PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 179, 324–330 (1985)

Measurement by Electrical Impedance Aggregometry of Porcine Platelets Response to Selected Physiological Agonists[1] (42104)

HENRY GEWIRTZ,[2] MANFRED STEINER, HARVEY SASKEN, AND ALBERT S. MOST

Departments of Medicine (Division of Cardiology) and Pathology, Rhode Island Hospital, Providence, Rhode Island 02902; Division of Hematology, Pawtucket Memorial Hospital, Pawtucket, Rhode Island 02860; and Program in Medicine, Brown University, Providence, Rhode Island 02903

Abstract. Although the domestic swine is commonly employed for physiological studies of the coronary circulation, there is relatively little data available concerning the responsiveness in whole blood of normal porcine platelets to standard physiological agonists. Such information is essential if the domestic swine is to be used as an animal model for studying potential interactions between platelets and the coronary circulation. Accordingly, the present study was undertaken to characterize the responses (aggregation and ATP release) observed in whole blood of normal porcine platelets to selected physiological agonists. The responses of platelets from 10 normal human volunteers also were studied with this system for comparison. Agents tested included ADP, arachidonic acid, collagen, epinephrine, norepinephrine, and thrombin. Studies were conducted with the Chronolog impedance aggregometer. The results demonstrate that platelets of domestic swine are reactive to ADP, arachidonic acid, and collagen. In contrast, neither epinephrine nor norepinephrine alone induced aggregation or release. Norepinephrine, however, caused modest potentiation of aggregation in response to ADP only. At 1 m*M* concentration each catecholamine inhibited the release response to collagen while at 10 m*M* each inhibited aggregation and release in response to either ADP or collagen. The data obtained indicate the domestic swine may be employed as a useful model to examine interactions between platelets and the coronary circulation.

The domestic pig is commonly used in laboratory investigation of myocardial blood flow because its coronary circulation is similar to that of humans (1). Since release of vasoactive substances from platelets may influence myocardial blood flow (2–6), it would be useful to establish the responsiveness of porcine platelets to various physiological stimuli to determine the utility of the domestic pig for experimental studies of interactions between platelets and the coronary circulation. To date, however, relatively little information has been accumulated in this regard. This is particularly so with respect to aggregation measurements made by electrical impedance technique in whole blood. In an earlier study the aggregatory but not ATP secretory responses of porcine platelets were measured by standard optical transmission methods (7). The present study was undertaken, therefore, in order to characterize simultaneously the aggregatory response and ATP secretory capacity of porcine platelets to several physiological stimuli. Studies were carried out in whole blood with a Chronolog dual channel electronic aggregometer (8). This device was employed in order to observe porcine platelet responses under conditions which were as physiological as possible. The responses of platelets from 10 normal human volunteers also were studied with this system for comparison.

Materials and Methods. *Blood collection.* All animals used in the study were premedicated with intramuscular injection of ketamine (25 mg/kg). Blood was then obtained either by direct puncture of the internal jugular vein or by cutdown over the femoral vein. In cases in which direct puncture was employed, care was taken to discard the initial 3 to 5 ml of blood obtained in order to avoid contamination of the sample with tissue thromboplastin. Approximately 10 to

[1] Supported in part by a grant from the NIH-NHLBI (HL-31664).

[2] To whom reprint requests should be addressed: Division of Cardiology, Rhode Island Hospital, Providence, R.I. 02902.

0037-9727/85 $1.50

15 ml of whole blood was collected directly into a syringe containing 3.8% sodium citrate. Nine parts whole blood were collected to one part sodium citrate solution. The hematocrit of each sample of whole blood was determined using a standard microcentrifuge. Platelet counts on whole blood were performed with the aid of a phase microscope according to standard laboratory technique (9). Manual platelet counts were compared and validated in three animals by comparison with an automated platelet counting device (Coulter S-Plus). Comparison between manual (mean value $4.87 \times 10^5/\mu l$) and automatic (mean value $4.78 \times 10^5/\mu l$) methods yielded a correlation coefficient that was 0.998 ($P < 0.01$) with slope = 0.95 and intercept = 33.3. After the hematocrit and platelet count of the whole blood sample had been determined, the sample was diluted with an equal volume of physiological saline and then kept in a water bath at 37°C throughout the duration of the study.

