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MODIFICATION, BY ASPIRIN AND INDOMETHACIN, OF THE HAEMODYNAMIC AND PROSTAGLANDIN RELEASING EFFECTS OF *E. coli* ENDOTOXIN IN THE DOG

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1 Dogs treated with aspirin (10 mg/kg) or indomethacin (1.5 mg/kg) 45 min before, and 3 h after, an LD_{50} dose (1 mg/kg) of *E. coli* endotoxin were alive 72 h later.

2 Although all dogs in both treated groups survived, only those treated with indomethacin were protected against the fall in blood pressure 1–2 min following endotoxin.

3 Endotoxin increased the level of prostaglandin $F_{2\alpha}$ in both the mixed venous and arterial blood. No increase was observed in the aspirin and indomethacin-treated groups.

4 Aspirin and indomethacin treatment did not modify thrombocytopaenia or blood coagulation parameters following endotoxin.

Introduction

Non-steroidal anti-inflammatory drugs decrease the haemodynamic responses in endotoxin shock and enhance survival (Northover & Subramanian, 1962; Hinshaw, Soloman, Erdos, Reins & Gunter, 1967; Culp, Erdos, Hinshaw & Holmes, 1971; Greenway & Murthy, 1971; Hall, Hodge, Irvine, Katic & Middleton, 1972; Parratt & Sturgess, 1974; 1975a,b,c; 1976; Anderson, Jubiz, Tsagaris & Kuida, 1975a,b; Fletcher, Ramwell & Herman, 1976). These drugs have a variety of actions. They competitively inhibit 5-hydroxytryptamine release from platelets (Evans, Packham, Nishizawa, Mustard & Murphy, 1968; deGaetano, Donati & Vermylen, 1971; Hall *et al.*, 1972), stabilize lysosomes (Miller & Smith, 1966), inhibit the platelet release reaction (Zucker & Peterson, 1970; deGaetano *et al.*, 1971), decrease vascular permeability (Hinshaw *et al.*, 1967), and inhibit prostaglandin synthesis and/or release (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971). The exact mechanism by which these drugs exert a beneficial effect in endotoxin shock is unknown. The drugs may act by different mechanisms at a number of sites or by influencing a single mechanism which could mediate the pathophysiology of endotoxin shock.

The prostaglandins are a group of possible mediators which are known to cause haemodynamic derangements (Kloeze, 1966; Sorells, Erdos & Massion, 1971; Raflo, Wangensteen, Glenn & Lefer, 1973), alterations in platelet functions (Kloeze, 1966), changes in vascular permeability (Arora, Lahiri & Sanyal, 1969), and influence the inflammatory process (Crunkhorn & Willis, 1969). Blood levels of these potent vasoactive fatty acids are increased in endotoxin shock (Anderson, Jubiz, Fralios, Tsagaris & Kuida, 1972; Kessler, Hughes, Bennett & Nadela, 1973; Anderson *et al.*, 1975a,b; Fletcher *et al.*, 1976), and are related in time to the haemodynamic events (Fletcher *et al.*, 1976).

There have been two main problems in implicating the prostaglandins as mediators of the haemodynamic events during endotoxin shock in dogs. First, in most studies the prostaglandin levels were determined at arbitrary time intervals after giving endotoxin, rather than at the time observable haemodynamic changes were present. There are no studies described in which the prostaglandin levels were assessed immediately after endotoxin injection or at the time when the first detectable haemodynamic changes were present. Second, the relative degree of stress introduced in the model has often been inadequately defined. For example, the reported studies frequently did not state the lethality of the model. As a consequence, the relationship of the prostaglandins to the pathophysiology of endotoxin shock is not clear.

This study was designed to (1) determine the levels of prostaglandins present in the mixed venous and

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systemic arterial blood as soon as haemodynamic changes were present following endotoxin injection in an LD_{50} dog model, (2) evaluate the effects of pre- and post-treatment with aspirin or indomethacin on prostaglandin release, on the haemodynamic events, and on the survival, (3) investigate the possible relation of changes in prostaglandin concentrations with the coagulation, platelet, and complement abnormalities present in endotoxin shock.

