

Acute Hypotension due to Platelet Serotonin-Induced Chemoreflexes after Intravenous Injection of Dextran Sulfate in the Rabbit

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SUMMARY. The hypotension and bradycardia observed after intravenous injection of dextran sulfate in rabbits was prevented by prior depletion of circulating platelets, but was not prevented by depletion of the third component of complement or Hageman factor. Dextran sulfate injection caused immediate thrombocytopenia with temporary localization of platelets within lungs. Morphological analysis revealed platelet aggregates in lung capillaries. The platelets had changed shape and were in the process of degranulating. Serotonin and histamine levels in blood increased approximately 5-fold and 7-fold, respectively, after dextran sulfate injection. The cardiovascular events following dextran sulfate injection were mimicked by intravenous serotonin but not by intravenous histamine injection, although a combination of serotonin and histamine reproduced the pattern of blood pressure changes better than did either agent alone. Quantification of platelets trapped in lung revealed that the potential release of serotonin from trapped platelets could account for the rise in plasma serotonin concentration and the hemodynamic changes observed. Both the dextran sulfate-induced cardiovascular effects and serotonin-induced hypotension were markedly diminished by cutting vagus and depressor nerves, and were virtually abolished by carotid ligation in addition to nerve section. These results support the concept that platelet activation within rabbit lungs may cause hypotension via serotonin-induced chemoreflexes. (*Circ Res* 57: 262-277, 1985)

ACUTE hypotension follows intravenous injections of numerous substances. This abrupt decrease in blood pressure may be mediated by direct effects of drugs on resistance vessels. On the other hand, similar events may be initiated by specific antigen recognition leading to activation of mast cells and basophils (anaphylaxis), or they may be induced by non-allergic reactions such as are seen after injection of contrast media (anaphylactoid). The precise chain of events responsible for acute hypotension in the latter situation is not fully understood, although histamine is thought to be an important mediator. In the rabbit model of IgE-anaphylaxis, acute hypotension has been shown to be platelet-dependent (Benveniste et al., 1972; Pinckard et al., 1977) and secondary to release of platelet-activating factor from basophils (McManus et al., 1979). In this study (also in the rabbit), the contributions of different mediator systems activated after intravenous injection of an anionic heparin-like polymer, dextran sulfate, have been analyzed. The results of this study show that a powerful chemoreflex mediated by platelet serotonin was responsible for causing the acute hypotension. A similar sequence of events could contribute to the anaphylactoid response, including acute hypotension, sometimes seen in humans immediately after injection of various sub-

stances, or after the hookup of extracorporeal circulation systems.

Methods

Male New Zealand white rabbits weighing 2-3 kg were used. They were fasted for 24-48 hours before study, although they had free access to water. Arterial pressure measurements were made directly with catheters inserted under local anesthetic into the femoral artery (Intramedic PE50). Infusions and venous pressure measurements were made via a catheter inserted into the femoral vein (Intramedic PE50). Both catheters were introduced to the approximate level of the diaphragm. For infusion into the right ventricle, the venous catheter was advanced until pressure recordings showed appropriate right ventricular pressure changes. In each case, the position of the catheter subsequently was checked by autopsy at the end of the study. Catheters sometimes were inserted the day prior to study. In this case, the catheters were filled with saline, knotted, and buried under the skin, with the skin closed by suture. The following day, access to catheters was obtained by cutting the sutures. For measurements of arterial pressure, rabbits were placed supine on boards and were secured by cords to the limbs. Since the response to dextran sulfate was not affected by barbiturate anesthesia, many of the studies described were performed under a barbiturate general anesthetic (sodium pentobarbitone, 10-15 mg/kg) administered as required via a catheter in the marginal ear vein). Arterial and venous pressure

was measured with a Statham pressure transducer (P37B) and a Statham SP1400 blood pressure monitor. For most studies, the data were expressed as the mean arterial pressure, which was either recorded directly from the monitor or was calculated (diastolic plus one-third of the difference between systolic and diastolic pressure) from the printout that had been calibrated previously.

Depletion of C3 in Rabbits using Cobra Factor

Cobra factor was purified from crude lyophilized venom from *Naja naja* as previously described (Ulevitch and Cochrane, 1977). Purified cobra factor (250 U) was injected intraperitoneally into three rabbits on two occasions (3 days and 2 days before study) as previously described (Ulevitch and Cochrane, 1977). Blood taken on the day of study showed C3 levels, as measured by radial immunodiffusion, to be less than 4% of the levels prior to cobra factor treatment in all animals.