The pigs employed in this study were either castrated males ($N = 18$) or females ($N = 17$). Weights ranged from 36 to 60 kg (Mean = 44.4). The animals were given an ordinary diet of Agway Pig Feed and observed daily in the animal care center of Rhode Island Hospital for 2 weeks prior to use. Agway Feed contains tylosin phosphate 20 g/ton. The drug is an antibiotic, active against mycoplasma, but is added to feed to stimulate the animal's appetite. All pigs (Yorkshire/Landrace mix breed) were obtained from a local hog farm and were 3–4 months old at the time of use.

Determination of platelet aggregatory and secretory responses to physiological stimuli. A Chronolog aggregometer with impedance electrode and photomultiplier tube was employed for these studies. Outputs from the impedance electrode and photomultiplier tube were directed to a dual channel recorder which permitted simultaneous measurements of aggregation and ATP release (Chronolume reagent–Luciferase-Luciferin method (10)). Studies were performed in the following fashion. Siliconized glass cuvettes were filled with 0.9 ml of diluted whole blood plus 0.1 ml Chronolume reagent and stored in a heating block at 37°C contained within the aggregometer. A Teflon-coated magnetic stir bar was used to stir the diluted whole blood mixture at a rate of 1000 rpm. Aggregating agents were added with precisely calibrated siliconized glass syringes capable of delivering microliter quantities (vol 1–10 μl) of agonist solution. Impedance and ATP secretory channels were monitored continuously for 5 min at the end of which time the run was terminated. All samples were done in duplicate and sometimes triplicate if more than 10% variation occurred between the first two samples. The peak response in terms of change in electrical impedance (ohms) and ATP secretion was recorded for each run. An average value for each parameter was calculated and used in analysis of the data. The amount of ATP released was determined by comparing the measured change in light output with that obtained when a known amount of ATP (2 μM final concentration) was added to the reaction mixture. Since the number of agonists employed was too large to permit completion of testing within a 4- to 5-hr period, several groups of animals were used in order to obtain at least seven sets of responses for each agonist employed. In double stimulant studies epinephrine (EPI) or norepinephrine (NE) was added to the cuvette 1 min prior to addition of ADP or collagen.

The following agonists were used: ADP 10 μM, collagen 4 μg/ml, arachidonic acid (0.5, 2.5, and 5.0 mM), epinephrine or norepinephrine (1 and 10 mM), and thrombin (1 unit/ml to assess maximal ATP secretion only). In eight animals impedance change and ATP release also were measured in response to collagen 1 and 4 μg/ml and collagen 1 μg/ml after 1-min preincubation with epinephrine (1 mM). All concentrations refer to final concentration of the reaction mixture. ADP and collagen were obtained from Chronolog (Havertown, Pa). Aracidonic acid in albumin was purchased from Nu-Check Prep (Elysian, Minn.). Solutions of epinephrine and norepinephrine were obtained from Sigma Chemicals (St. Louis, Mo.). Thrombin was acquired from Parke-Davis (Morris Plains, N.J.).

Platelets from healthy, normal human volunteers (six male, four female; ages 27 to 40 years; mean = 33 years) were studied in the same fashion as those from pigs with the

following exceptions. First, responses to 5 and 10 μM ADP and to 1 and 4 μg/ml collagen were measured. Second, only 0.5 and 5.0 mM arachidonic acid and 1.0 mM epinephrine stimulation was tested. Third, the response to collagen 1 μg/ml after 1 min preincubation with epinephrine (1 mM) was evaluated. Stimulation with norepinephrine was not performed so that all responses could be measured within 4 hr of obtaining blood for testing. The order in which stimuli were tested was randomized. None of the subjects smoked, nor were any taking any medications for at least 2 weeks prior to the study.

Statistical methods. All results are expressed as means ± 1 SD. The significance of differences between group mean values was assessed by blocked one-way analysis of variance when appropriate (11). P values less than 0.05 were considered statistically significant.

Results. *Hematocrit and platelet count.* A total of 35 animals were employed in the protocols described above. Mean hematocrit of undiluted whole blood was 30.1 ± 4.6 (range 24–42). Platelet counts, however, exhibited greater variability (mean 643,000 ± 288,000 ml). Platelet counts obtained on a daily basis (×4 days) in four additional pigs demonstrated minimal day to day variation in counts (average coefficient of variation = 5.2%, range = 2.5–7.0%), thereby indicating that technical problems related to obtaining blood were unlikely to be responsible for animal to animal variability in platelet counts.