Methods

Adult male mongrel dogs (15–23 kg) were anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.), intubated and allowed to breathe spontaneously throughout the study. Indwelling catheters were inserted into the femoral and the pulmonary arteries through a cut-down in the right groin. The position of the pulmonary artery catheter (Swan-Ganz thermodilution) was monitored by pressure tracings and the femoral artery catheter was advanced into the descending aorta. The animals were kept in a supine position during the study. Cardiac outputs were measured by the thermal dilution method and calculated on a computer programme utilizing a PDP-12 computer (Digital Equipment Corporation). Systemic and pulmonary artery pressures were measured with transducers (Sanborn models 267AC and 267BC) and an 8-channel recorder (Sanborn model 958–100). All parameters were measured just before giving endotoxin and then 1–2, 15, 60, 120, and 240 min after the injection of endotoxin.

Blood analysis

Blood samples for prostaglandin analysis were collected from the femoral and pulmonary arteries simultaneously, and then immediately centrifuged at 4°C at 2450 rev/minute. The plasma was then removed and frozen (–20°C) until analysed. The thawed plasma (2 ml) was extracted with redistilled ethyl acetate (2 x 1 vol x 2) at pH 4.0. The dried ethyl acetate extract was reconstituted in benzene, ethyl acetate, methanol (60:40:10) and separated on a silicic acid column (0.5 gram). The prostaglandin F fractions were eluted with 5 ml benzene, ethyl acetate, methanol (60:40:30). Recovery of prostaglandin tracers added to plasma was 75–85% for prostaglandin $F_{2\alpha}$. Prostaglandin $F_{2\alpha}$ antisera was obtained from Dr H. Behrman (Yale, New Haven, Conn. U.S.A.). Radioimmunoassay was performed on the prostaglandin F fractions at two dilutions. Plasma standards were extracted with each group of unknown samples. The coefficient of variation for between and within assay reproducibility was required to be no greater than 10%. Data were calculated by the Rodbard and Hutt computer programme and expressed as picograms/ml plasma.

Arterial and mixed venous platelet counts were determined by an electronic particle counter (Coulter, Model ZBI). The one-stage method of Quick (1966) was used to assess the prothrombin time. The activated partial thromboplastin time was performed with Celite-activated phospholipid (Nye & Graham, 1962) and fibrinogen was measured turbidometrically (Parfentjev, Johnson & Clifton, 1953). Quantitation of the fibrin-split products was by means of the Staphylococcal clumping test (Hawiger, Niewearoski, Gusewich & Thomas, 1970). Total haemolytic complement (CH50) was determined as the greatest dilution of serum that produced 50% haemolysis in a standard sheep tanned erythrocyte microtitre plate system.

Intravenous injections

Endotoxin (*E. coli* 0 111:B4, Difco) was reconstituted in Ringer lactate solution on the day of the experiment. Aspirin (Mallinckrodt) was dissolved in sterile Ringer lactate solution with the aid of 0.1 N NaOH solution. Indomethacin (100 mg) was prepared in sterile 0.9% w/v NaCl solution (saline) by adding anhydrous sodium carbonate (37.5 mg) on the day of the experiment. Endotoxin, aspirin, and indomethacin were given intravenously.

Three groups of animals were studied: Group I (11 dogs) was given only an LD_{50} dose (1 mg/kg) of endotoxin; Group II (10 animals) was treated with aspirin (10 mg/kg), 45 min before, and 3 h after, endotoxin; Group III (10 animals) received intravenous indomethacin (1.5 mg/kg) 45 min before, and 3 h after, endotoxin.

No attempts were made to resuscitate the animals after endotoxin. All animals received a total of 400 ml of Ringer lactate solution during the study. Upon completion of the experiment, the dogs were placed in a recovery room, observed for 72 h and considered to be survivors if they were alive and well at this time.

Each dog was observed during a control period of 45 min before the administration of the endotoxin. At the end of the control period, baseline values were determined and endotoxin was injected into a peripheral vein. At the time of the acute fall in blood pressure, usually 1–2 min after endotoxin, measurements were taken and haemodynamic parameters assessed. In the aspirin (Group II) or indomethacin (Group III)-treated animals, there was an additional 45 min time period between giving the drugs and the administration of the endotoxin. Statistical analysis was performed by the paired two-tailed Student's t test for the difference between the baseline values and the experimental values in the same animals and the unpaired two-tailed t test for the difference between the groups. Chi square analysis was used to compare the survival data between the groups.