Depletion of Hageman Factor with Goat Antirabbit HF IgG

Hageman factor (HF) was purified from rabbit plasma by a method similar to that described for human HF (Griffin and Cochrane, 1976). Goats were immunized with purified HF in complete Freund's adjuvant every 2–3 weeks for 6 months. The serum collected showed a single precipitation band against purified rabbit HF and rabbit plasma by immunodiffusion analysis. Purification of the IgG was performed as previously described (Wiggins et al., 1979). Rabbits were depleted of circulating HF by an initial intravenous injection of goat antiHF IgG (240 mg) followed by infusion of the IgG at 32 mg/hr, intravenously, for the duration of the experiment. After the initial injection, the rabbit shivered for 2–3 minutes, and, thereafter, had no obvious ill effects from the infusion. No effect of antiHF IgG infusion on circulating levels of Factor XI, prekallikrein, high molecular weight kininogen, or Factor IX was observed. The C3 level fell to approximately 60% of normal within 1 hour of infusion of goat antirabbit HF. This fall in C3 subsequently was shown to be due to aggregates of IgG in the goat antiHF preparation. For these studies, proteins were measured by the following methods: prekallikrein and C3 by radial immunodiffusion. HF, Factor XI and high molecular weight kininogen were measured as procoagulant activity using human HF, Factor XI, and high molecular weight kininogen-deficient plasmas in a kaolin-activated partial thromboplastin time assay (Bouma and Griffin, 1977). Depletion of HF to less than 0.1% of control levels for up to 8 hours was achieved.

⁵¹Cr-Labeling of Platelets

Rabbit platelet-rich plasma from 200 ml of citrated rabbit blood was incubated with ⁵¹Cr (500 μ Ci) for 30 minutes at 37°C. The platelets then were washed twice by centrifugation in platelet-poor citrated plasma. ⁵¹Cr platelets (0.5–1.2 μ Ci) were then injected into each of six rabbits 18 hours prior to study.

Platelet Depletion

Sheep antirabbit platelet antiserum, which was kindly supplied by Dr. C. Cochrane, Department of Immunopathology, Research Institute of Scripps Clinic, was used to deplete rabbits of circulating platelets. Preliminary studies showed that intraperitoneal injection of 5 ml of antiserum resulted in a decrease in circulating platelet levels

to less than 10% of the starting level within 1 hour, and that this extent of platelet depletion lasted for at least 5 hours. Furthermore, in association with the depletion of platelets, there were no significant changes in circulating neutrophil leukocytes or monocytes in the pilot study.

For the platelet-depletion studies described, three rabbits were depleted by intraperitoneal injection of 5 ml of sheep antirabbit platelet antiserum. Three hours after administration of the antiserum, the platelet count had fallen to 3.2, 9.8, and 4.8% of initial values. Three control rabbits that received non-immune serum had platelet counts of 89.4, 109.4, and 110.1% of initial values. Counts for mononuclear cells were 66.7, 97.6, and 75.6% in the platelet-depleted animals compared with 97.0, 76.7, and 160% in the control animals 3 hours after antiserum injection. Neutrophil counts were 79.3, 83.5, and 72.3% in platelet-depleted rabbits compared with 75.4, 120.0, and 268% in control animals. The third control rabbit had a marked increase in both blood mononuclear and neutrophil levels in the 3-hour period after non-immune serum injection. Otherwise, values obtained with the control animals were comparable to those of the experimental rabbits. Dextran sulfate (20 mg/kg intravenously) was administered to both groups of animals 3 hours after they had received either antiplatelet antiserum or non-immune globulin.

Platelet Distribution Study

⁵¹Cr-Labeled platelets (0.9 μ Ci) were injected into each of six rabbits 18 hours before study. Dextran sulfate (DxSO₄, 20 mg/kg in 0.15 M NaCl) was injected into three rabbits, while three rabbits received 0.15 M NaCl alone. One minute after DxSO₄ (or saline) injection, each rabbit received ¹²⁵I-albumin (1 μ Ci) injected in 5 ml 0.15 M NaCl into the inferior vena cava via a femoral vein catheter. After another minute, rabbits were killed by intravenous injection of sodium pentobarbitone (120 mg). Blood was taken before injection of DxSO₄ for counting ⁵¹Cr and platelets. After sacrifice, tissues (lungs, heart, liver, spleen, kidneys, fat, muscle, skin, stomach, and intestine) were removed, weighed, and counted for radioactivity. With the ratio of ⁵¹Cr to ¹²⁵I in the blood and the amount of ¹²⁵I in each organ known, it was possible to calculate the expected ⁵¹Cr in each organ based on that organ's blood content. This calculated value was subtracted from the observed value and corrected for organ weight to give the value in Table 1. The high levels in liver and spleen represent platelet destruction in the 18 hours between ⁵¹Cr-platelet injection and death of the animal, and are similar in the DxSO₄-treated and control animals. The major difference between the groups of animals was in the lung. Since the blood platelet counts before injection of DxSO₄ were known, and corresponded to a ⁵¹Cr count in the blood, it was possible to calculate the excess number of platelets per gram of lung present in the DxSO₄-treated animals. As the total lung weights for each animal were also measured, it was possible to estimate the total number of platelets trapped in the lung 2 minutes after DxSO₄ injection.