Aggregation and ATP secretion. (a) ADP (10 μM) and collagen (4 μg/ml); N = 8 animals. In response to ADP, electrical impedance increased 14.3 ± 3.8 ohms. At the same time maximal ATP concentration increased to 0.85 ± 0.54 μM. Since the final reaction volume was just over 1.0 ml this corresponds to release of 0.85 ± 0.54 nmol of ATP. Collagen was found to be a more potent stimulant to both platelet aggregation and ATP release. Thus, impedance increased by 20.4 ± 3.1 ohms while maximal ATP concentration increased to 3.50 ± 1.84 μM, after addition of collagen.

(b) Epinephrine and norepinephrine (1 and 10 mM); N = 9 animals (Table I). In pilot studies of three pigs, no aggregatory or secretory response to epinephrine or norepinephrine was observed at agonist concentrations between 20 μM and 1.0 mM. The addition of 1 mM epinephrine did not potentiate the aggregatory response to 10 μM ADP (12.3 ± 2.0 versus 13.0 ± 3.1) or 4 μg collagen (21.8 ± 2.6 versus 23.3 ± 2.5). Furthermore, addition of 10 mM epinephrine caused a significant ($P < 0.01$) reduction in aggregatory response to either ADP (2.8 ± 2.1) or collagen (15.0 ± 5.0). ATP release in the presence of 10 mM epinephrine paralleled the aggregatory response. Addition of 10 mM epinephrine caused a significant ($P < 0.01$) reduction in

TABLE I. IMPEDANCE CHANGE AND ATP RELEASE TO EPINEPHRINE AND NOREPINEPHRINE (MEAN ± 1 SD)

Agonist	Impedance		
	Maximum change (ohms)	Maximum slope (ohms/min)	ATP release (nmole)
Saline + ADP (10 μM)	12.3 ± 2.0	12.4 ± 3.1	0.30 ± 0.17
Saline + collagen (4 μg)	21.8 ± 2.6	15.0 ± 2.6	4.00 ± 0.93
EPI (1 mM) + ADP	13.0 ± 3.1	12.6 ± 3.1	0.31 ± 0.18
EPI (10 mM) + ADP	2.8 ± 2.1*	3.1 ± 1.9*	0.17 ± 0.06*
EPI (1 mM) + collagen	23.3 ± 2.5	15.7 ± 2.7	3.61 ± 0.93*
EPI (10 mM) + collagen	15.0 ± 5.0*	6.9 ± 2.5*	1.18 ± 0.29*
NE (1 mM) + ADP	14.1 ± 2.5**	13.8 ± 4.4	0.31 ± 0.19
NE (10 mM) + ADP	6.3 ± 2.6*	6.1 ± 2.4*	0.24 ± 0.13**
NE (1 mM) + collagen	23.6 ± 1.6	14.9 ± 2.2	3.26 ± 0.99*
NE (10 mM) + collagen	19.7 ± 1.7**	10.5 ± 1.6*	2.04 ± 0.38*

Note. EPI, epinephrine; NE, norepinephrine.
* $P < 0.01$ versus ADP or collagen + saline.
** $P < 0.05$ versus ADP + saline.

ATP release following stimulation with either ADP (0.17 ± 0.06) or collagen (1.18 ± 0.29). Although no change was observed versus control in maximal ATP concentration in the presence of 1 m*M* epinephrine upon stimulation with 10 μM ADP (0.30 ± 0.17 versus 0.31 ± 0.18), ATP release upon stimulation with 4 μg collagen (3.61 ± 0.93) was reduced ($P < 0.01$) versus control (4.00 ± 0.93).