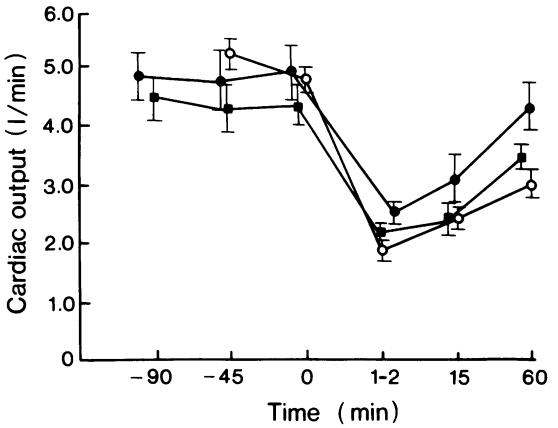

Figure 1 Mean cardiac outputs during endotoxin shock. Group I (○) consisted of 11 dogs given endotoxin alone. Group II (■) consisted of 10 dogs treated with aspirin (10 mg/kg) 45 min before, and 3 h after, an LD_{50} dose of endotoxin. Group III (●) consisted of 10 dogs treated with indomethacin (1.5 mg/kg) 45 min before, and 3 h after, an LD_{50} dose of endotoxin. The times indicate the following: −90 min (measurements taken immediately after surgical manipulation); −45 min (baseline measurements for all animals); 0 min (measurements in all groups immediately before endotoxin; in Groups II and III, the values indicate the direct effects of aspirin or indomethacin on cardiac output). The baseline cardiac output (5.0 ± 0.3 l/min) is the mean ± s.e. from all animals at −45 minutes. The other values represent mean values from the animals in each group. Vertical lines show s.e. mean. Values at 120 min and 240 min were not different from values at 60 minutes.

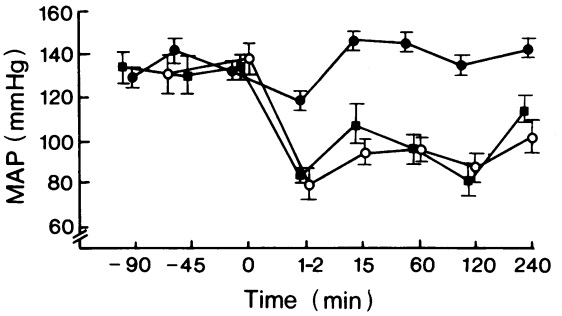

Figure 2 Mean systemic arterial blood pressures (MAP, mmHg) in dogs given only endotoxin (○) or given endotoxin and aspirin (■) or endotoxin and indomethacin (●). Endotoxin was given to each group at time 0 minutes. Vertical lines show s.e. mean. Baseline systemic arterial blood pressure was 130 ± 7 mmHg (mean ± s.e. mean).

Results

Survival

All (100%) animals that received aspirin (Group II) or indomethacin (Group III) survived, compared with only 46% in the endotoxin alone group. The survival rates were significantly ($P < 0.05$) different between the control group (I) and the treated groups (II and III).

Haemodynamic parameters

Cardiac outputs for the three groups are shown in Figure 1. Neither aspirin nor indomethacin had any significant effect on cardiac output. All experimental values after endotoxin were significantly less ($P < 0.05$) than the baseline values except for the 60 min value in Group III. Cardiac output values after endotoxin for Group III were significantly ($P < 0.05$) higher than for Group I. The greatest decrease in cardiac output was observed in all groups 1–2 min following endotoxin. Interestingly, dogs in Group I that died (54%) had significantly ($P < 0.01$) decreased cardiac outputs (1.41 ± 0.09 l/min) only at 1–2 min after the administration of endotoxin in comparison with those that survived (2.39 ± 0.23 l/min). The animals that survived in this group (I) had cardiac outputs that were not different from the aspirin or indomethacin-treated dogs at any time.