Morphological Studies

Lung samples for transmission electron microscopic (TEM) and light microscopic (LM) evaluation were obtained from six New Zealand white rabbits. Rabbits were killed 2, 3, 20, and 30 minutes after DxSO₄ injection. Two rabbits were used as controls. For control and 2- or 3-

minute studies rabbits were anesthetized with sodium pentobarbitone, then given either normal saline (control) or DxSO_4 (20 mg/kg) intravenously. For the 20- and 30-minute studies DxSO_4 was injected prior to anesthesia. Rabbits were killed by sodium pentobarbital injection (170 mg) given intravenously over 30 seconds. The thoracic cavity was immediately exposed. The trachea was cannulated, and the lung was inflated in situ with approximately 50 ml of cold 4% glutaraldehyde buffered with 0.1 M cacodylate, pH 7.3, (300 mOsm/liter). The entire lung was excised and placed in cold 4% buffered glutaraldehyde. Fifteen minutes later random sections (1 mm³) were cut for TEM evaluation, and adjoining tissue was taken for LM histology. The lung pieces for TEM were fixed overnight at 4°C in the buffered glutaraldehyde, then rinsed well with 0.1 M cacodylate buffer. The samples were postfixed in 2% OsO_4 (buffered with 0.1 M cacodylate, pH 7.3) for 1 hr at 4°C, dehydrated in graded alcohols to propylene oxide, infiltrated with epon/araldite:propylene oxide mixture, embedded in epon/araldite resin, and polymerized overnight at 65°C. Ultra-thin sections were cut, stained with 2% uranyl acetate and lead citrate, and examined on a Philips 201 transmission electron microscope.

Plasma Serotonin Measurement

Blood (1.8 ml) was drawn from a femoral arterial catheter and immediately added to 200 μl of acid-citrate-dextrose anticoagulant. After being gently mixed, 1 ml of blood was added to 200 μl of EDTA (4 mg/ml) in a polypropylene conical centrifuge tube and centrifuged on a Beckman microfuge (Beckman Instruments, Inc.). Plasma was removed and stored frozen at -30°C. Measurement of serotonin was performed by the method described by Parbtani and Cameron (1979). This method involves the formation of a fluorophore by serotonin and *O*-phthalaldehyde (OPT) under acid conditions. Fluorescence emitted at 475 nm while exciting at 360 nm was measured on an SF 330 spectrofluorometer (Varian Instruments). This method assumes that the major substance in plasma which will fluoresce under these conditions is serotonin. In control studies, DxSO_4 and histamine did not induce fluorescence, and the spectra for serotonin and plasma were similar, indicating that serotonin was the major substance giving rise to fluorescence under the conditions of study. The recovery of serotonin added back to platelet poor

plasma was 71%. The limit of sensitivity of the assay was 10 ng/ml ($n = 10$). The coefficient of variation was 19% (standard deviation $\times 100$ divided by the mean for 10 measurements).

To confirm that serotonin was present in plasma, we also performed high pressure liquid chromatographic (HPLC) analyses of plasma. These analyses were performed on plasma samples from which the protein had been precipitated with trichloroacetic acid. Twenty microliters of trichloroacetic acid (200 g/100 ml) were added to 200 μl of plasma and allowed to stand in ice for 30 minutes. The sample was centrifuged, and the supernatant was washed with 500 μl of diethyl ether. One hundred microliters of sample were applied to a 30 cm \times 4 mm MCH 10 reverse phase column (Varian Instruments) and run on an LC 5000 liquid chromatograph (Varian Instruments). The effluent was monitored at 210 nm with a variable wavelength UV 50 detector (Varian Instruments). The buffer consisted of 12% acetonitrile containing 0.1% trifluoroacetic acid throughout. The retention time for serotonin was 9.5 minutes.

Plasma Histamine Measurement

Blood was drawn into anti-coagulant, centrifuged, separated as described for serotonin, and then stored at -70°C for assay. A specific histamine assay using [³H]-*S*-adenosyl methionine to methylate histamine in the presence of histamine *N*-methyltransferase, as described by Dyer et al. (1982), was used. Briefly, the isotope, enzyme, and sample were incubated at 37°C for 1.5 hours. The reaction was stopped by the addition of perchloric acid. After centrifugation, the supernatant was extracted with chloroform. The extracted material was dried under nitrogen, then reconstituted in chloroform and spiked with unlabeled methyl histamine. This mixture was spotted onto thin layer plates and run in a 95:10 (vol/vol) acetone:ammonium hydroxide system. The plates then were dried in an oven, sprayed with *O*-phthalaldehyde, redried, and the spots were visualized by ultraviolet light. The spots were scraped off into a scintillation vial, extracted with 0.1 N HCl, and, after addition of scintillant, were counted on a β -counter. All samples were done in duplicate and were compared to a standard curve constructed by adding known amounts of histamine to dialyzed rabbit plasma. The coefficient of variation of the assay was 32%, and the limit of sensitivity was 200 pg/ml.

Nerve Section and Carotid Ligation

In more than 20 rabbits studied in which femoral artery catheters had been placed under local anesthetic (2% lidocaine), and in more than 30 rabbits that received sodium pentobarbitone anesthesia, we noted no difference in cardiovascular response to serotonin, histamine, or DxSO_4 injection. Therefore, to minimize discomfort to animals, we studied the rabbits under sodium pentobarbitone general anesthesia (10–15 mg/kg, intravenously, as required). Femoral arterial and venous catheters were placed in each rabbit to approximately the level of the diaphragm. A midline incision was made in the skin from the jaw to the manubrium, and the pretracheal fascia was split in the midline. Muscles lying in front of the trachea were cut, and a tracheal cannula (5-cm portion of a 2-ml plastic pipette) was secured with a silk thread. A longitudinal incision through the strap muscles was made 0.5 cm from the midline according to the method of Kremer et al. (1933). The carotid artery, vagus, and depressor nerves were identified and isolated by gentle blunt dissection.