The response to norepinephrine 1 m*M* differed with respect to impedance changes for ADP and collagen. Thus, norepinephrine caused modest potentiation of aggregation in response to ADP (12.3 ± 2.0 versus 14.4 ± 2.5, $P < 0.05$) but no change in response to collagen (21.8 ± 2.6 versus 23.6 ± 1.6). Although ATP release was unchanged versus control in response to ADP plus 1 m*M* norepinephrine, it was reduced significantly ($P < 0.01$) in response to collagen plus 1 m*M* norepinephrine (4.00 ± 0.93 versus 3.26 ± 0.99). Norepinephrine 10 m*M* also resulted in significant reductions in aggregation upon stimulation with either ADP (6.3 ± 2.6, $P < 0.01$) or collagen (19.7 ± 1.7, $P < 0.05$). Similarly, maximal ATP release following either ADP or collagen stimulation was reduced versus control in the presence of 10 m*M* norepinephrine (0.24 ± 0.13, $P < 0.05$ and 2.04 ± 0.38, $P < 0.01$, respectively). Finally, analysis of the maximal rate of change of the impedance curves (ohms/min) provided much the same information as that obtained from analysis of maximal impedance response without regard to time (Table I). The only exception being that the maximal slope of the 1 m*M* norepinephrine + ADP curve was not increased versus control whereas the maximal change in impedance was.

(c) Arachidonic acid (0.5, 1.0, 5.0 mM); N = 10 animals (Table II). Aggregation and ATP secretion by platelets in response to 0.5 m*M* arachidonic acid were variable from animal to animal. Some exhibited rather vigorous responses while others exhibited no response at all. The mean impedance change was 7.3 ± 6.2 and mean ATP release 0.34 ± 0.42. The response to 2.5 m*M* arachidonic acid was more consistent from animal to animal. Mean impedance change was 13.1 ± 2.9 and mean ATP release 1.10 ± 0.55 ($P < 0.01$ versus 0.5 m*M* arachidonic acid for each). Finally, addition of arachidonic acid 5.0 m*M* did not augment the response observed versus 2.5 m*M* either for change in impedance (13.7 ± 3.2) or ATP release (1.21 ± 0.52).

TABLE II. MAXIMAL IMPEDANCE CHANGE AND ATP RELEASE TO ARACHIDONIC ACID (MEAN ± 1 SD)

Arachidonic acid	Impedance (ohms)	ATP release (nmole)
0.5 m*M*	7.3 ± 6.2	0.34 ± 0.42
2.5 m*M*	13.1 ± 2.9*	1.10 ± 0.55*
5.0 m*M*	13.7 ± 3.2*	1.21 ± 0.52*

* $P < 0.01$ versus 0.5 m*M* arachidonic acid.

(d) Thrombin stimulation; N = 7 animals. Maximal ATP release in response to thrombin (1.0 U/ml) was reasonably consistent from animal to animal (range 2.6–6.6 μM, mean 4.8 ± 1.4). It should also be noted that platelet count of undiluted blood did not correlate with maximal ATP release ($r = 0.33$, $P = 0.50$). Similar results have been obtained by others in studies with human platelets (12) and have been attributed to individual variation in platelet ATP content since serial dilution of any given blood sample has been shown to result in proportionate reduction in total ATP release (13). It is likely that similar conclusions apply to porcine platelets. Finally, the value observed did not differ significantly from that recorded with collagen (4 μg/ml). This likely reflects the fact that a very potent form of collagen (primarily from equine tendon) was employed and produced near maximal aggregatory response in these studies.

(e) Collagen–epinephrine protocol; N = 8 animals. In response to 1 μg/ml collagen impedance increased 4.8 ± 3.0 ohms while ATP released was 0.47 ± 0.11 μM. Stimulation with 4 μg/ml collagen increased impedance to 16.3 ± 1.7 ($P < 0.01$ versus 1 μg/ml) and ATP released to 2.49 ± 0.58 ($P < 0.01$ versus 1 μg/ml). Preincubation of platelets with 1 m*M* epinephrine resulted in a modest but statistically significant ($P < 0.05$) decline in impedance (3.4 ± 2.4) versus 1 μg/ml collagen alone but no change in ATP released (0.41 ± 0.14). In 5/5 animals tested 1 m*M* epinephrine alone failed to induce aggregation or release.

(f) Human platelet responses; (N = 10 volunteers). Platelet counts in these subjects

ranged between 137,500 and 355,000/μl (mean = 273,900). The mean hematocrit was 37.9 ± 2.5.