Systemic mean arterial pressures (MAP) for all groups are shown in Figure 2. Neither aspirin nor indomethacin alone had any significant effect on MAP. Following endotoxin, mean arterial pressure values in the control (I) group of animals and the aspirin-treated (II) dogs were similar and were all significantly ($P < 0.01$) less than the baseline values. In contrast, after endotoxin the indomethacin-treated dogs (III) had MAP values that were not different from the baseline values and were significantly ($P < 0.01$) greater than the control (I) and aspirin-treated (II) groups. Group I dogs that died had significantly ($P < 0.01$) decreased pressures only at the 1–2 min interval (63 ± 12 mmHg; mean ± s.e.) when compared to those that survived (121 ± 13 mmHg).

Pulmonary arterial pressures (PAP) are shown in Figure 3. Neither aspirin nor indomethacin alone had any significant effect on PAP. Only at the 1–2 min sampling time in the control (endotoxin alone) group did the PAP values increase significantly ($P < 0.05$) from the baseline values. Neither the aspirin (II) nor the indomethacin (III) group exhibited an acute rise in PAP as observed in the control group (I) at 1–2 minutes.

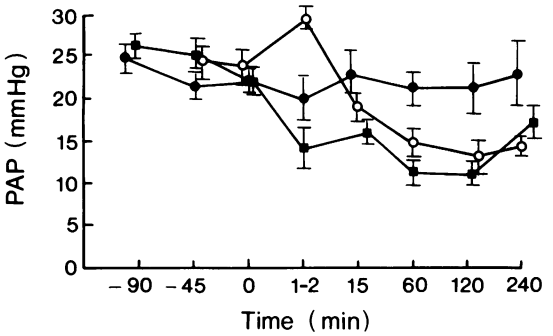

Figure 3 Mean pulmonary arterial pressures (PAP, mmHg) in dogs given only endotoxin (○) or given endotoxin and aspirin (■) or endotoxin and indomethacin (●). Endotoxin was given at time 0. Vertical lines show s.e. mean. Baseline pulmonary arterial pressure was 23 ± 2 mmHg (mean ± s.e. mean).

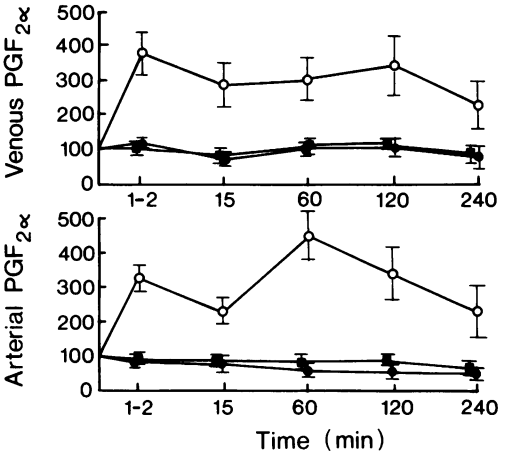

Figure 4 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels following the administration of endotoxin alone (○), of endotoxin following aspirin (■) and of endotoxin following indomethacin (●). Values are % of baseline values. Vertical lines show s.e. mean. Baseline value for venous prostaglandin $F_{2\alpha}$ was 242 ± 31 pg/ml; baseline value for arterial prostaglandin $F_{2\alpha}$ was 148 ± 20 pg/ml (means ± s.e. mean). For details of treatment see text. Treatment with either aspirin or indomethacin completely prevented the increase in circulating prostaglandin $F_{2\alpha}$ that resulted from endotoxin administration.

Platelets, coagulation and complement

The arterial and venous platelet counts were significantly ($P < 0.001$) decreased after endotoxin administration at all sampling times and in all groups; there were no significant differences between the mixed venous and arterial platelet counts in any group for the entire study. Coagulation tests demonstrated similar prolongations of the prothrombin time and activated partial thromboplastin time and there was a decrease in the fibrinogen concentration. Fibrin-split products were increased in all groups from the 60 min sampling time throughout the remainder of the study. There were no significant differences between the groups for these parameters at any sampling time.

Indomethacin alone (III) significantly ($P < 0.01$) decreased the total haemolytic complement levels when compared to the control (I) and the aspirin (II) treated groups. In addition, total haemolytic complement levels were significantly ($P < 0.01$) less at the 15 and 60 min sampling times in the indomethacin-treated (III) group when compared to the control (I) and the aspirin-treated (II) groups.