TABLE 1
⁵¹Cr-Platelet Accumulation in Various Organs 1 Minute after Injection of DxSO_4 or Saline (control)

	⁵¹ Cr in excess of blood contribution (counts/min per g tissue) ($n = 3$)	
	Control	Dextran sulfate
Lung	1,307 \pm 351	5,572 \pm 863*
Heart	476 \pm 109	223 \pm 124
Liver	2,438 \pm 594	2,244 \pm 943
Spleen	43,058 \pm 10,643	36,562 \pm 9,850
Kidney	85 \pm 45	144 \pm 73
Skin	320 \pm 163	475 \pm 235
Muscle	93 \pm 62	27 \pm 10
Fat	15 \pm 8	11 \pm 4
Stomach	86 \pm 54	34 \pm 18
Intestine	25 \pm 21	50 \pm 38

Results are expressed as mean \pm SEM.

* $P < 0.05$.

For the study described (Figs. 12 and 13), both vagus and depressor nerves were cut on both sides in three rabbits. In the control animals, the nerves were identified and isolated but were not cut (sham-operated). After nerve section, there was an increase in mean arterial pressure of approximately 5–15%, and respiration became slower and deeper. A 30-minute interval was allowed between nerve section and $DxSO_4$ injections. At the end of this time, the mean arterial pressure was still approximately 5–15% above pre-section levels. In one rabbit, the response to bradykinin was measured before and after nerve section. The change in mean arterial pressure to 40, 200, and 1000 ng bradykinin were 0, 7, and 19 mm Hg before section and 0, 15, and 40 mm Hg after nerve section. Thus, the response to bradykinin remained intact after nerve section.

In the experiment in which both internal carotid arteries were tied (Figs. 14 and 15), the rabbits first were treated by nerve section, as described above. Clamping of one or both internal carotid arteries produced an immediate increase in mean arterial pressure of about 30%. If we allowed an interval of 60 minutes, mean arterial pressure fell to approximately 10% above the initial levels. For the experiment described in Figures 14 and 15, 60-minutes elapsed between carotid ligation and rechallenge with serotonin and the $DxSO_4$ injection.

For the nitroprusside/bradykinin study (Fig. 19), femoral arterial catheters were placed in the rabbits, under barbiturate anesthetic. The drugs to be tested (serotonin 22 μ g, bradykinin 1 μ g, or sodium nitroprusside 50 μ g) were injected as a bolus into an ear vein in 0.5 ml of 0.15 M NaCl. Each drug was tested in duplicate at each stage of the experiment in four rabbits. After the control studies, the depressor and vagus nerves were in turn identified and cut as described above. After 10 minutes, the drugs were tested. Both carotid arteries then were tied. After 15 minutes, the drugs were tested again.

Statistical Analysis

Paired *t*-test was used for this analysis.

Miscellaneous

Human deficient plasmas were purchased from George King Biomedical, Inc. Angiotensin-converting enzyme inhibitor (SQ 20881) was kindly provided by Dr. W.B. McDowell, of the Squibb Institute of Medical Research. Dextran sulfate (mol wt 500,000) was purchased from Pharmacia, Fine Chemicals, and was dissolved in sterile 0.15 M NaCl; serotonin creatine sulfate, histamine dihydrochloride, and bradykinin were purchased from Sigma Chemical Co.; chromium-51 was purchased from New England Nuclear; cobra venom was purchased from Biologicals Unlimited, Inc.; and cimetidine (Tagamet) came from Smith, Kline and French Laboratories. Methysergide maleate was kindly provided by Ms. K.D. Roskaz, Sandoz Inc. Ketanserin tartrate (kindly provided by Dr. Clinton Webb, Department of Physiology, University of Michigan) came from Janssen Pharmaceutica (Janssen R & D Inc.) and sodium nitroprusside was from Abbott Laboratories.

Results

Pathophysiological Consequences of Dextran Sulfate Injection

Dextran sulfate (mol wt 500,000) injection (20 mg/kg, intravenously, given over a 20-second period) had no immediate adverse effect on the rabbit. After a 45- to 60-second interval, there was a slight

rise in systemic arterial pressure, followed immediately by a profound decrease in arterial pressure of about 30–40% accompanied by bradycardia (Fig. 1). This hypotension was associated with transient irregular respiration and, in some rabbits, mild wheezing, defecation, or micturition. Large waves of peristaltic bowel movements were visible through the abdominal wall. These changes lasted approximately 1 minute. Over the next 5–15 minutes, the arterial pressure returned toward the preinjection values, but never attained those values, remaining at 90–95%. There were no apparent aftereffects, with the exception of a severe tendency to bleed. At lower doses of injected $DxSO_4$, some rabbits became hypotensive and some did not. The response tended to be all-or-nothing. Increasing the amount of $DxSO_4$ injected did not increase the hypotension.