The volunteer's platelets exhibited neither aggregation nor release of ATP in response to 1 m*M* epinephrine. Stimulation with collagen 1 μg/ml increased impedance to 9.3 ± 8.0 and ATP released to 0.30 ± 0.22 μ*M*. Collagen 4 μg/ml caused significant ($P < 0.01$ versus 1 μg/ml) augmentation of impedance (23.0 ± 8.1) and ATP release (0.89 ± 0.43). Preincubation of platelets with 1 m*M* epinephrine also resulted in increased impedance (14.0 ± 6.9) and ATP release (0.57 ± 0.31) in response to 1 μg/ml collagen versus collagen 1 μg/ml alone (both $P < 0.01$).

Arachidonic acid 0.5 m*M* gave variable impedance (5.3 ± 6.4) and ATP release responses (0.30 ± 0.46). In contrast the response to 5.0 m*M* arachidonic acid was more consistent. Impedance increased to 14.0 ± 7.5 ($P < 0.05$ versus 0.5 m*M* arachidonic acid) while ATP released increased to 1.29 ± 0.49 μ*M* ($P < 0.01$ versus 0.5 m*M* arachidonic acid).

Electrical impedance was 9.6 ± 6.3 and ATP released 0.30 ± 0.34 in response to ADP 5 μ*M*. Stimulation with 10 μ*M* ADP caused a significant ($P < 0.05$) increase verus 5 μ*M* ADP in impedance (13.0 ± 7.0) but no change in ATP released (0.45 ± 0.30).

Discussion. The present investigation was designed to evaluate the responsiveness in whole blood of porcine platelet to selected physiological stimuli. The Chronolog whole blood aggregometer was employed for these studies so that porcine platelet responses could be determined under conditions which were as physiological as possible. The advantages and disadvantages of this approach have been described previously (8, 14). It also should be noted that an effort was made in these studies to find the lowest dose of ADP and collagen which would produce a consistent, easily measured aggregatory response (arbitrarily chosen as ⩾10 ohms). This was done in order to facilitate future studies in which platelet responsiveness (in terms of aggregation and ATP release) could be compared before and after a given intervention.

The data obtained demonstrate that porcine platelets respond vigorously to collagen in terms of both aggregation and ATP release. This observation is particularly important from the point of view of studies involving coronary blood flow in the setting of a coronary artery stenosis. Both artificial and induced atherosclerotic coronary artery stenoses have been employed in porcine models of human ischemic heart disease (15, 16). The fact that porcine platelets respond vigorously when exposed to a "foreign" surface such as collagen indicates the porcine model should be useful for studies concerned with potential interactions between platelets and the stenosed coronary circulation. This conclusion is supported by the observation in the present study (Table III) that stimulation of human platelets with 1 or 4 μg/ml collagen produced aggregation responses which were similar to those exhibited by porcine platelets.

Porcine platelets also were found to be responsive to ADP in this study. Aggregation of porcine platelets upon exposure to ADP has been demonstrated in the past both *in vivo* (17) and *in vitro* (7). However, since different methodologies were employed, it is not possible to directly compare the ADP responsiveness of porcine platelets observed in this study with that reported earlier. Human platelet aggregation in response to ADP has been studied by Ingerman-Wojenski *et al.* with electrical impedance technique (12, 14, 18). In one of their studies (14) of normal human platelets a change in electrical impedance comparable to that observed in the present study was produced with 5 μ*M* ADP. In an earlier study, however, an ADP dose of 10 μ*M* was used to produce an approximate 15 ohm change in electrical impedance (18). Human and porcine platelets exhibited comparable responses (both in the range of 15 ohms) to 10 μ*M* ADP in the present study (Table III). Therefore, when aggregation in response to ADP is measured by electrical impedance technique in diluted whole blood, porcine platelet responsiveness is very similar to that of humans.