Prostaglandins

The systemic arterial and mixed venous prostaglandin $F_{2\alpha}$ values are shown in Figure 4. Before the administration of aspirin and indomethacin they were 126 ± 10 and 220 ± 34 pg/ml, respectively. Following

Table 1 Prostaglandin $F_{2\alpha}$ levels in control animals after endotoxin

Time (min)	*0*	*1–2*	*15*	*60*	*120*	*240*
Mixed venous						
Lived	209 ± 69 pg/ml	345 ± 94	233 ± 60	287 ± 136	341 ± 112	241 ± 109
Died	219 ± 60 pg/ml	382 ± 51	268 ± 80	266 ± 84	260 ± 118	218 ± 85
Arterial						
Lived	208 ± 20 pg/ml	315 ± 30	181 ± 35	362 ± 162	344 ± 180	238 ± 171
Died	209 ± 59 pg/ml	277 ± 82	201 ± 45	463 ± 200	242 ± 149	182 ± 50

Values are % of baseline values (mean with s.e. mean)
No significant differences noted between those animals that lived or died.

aspirin or indomethacin the arterial prostaglandin $F_{2\alpha}$ levels were 183 ± 30 pg/ml and 159 ± 20 pg/ml, and the mixed venous prostaglandin $F_{2\alpha}$ levels were 284 ± 30 and 213 ± 46 pg/ml, respectively.

The arterial and mixed venous prostaglandin $F_{2\alpha}$ levels were significantly ($P < 0.02$, at all sampling times) increased by endotoxin only in the control group (Figure 4), but there were no significant differences in either the arterial or the mixed venous prostaglandin $F_{2\alpha}$ concentrations between those animals that lived or died (see Table 1). The greatest increase in the mixed venous prostaglandin $F_{2\alpha}$ level was within 1–2 min after the injection of endotoxin whereas the peak of the arterial prostaglandin values was at 60 minutes.

Discussion

The important findings in this study are: (i) Therapeutic rather than pharmacological, doses of aspirin or indomethacin are equally effective in improving survival during endotoxin shock. (ii) That prostaglandin levels were elevated within 1–2 min after the injection of endotoxin and that this coincided with the first haemodynamic changes. (iii) Aspirin and indomethacin afforded significantly different degrees of attenuation of the haemodynamic events even though both were equally effective inhibitors of the increase in circulating prostaglandin $F_{2\alpha}$ evoked by endotoxin. (iv) That aspirin and indomethacin did not modify the thrombocytopaenia or the coagulation changes during endotoxin shock. (v) Animals that died in the control group did not have significantly higher levels of circulating prostaglandin $F_{2\alpha}$ than those animals that survived.

All previously reported studies (Northover & Subramanian, 1962; Hinshaw *et al.*, 1967; Parratt & Sturgess, 1974; 1975a,b,c) using aspirin or indomethacin-treatment employed varying doses (aspirin 20–200 mg/kg, indomethacin 10–20 mg/kg) given at various times (30 min before to 2 h after endotoxin administration). Pharmacological doses of these drugs have a variety of effects as described earlier. Since aspirin (10 mg/kg) and indomethacin (1.5 mg/kg) have been shown to be effective inhibitors of the synthesis of prostaglandins as early as 1 h after oral administration (Kocsis, Hernandovich, Silver, Smith & Ingerman, 1973), these lower doses were selected in the present study in the hope that they would be more specific inhibitors of the synthesis of prostaglandins (Flower, 1974). The times chosen for these doses (4 h apart) were based on the fact that the half-life of intravenously administered indomethacin in the dog is 3.6 h (Hucker, Zacchei, Cox, Broadie & Cantwell, 1966).