Blood Cell Changes following $DxSO_4$ Injection

Platelets

In three separate experiments, intravenous injection of $DxSO_4$ (20 mg/kg) was immediately followed by disappearance of between 50 and 90% of the platelets from blood within 5 minutes of $DxSO_4$ injection. By 60 minutes, platelet counts were returning toward preinjection levels. This is illustrated in Figure 2, in which ^{51}Cr platelets were used in a group of three rabbits. Of note is the fact that the level of ^{51}Cr platelets at 4 hours is approximately the same as the level that would be predicted from the slope of disappearance of ^{51}Cr if no $DxSO_4$ had been injected. Similar results were found when platelets were counted directly in other experiments (data not shown). This implies that platelets were sequestered somewhere in the animal following $DxSO_4$ injection, but that they returned to the circulation and were not removed and destroyed by

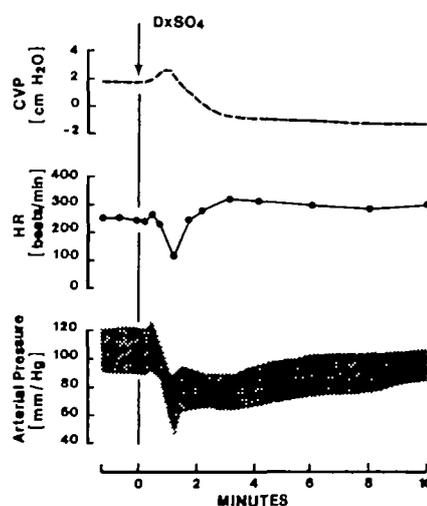


FIGURE 1. Representative cardiovascular changes following $DxSO_4$ injection (20 mg/kg) in a rabbit. There is an initial lag period of 30–45 seconds followed by bradycardia, hypotension, and a rise in the central venous pressure (CVP). By 2 minutes, a tachycardia supervenes, accompanied by a fall in CVP. The arterial pressure gradually rises toward normal over a 15- to 20-minute period.

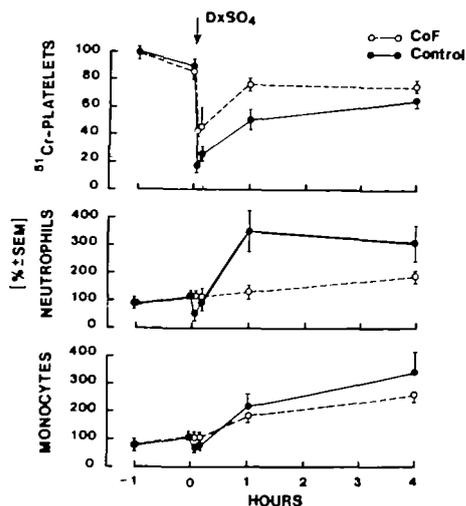


FIGURE 2. Comparison of changes in blood platelets (^{51}Cr platelets) and neutrophils and monocytes (counted in a counting chamber) from controls and from rabbits that had been injected with DxSO_4 (20 mg/kg) which had been depleted of C3 with cobra factor (CoF) ($n = 3$ rabbits in each group). During the hour preceding DxSO_4 injection, there was a 4–5% fall in circulating ^{51}Cr platelets. The responses were not statistically significantly different ($P = 0.13$ at 5 minutes) except at the 4-hour point ($P = 0.03$). Both groups of animals showed an immediate unequivocal decrease in blood platelets following DxSO_4 injection. By 4 hours after DxSO_4 injection, the ^{51}Cr platelets had returned to the circulation at approximately the predicted level, indicating that extensive platelet lysis had not occurred. The blood neutrophil count fell to 40% of control following DxSO_4 injection, but by 1 hour after DxSO_4 injection had rebounded to 340% of initial values. These changes were prevented by cobra factor treatment ($P < 0.05$ at both 5 minutes and 1 hour; $P > 0.05$ at 4 hours), indicating that C3 was necessary for the changes in blood neutrophil count. The apparent fall in blood monocyte count to 46% of control at 5 minutes was prevented by prior treatment with cobra factor. None of the differences were statistically significant.

the spleen. In other words, the thrombocytopenia was not due to thrombolysis.

In a parallel experiment in which three rabbits were depleted of C3 by prior injection of cobra factor (see Methods), DxSO_4 injection induced a fall of ^{51}Cr platelets which was not statistically significantly different from that seen in the control rabbits over the first hour (Fig. 2). These results suggest that the thrombocytopenia was not completely C3-dependent, although complement activation may have contributed to thrombocytopenia.

Neutrophils

Within 5 minutes of DxSO_4 injection, there was a fall in neutrophil count to 36% of the starting values (Fig. 2). This was followed by a neutrophilia of 340% by 60 minutes which persisted until 4 hours after DxSO_4 injection (Fig. 2). These changes in neutrophil count were prevented by prior depletion of C3 by cobra factor injection (Fig. 2). These data suggest that complement activation occurred following DxSO_4 injection and was responsible for the initial neutropenia followed by a rebound neutrophilia.

Mononuclear Cells

The mononuclear cell count (monocytes and lymphocytes) fell by 46% 5 minutes after DxSO_4 injection. This fall was also prevented by prior cobra factor treatment (Fig. 2).