The data obtained also are consistent with a previous report in which it was demonstrated that porcine platelets do not aggregate in response to epinephrine (6). Further, epinephrine (1 m*M*) failed to potentiate aggregation or ATP release in response to ADP

TABLE III. COMPARISON OF HUMAN AND PORCINE PLATELET RESPONSES (MEAN ± 1 SD) TO SELECTED PHYSIOLOGICAL AGONISTS

	Impedance (ohms)					ATP release (nmole)					
	ADP (10 μM)	AA (5 mM)	Collag (1 μg)	Collag (4 μg)	Collag + (EPI*)	EPI (1 mM)	ADP (10 μM)	AA (5 mM)	Collag (1 μg)	Collag (4 μg)	Collag + (EPI*)	EPI (1 mM)
Humans	13.0 ± 7.0	14.0 ± 7.5	9.3 ± 8.0	23.0 ± 8.1	14.0 ± 6.9	NR	0.45 ± 0.30	1.29 ± 0.49	0.30 ± 0.22	0.89 ± 0.43	0.57 ± 0.31	NR
N	10	10	10	10	10	10	10	10	10	10	10	10
Pigs	14.3 ± 3.8	13.7 ± 3.2	4.8 ± 3.0	18.3 ± 3.2	3.4 ± 2.4	NR	0.85 ± 0.54	1.21 ± 0.52	0.47 ± 0.11	2.99 ± 1.42	0.41 ± 0.14	NR
N	8	10	8	16	8	8	8	10	8	16	8	8

Note. Abbreviations: ADP, adenosine diphosphate; AA, arachidonic acid; Collag, collagen; EPI, epinephrine; N, number of subjects; NR, no reaction.
* Collagen (1 μg/ml) + epinephrine (1 mM).

or collagen. Indeed, at 1 mM concentration both epinephrine and norepinephrine caused modest but statistically significant reduction in ATP release in response to collagen (4 μg/ml) stimulation. Pretreatment with epinephrine (1 mM) also failed to enhance aggregation and in fact inhibited it modestly in response to collagen 1 μg/ml. At a higher dose (10 mM) epinephrine consistently antagonized both the aggregatory and ATP release responses to either ADP or collagen (4 μg/ml). The same was true of norepinephrine (10 mM). The mechanism involved cannot be stated with certainty based on the data obtained. However, it is possible in high concentration that either epinephrine or norepinephrine could have stimulated cAMP production by platelets or lymphocytes in blood and thereby inhibited aggregation and ATP release responses (19).

The fact that norepinephrine (1 mM) produced modest enhancement of platelet aggregation in response to ADP stimulation, while epinephrine (1 mM) did not, is surprising since previous studies in humans indicate epinephrine is a more potent α-2 agonist than norepinephrine (20). Species differences may be responsible for these findings. In any case the fact that norepinephrine potentiated aggregation with ADP suggests that porcine platelets may possess α-2 receptors. Finally, the data also indicate that human and porcine platelets differ in their responsiveness to epinephrine. Human platelets exhibit potentiation of aggregation in response to low-dose collagen stimulation (i.e.; 1 μg/ml) following pretreatment with epinephrine whereas porcine platelet aggregation is inhibited by epinephrine (Table III).

Porcine platelets demonstrated aggregation and release of ATP in response to arachidonic acid stimulation. The dose required to produce a consistent response was comparable to that observed for human platelets studied in similar fashion (Table III). The fact that porcine platelets exhibit aggregation and release in response to arachidonic acid indicates the cyclooxygenase pathway is present in their platelets and that they are capable of synthesizing and responding to thromboxane-A2 (21). It should be noted in this regard that we have measured thromboxane-B2 concentration (I-125, radioimmu-

noassay method) in coronary venous plasma of swine ($N = 8$) during thrombotic occlusion of an artificial coronary arterial stenosis. Concentrations ranging between 134.6 and 935.0 pg/ml (mean = 466.0) were observed in these animals (22).

In conclusion porcine platelets respond vigorously in terms of aggregation and ATP release to stimulation with collagen. They also exhibit clearcut aggregatory and release responses to either ADP or arachidonic acid. In addition, porcine platelets exhibit modest potentiation of aggregation in response to ADP when pretreated with 1 m*M* norepinephrine. Thus, the data indicate the porcine model is a suitable one for studying a variety of potential interactions between platelets and the coronary circulation. The only exception to this generalization concerns the differing responses of porcine and human platelets to collagen stimulation (1 μg/ml) following pretreatment with epinephrine (1 m*M*); human platelet aggregation is potentiated whereas porcine platelet aggregation is inhibited.

The authors are grateful to Mr. Ronald Stewart for expert performance of platelet aggregation studies. Mrs. Christine Abatiello and Miss Janice Bordieri assisted in the preparation of the manuscript.

