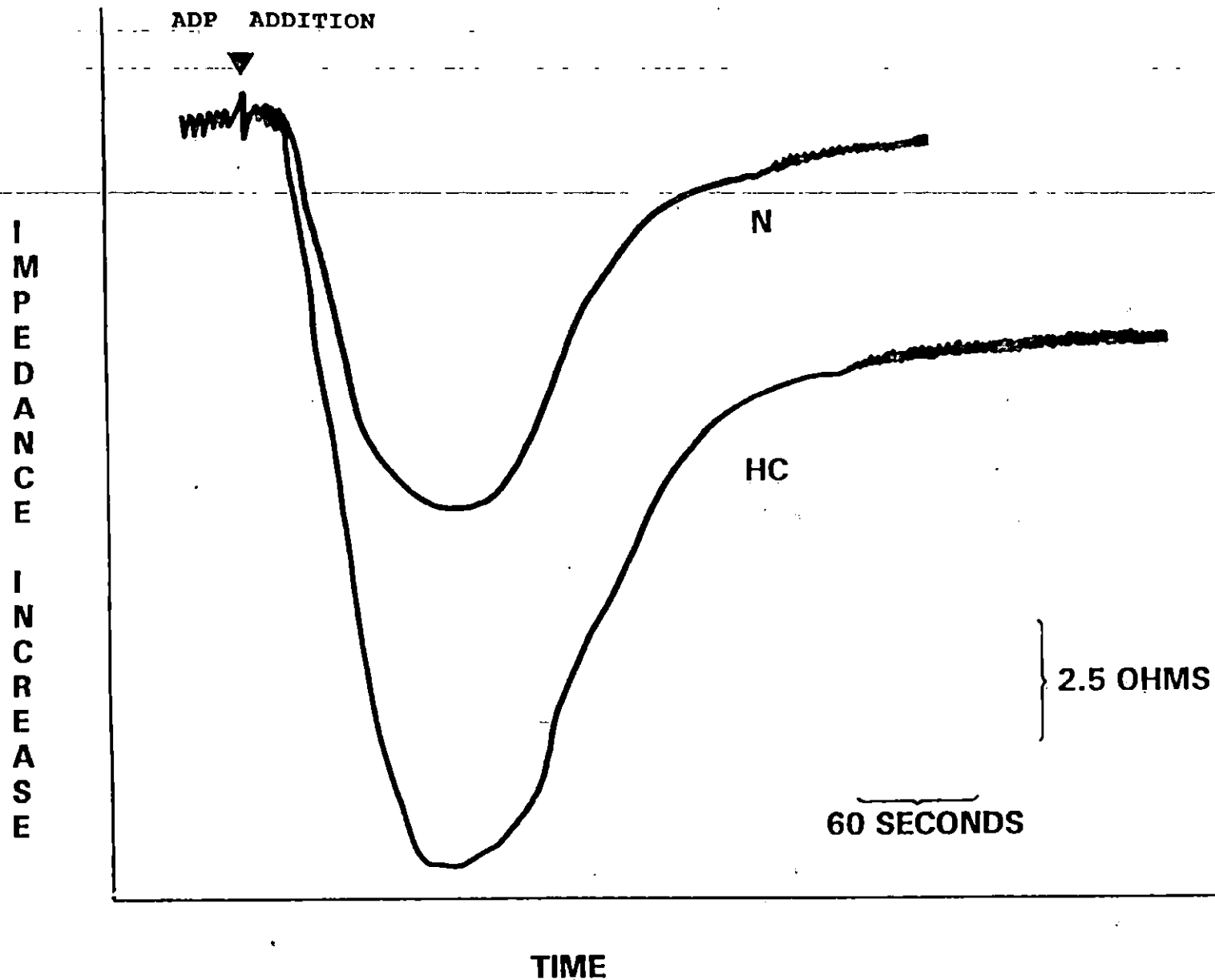


FIGURE 1: TYPICAL AGGREGATION RESPONSES TO 0.5 μ M ADP BY PLATELETS IN 0.5 ML CITRATED BLOOD + 0.5 ML TYRODE BUFFER, pH 7.4. N = NORMOCHOLESTEROLEMIC BLOOD, HC = HYPERCHOLESTEROLEMIC BLOOD.

CONCLUSIONS

HYPERCHOLESTEROLEMIA DOES APPEAR TO HAVE A ROLE IN ALTERING THE PLATELET RESPONSE OF ADP-INDUCED AGGREGATION AS MEASURED IN WHOLE BLOOD USING IMPEDANCE AGGREGOMETRY.

THE ALTERATIONS IN PLATELET RESPONSE TO 0.5 μ M ADP INCLUDE:

- 1. A SIGNIFICANTLY GREATER MAXIMUM AGGREGATION RESPONSE BY PLATELETS OF HYPERCHOLESTEROLEMIC RATS AND**
- 2. A SIGNIFICANTLY GREATER RATE OF AGGREGATION BY THESE THESE HYPERCHOLESTEROLEMIC PLATELETS.**

THESE ALTERATIONS BY HYPERCHOLESTEROLEMIA IN REGARD TO PLATELET RESPONSES TO ADP ARE IMPORTANT SINCE ADP DERIVED FROM ERYTHROCYTES AND ENDOTHELIAL CELLS MAY BE AN AGONIST FOR PLATELET AGGREGATION AND BECAUSE ADP IS ALSO RELEASED FROM DENSE GRANULES AS A PART OF A POSITIVE FEEDBACK LOOP IN THE BASIC PLATELET REACTION TO A VARIETY OF AGONISTS.