Basophils

Basophils were counted using a metachromatic stain (Meng et al., 1973). Two minutes after DxSO_4 injection, the metachromatically stained cells in blood had fallen to 18% of initial levels. By 30 minutes, the level had increased to 84% of starting values (data not shown).

Hematocrit

There was a small rise in hematocrit of 2–3% 5 minutes after DxSO_4 injection (data not shown).

Effect of Depletion of C3 upon DxSO_4 -Induced Hypotension

DxSO_4 is known to activate complement. Therefore, to determine whether the complement system was playing a major role in the hemodynamic changes seen after DxSO_4 injection, three rabbits were depleted of C3 with cobra factor to less than 4% of control levels (see Methods and Fig. 2). After DxSO_4 injection, both cobra factor-treated and control rabbits exhibited brisk hypotensive responses which were not significantly different (Fig. 3). There was therefore no evidence that C3 was essential to mediation of the cardiovascular response to DxSO_4 .

Effect of Depletion of Hageman Factor with Goat Anti-HF IgG

DxSO_4 is known to activate the HF system, and activation of HF in plasma by DxSO_4 leads to bradykinin generation (Kluft, 1978). Since bradykinin injection in the rabbit can cause bradycardia and hypotension similar to that seen after DxSO_4 administration, studies were performed to examine the possibility that HF-mediated bradykinin formation was responsible for the observed cardiovascular changes. The rabbits were depleted of HF to less than 0.1% of initial circulating HF level by infusion of goat anti HF IgG (see Methods). After DxSO_4 injection, there was a brisk hypotensive response in both rabbits associated with bradycardia similar to that seen in a rabbit receiving control non-immune IgG (Fig. 4). This implies that the HF was not essential for the cardiovascular response to DxSO_4 injection.

Effect of Inhibition of Angiotensin-Converting Enzyme on DxSO_4 -Induced Hypotension

To investigate further whether bradykinin generated by a different mechanism could be mediating cardiovascular events following DxSO_4 injection, the effect of prevention of bradykinin destruction by

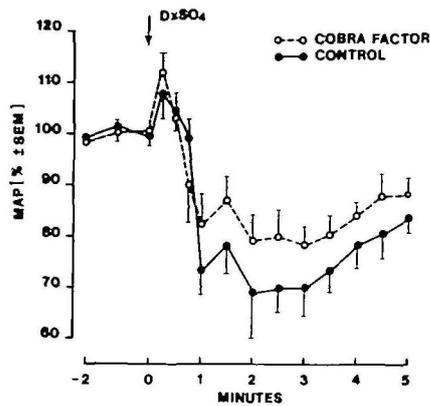


FIGURE 3. Changes in mean arterial pressure (MAP) following $DxSO_4$ injection (20 mg/kg) in rabbits depleted of C3 with cobra factor ($n = 3$) and in control animals ($n = 3$). After $DxSO_4$ injection, there was an initial rise in MAP followed by a fall to approximately 70 and 80% in both the control and cobra factor-treated animals, respectively. These differences were not significantly different. The cardiovascular changes in response to $DxSO_4$ were therefore not prevented by prior depletion of C3.

inhibition of kinase II (the angiotensin-converting enzyme) was studied. SQ 20881 (5 mg/kg) was injected into three rabbits 10 minutes before $DxSO_4$ injection. (In parallel studies, this dose of SQ 20881 potentiated bradykinin-induced hypotension more than 10-fold.) The results are shown in Figure 5. SQ 20881 actually inhibited the $DxSO_4$ -induced hypotension and bradycardia. The reason for this is not known. However, the result is the opposite of that which would be expected if bradykinin were a mediator of the $DxSO_4$ -induced cardiovascular effects. These data make it very unlikely that kinin generation was contributing to $DxSO_4$ -induced hypotension and bradycardia in these experiments.

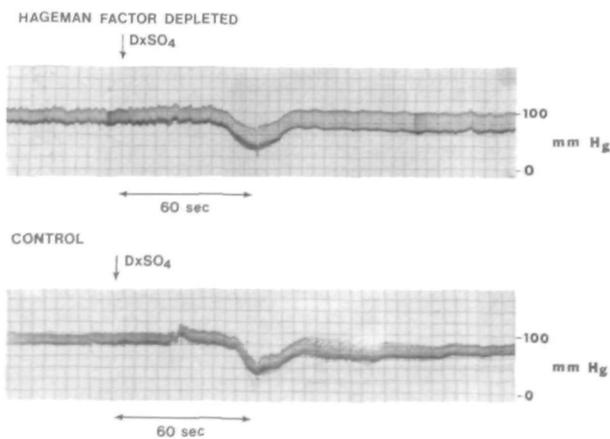


FIGURE 4. Arterial pressure recordings from a Hageman factor (HF)-depleted rabbit (see Methods) and a control rabbit. In both cases, the hypotension and bradycardia began approximately 45 seconds after $DxSO_4$ injection. These findings show that HF was not required for the initial fall in MAP.