1. Jonsson L, Johansson G, Lannek N, Lindberg P. Intramural blood supply of the porcine heart. A postmortem angiographic study. Anat Rec **178**:647–656, 1974.
2. Hirsh PD, Hillis LD, Campbell WB, Firth BG, Willerson JT. Release of prostaglandins and thromboxane into the coronary circulation in patients with ischemic heart disease. New Engl J Med **304**:685–691, 1981.
3. Needleman P, Kaley G. Cardiac and coronary prostaglandin synthesis and function. New Engl J Med **298**:1122–1128, 1978.
4. Weiss HJ. Platelets and ischemic heart disease (Editorial). New Engl J Med **302**:225–226, 1980.
5. Needleman P, Kulkarni PS, Raz A. Coronary tone modulation: Formation and actions of prostaglandins, endoperoxides, and thromboxanes. Science (Washington, DC) **195**:409–412, 1977.
6. Green LH, Seroppian E, Handin RI. Platelet activation during exercise induced myocardial ischemia. New Engl J Med **302**:193–197, 1980.
7. Addonizio VP, Edmunds LH, Jr, Coleman RW. The function of monkey (M. Mullatta) platelets compared to platelets of pigs, sheep and man. J Lab Clin Med **91**:989–997, 1978.
8. Cardinal DC, Flower RJ. The electronic aggregometer: A novel device for assessing platelet behavior in blood. J Pharmacol Methods **3**:135–158, 1980.
9. Todd-Sanford. Clinical Diagnosis by Laboratory Methods. Davidshon S, Henry JB, eds., 5th ed. Philadelphia, Saunders, p126, 1974.
10. Detwiler TC, Feinman RD. Kinetics of the thrombin-induced release of adenosine triphosphate by platelets. Comparison with release of calcium. Biochemistry **12**:2462–2468, 1973.
11. Winer BJ. Statistical Principles in Experimental Design. New York, McGraw-Hill, 1971, p191.
12. Ingerman-Wojenski C, Silver MJ. A quick method for screening platelet dysfunctions using the whole blood lumi-aggregometer. Thromb Haemostasis **51**: 154–156, 1984.
13. Ingerman C, Smith JB, Silver MJ. Direct measurement of platelet secretion in whole blood. Thromb Res **16**:335–344, 1979.
14. Ingerman-Wojenski C, Smith JB, Silver MJ. Evaluation of electrical aggregometry: Comparison with optical aggregometry, secretion of ATP, and accumulation of radiolabeled platelets. J Lab Clin Med **101**:44–52, 1983.
15. Gewirtz H, Brautigan DL, Olsson RA, Brown P, Most AS. Role of adenosine in the maintenance of coronary vasodilation distal to a severe coronary artery stenosis: Observations in conscious domestic swine. Circ Res **53**:42–51, 1983.
16. Gewirtz H, Most AS. Production of a critical coronary artery stenosis in closed chest laboratory animals: Description of a new non-surgical method based on standard cardiac catheterization techniques. Amer J Cardiol **47**:589–596, 1981.
17. Jorgensen L, Rowsell HC, Hoving T, Glynn MF, Mustard JF. Adenosine diphosphate-induced platelet aggregation and myocardial infarction in swine. Lab Invest **17**:616–644, 1967.
18. Ingerman-Wojenski C, Smith JB, Silver MJ. Use of whole-blood aggregometer to study the aggregation of "giant" platelets. Thromb Res **27**:371–376, 1982.
19. Moncada S, Vane JR. Arachidonic acid metabolites and interactions between platelets and blood vessel walls. New Engl J Med **300**:1142–1147, 1979.
20. Motulsky HJ, Insel PA. Adrenergic receptors in man. Direct identification, physiologic regulation, and clinical alterations. New Engl J Med **307**:18–29, 1982.
21. Harlan JM, Harker LA. Hemostasis, thrombosis and thromboembolic disorders. The role of arachidonic acid metabolites in platelet-vessel wall interaction. Med Clin N Amer **65**:855–880, 1981.
22. Gewirtz H, Sasken H, Steiner M, Most AS: Impaired arteriolar vasodilation induced by thrombosis of a coronary artery stenosis. Submitted for publication.

Received October 22, 1984. P.S.E.B.M. 1985, Vol. 179.
Accepted March 14, 1985.