Treatment of endotoxin shock with anti-inflammatory drugs was first introduced by Northover & Subramanian (1962). They showed that the administration of anti-inflammatory drugs either before or after endotoxin resulted in improved circulatory function. Others (Hinshaw *et al.*, 1967; Greenway & Murthy, 1971; Culp *et al.*, 1971; Parratt & Sturgess, 1974; 1975a,b,c; 1976) have demonstrated improved haemodynamic function as well as increased survival by pretreatment with anti-inflammatory drugs. In contrast, Hinshaw *et al.* (1967) and Parratt & Sturgess (1975d) were unable to show increased survival when analgesic-antipyretic drugs were administered only after endotoxin. These studies appeared to indicate that pretreatment was necessary to improve survival. However, one must remember that these authors were investigating different species (cats and dogs) and that the degree of shock was severe (LD_{80}). On the basis of their studies it can not be concluded necessarily that treatment with aspirin or indomethacin in endotoxin shock will not improve survival. In our previous study (Fletcher *et al.*, 1976) we demonstrated that pre- and post-treatment with aspirin or indomethacin were not effective in improving survival in an LD_{100} endotoxin shock baboon model. We concluded that the model was too severe for determining the effects of drugs on survival. The present study was designed to evaluate the effects of pre- and post-treatment in a less severe (LD_{50}) shock model and was a logical step in determining the role of prostaglandins in the pathophysiology of endotoxin shock.

The idea that the arachidonic acid – prostaglandin system participates in the pathophysiology of endotoxin shock is well founded. Thus, several investigators (Anderson *et al.*, 1972; Collier, Herman & Vane, 1973; Kessler *et al.*, 1973) have shown increased levels of prostaglandins following the administration of endotoxin. However, in these studies, prostaglandin levels were determined at arbitrary time intervals which were not necessarily related to changing circulatory function. In addition, the relative degree of haemodynamic stress on the animal in these studies often was not stated. The association of the prostaglandins with events after endotoxin has been best demonstrated by Parratt & Sturgess (1975a) in cats and Anderson *et al.* (1975) in calves in which they showed that the rise in pulmonary artery pressure was closely related to the presence of prostaglandin $F_{2\alpha}$. The calf, however, is exquisitely sensitive to endotoxin (Anderson *et al.*, 1975) and thus may not be a good model to determine pathophysiological mechanisms. Nevertheless, the above studies all support the concept that prostaglandins are elevated in endotoxin shock. Our first study (Fletcher *et al.*, 1976), and the present one, clearly demonstrate that plasma prostaglandin F levels are significantly increased at times when haemodynamic changes are

occurring. The inhibition of prostaglandin synthesis by indomethacin in this study suggests that the prostaglandins may mediate the early haemodynamic events in endotoxin shock.

The most interesting new observation to emerge from the present work was that indomethacin-treatment clearly improved the haemodynamic status after endotoxin more than did aspirin-treatment. In a review of the data from our lethal study in baboons, aspirin did not improve the early haemodynamic changes as well as indomethacin, although both were effective as inhibitors of the increase in the concentrations of prostaglandins. Aspirin and indomethacin may alter vascular smooth muscle sensitivity to circulating vasopressor agents (Aiken & Vane, 1973; Zimmerman, Ryan, Gomer & Kraft, 1973; Jackson, Johnson, Ng, Pye & Hall, 1974; Kadowitz, Joiner & Hyman, 1975). In addition, indomethacin could eliminate the postulated prostaglandin-mediated negative feedback inhibition of sympathetic neural activity (Hedqvist, 1970) as suggested by Parratt & Sturgess (1974). The increased levels of catecholamines present in endotoxin shock (Vick, 1964) might effect a greater vasoconstriction in the presence of indomethacin or aspirin. In contrast to the previous studies, the present study did not show improved circulatory function with aspirin treatment. The explanation may be that the dose used was 4 to 10-fold less and it was given at different times. Nevertheless, aspirin was effective in preventing prostaglandin release.

In this study we could not demonstrate any effect of aspirin or indomethacin on the thrombocytopaenia or in the disorders of coagulation seen in endotoxin shock. It is possible that the stimulus to aggregation of platelets by endotoxin was much greater than any protective effect aspirin or indomethacin might have had at these doses. The reason why indomethacin alone decreased the total haemolytic complement levels in this study is unexplained.

The only haemodynamic observations that correlated ($r = +0.96$) with death in the control animals were those that occurred 1–2 min after endotoxin administration. Although the prostaglandins were elevated at this time, there were no differences between those animals that lived or died. It may be that the prostaglandins are not important for survival, but are elevated coincidentally with the early haemodynamic events. This study also suggests that the early haemodynamic events in the dog may not necessarily be related to survival since the aspirin treatment did not alter these events despite the fact that all the animals survived. In consequence, the primary factor in survival in this model may not be the inhibition of the prostaglandin synthesis, but may be related to other actions of aspirin and indomethacin.

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