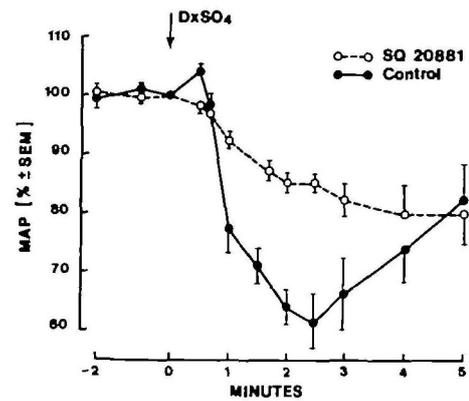


FIGURE 5. Comparison of arterial pressure changes in control animals and in rabbits treated with angiotensin-converting enzyme inhibitor (SQ 20881) ($n = 3$ for both groups). The SQ 20881 prevented rather than enhanced the initial fall in MAP seen following $DxSO_4$ injection.

Effect of Depletion of Blood Platelets

Depletion of blood platelets to less than 10% of normal with antiplatelet serum (see Methods) prevented the hypotension and bradycardia in response to $DxSO_4$ injection (Fig. 6). These data imply that platelets play an essential role in mediating the response.

Distribution of Blood Platelets after $DxSO_4$ Injection

^{51}Cr -Labeled platelets were used to determine what had happened to the platelets which had disappeared from the blood. Rabbits were injected with a blood marker (^{125}I -albumin) 1 minute after $DxSO_4$ injection and were killed 1 minute later. From the ratio of ^{125}I -albumin to ^{51}Cr platelets in the blood and organs, it was possible to calculate the ^{51}Cr present in each organ contributed by blood alone (see Methods). This calculated amount of ^{51}Cr was subtracted from the measured ^{51}Cr in each organ to give the values in Table 1. The principal difference between the control and $DxSO_4$ -treated animals was the excess ^{51}Cr in the lung 2 minutes after $DxSO_4$ injection. The high levels of ^{51}Cr in the spleen and liver in both control and $DxSO_4$ animals reflect

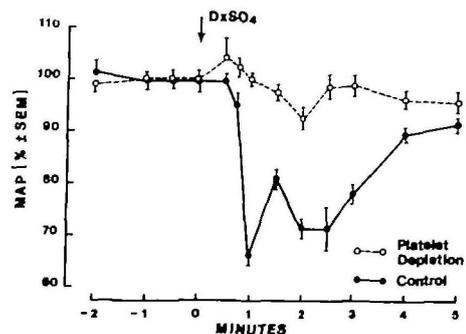


FIGURE 6. The effect of platelet depletion (see Methods) on the cardiovascular response to $DxSO_4$ injection ($n = 3$ for both groups). Depletion of platelets prevented the initial fall in MAP.

platelet breakdown in the 18 hours during which ^{51}Cr -platelets were circulating prior to study.

Quantification of Platelets in the Lung 2 Minutes after DxSO_4 Injection

The actual number of platelets trapped in the lung after DxSO_4 injection was calculated from (1) the mean difference in ^{51}Cr counts between experimental and control rabbits (4265 counts/min ^{51}Cr /mg lung tissue), (2) the mean total lung weight (11.0 g), and (3) the mean blood platelet count and corresponding ^{51}Cr level before DxSO_4 injection (3.8×10^8 platelets/ml = 2558 counts/min ^{51}Cr /ml). Approximately 7.0×10^9 more platelets were estimated to be in the DxSO_4 -treated rabbit lungs than in control rabbit lungs. This number corresponds to only 11% of the total number of circulating platelets. Since 50–90% of the platelets disappeared from the circulation after DxSO_4 injection in three separate experiments and the lung seemed to be the only site of platelet accumulation after DxSO_4 injection, this leaves a large number of platelets unaccounted for. This could be explained by platelet lysis, but such an explanation does not seem likely in view of the data shown in Figure 2, where the level of ^{51}Cr platelets 4 hours after DxSO_4 injection was close to the predicted value. In other words, there was no evidence for net permanent loss of platelets. An alternative explanation is that increased lung permeability occurs after DxSO_4 injection. The fact that increased permeability did occur was shown by finding increased ^{125}I -albumin in lungs (6387 ± 1856 counts/min per g in DxSO_4 -treated group compared with 1418 ± 423 counts/min per g in control group, $P < 0.05$) in addition to intraalveolar hemorrhage immediately after DxSO_4 injection (Fig. 7). If ^{125}I -albumin was leaking out of the vascular compartment into the lungs after DxSO_4 injection, then the use of ^{125}I -albumin as a blood marker would result in falsely low calculated values for platelet trapping in the lung. The calculation that 7×10^9 platelets

were trapped in the lung therefore is probably an underestimate.

Ultrastructural Analysis of Lungs after DxSO_4 Injection

Lung tissue was examined ultrastructurally from a control rabbit and rabbits sacrificed 2, 3, and 30 minutes after DxSO_4 injection (see Methods).

In normal rabbit lung (Fig. 7A), platelets were rarely seen, and, when present, were rod-shaped and contained numerous dense granules. Only an occasional neutrophil was seen.

Within 2 or 3 minutes of DxSO_4 injection, many changes had occurred (Fig. 7, B and C): The lumina of some capillaries were filled with granular amorphous material which was probably DxSO_4 or a plasma- DxSO_4 mixture. Within this amorphous material were many platelets which were irregularly shaped, contained vacuolar structures, and had fewer dense granules than control platelets. Higher magnification revealed what appeared to be granules in the process of passing into vacuolar structures that were in direct communication with the exterior of the cell (Fig. 8). In addition to the platelet changes, other types of cells had appeared in the lung (Fig. 7, B and C). These were (1) numerous neutrophils that were present both in capillaries which did and which did not contain amorphous material, (2) numerous eosinophils, and (3) cells with multilobed nuclei whose cytoplasm contained patchy dense granule-like material. These cells often appeared to lie beside eosinophils (Fig. 7, B and C) within lung capillaries, and are probably degranulated basophils. Erythrocytes were present in alveolar spaces (Fig. 7C).

Thirty minutes after DxSO_4 injection (Fig. 7D), most of the changes noted at 2 and 3 minutes had disappeared. No erythrocytes were seen in alveolae, and no amorphous material was present in capillaries. Neutrophils still were present, but the eosinophils and basophils had disappeared. Many more

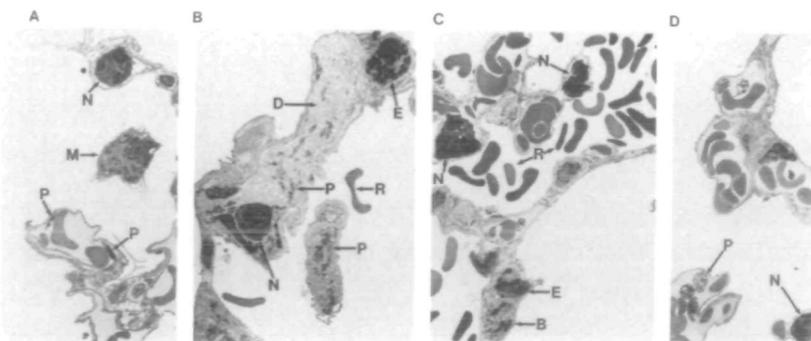


FIGURE 7. Ultrastructural analysis of rabbit lung removed before (panel A), 2–3 minutes after (panels B and C), and 30 minutes after (panel D) DxSO_4 injection. In panel A, a few rod-shaped platelets (P) containing dense granules are seen, with only an occasional intravascular neutrophil (N) and intralveolar macrophage (M). Immediately after DxSO_4 injection (panels B and C), capillaries are filled with amorphous material (D) which is probably DxSO_4 . There are numerous irregularly shaped platelets (P) within the amorphous material. There are also numerous neutrophils (N), eosinophils (E), and cells which may be degranulated basophils (B). In addition, red cells (R) have extravasated into the alveolar space. By 30 minutes after DxSO_4 injection (panel D), the platelets have not yet returned to their original shape and are still plentiful in the lung capillaries, but otherwise the lung architecture appears normal. 1200 \times .

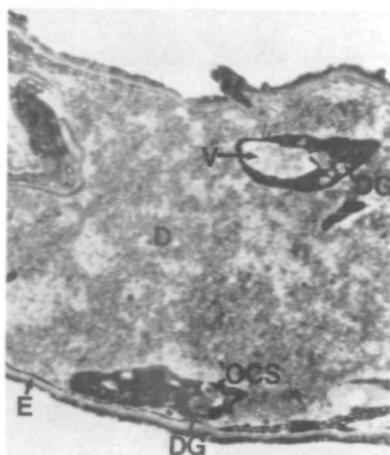


FIGURE 8. Ultrastructural analysis of a single capillary containing granular amorphous material D (probably $DxSO_4$). Two platelets are seen. The upper platelet contains a large vacuolar structure (V) and a dense granule (DG) which appears to be in the process of fusing with a smaller vacuole. The lower platelet appears to be attached to endothelium (E) by spike-like projections. A dense granule (DG) appears to be in the process of dissolving within a vacuole which is in continuity with the outside of the cell ("open canalicular system," OCS). 11,250 \times .

platelets were present than in normal lung. Some were large (up to half the diameter of an erythrocyte). They were rounded, contained many vacuole-like structures, and few or no dense granules.

These observations are compatible with the concept that $DxSO_4$ accumulates in lung capillaries after intravenous injection and remains there for at least 3 minutes. Platelets became trapped and activated in these capillaries. Dense granular contents were released from platelets via an open canalicular system, as has previously been described (White, 1974). Other circulating cells (neutrophils, eosinophils, and probably also basophils) accumulate in the lung capillaries. There was a leak of erythrocytes out of capillaries into alveolar space. By 30 minutes after $DxSO_4$ injection, the lung anatomy was returning toward normal with platelets still showing signs of having been activated.

Amount of Histamine and Serotonin Released from Platelets in Lung

Dense granules from rabbit platelets contain histamine and serotonin. The amounts of serotonin and histamine present in rabbit platelets have been measured previously [serotonin 38.5, and histamine 26.2 nmol/mg protein, respectively (Da Prada et al., 1981)]. With this information, and knowing the number of platelets trapped in the lung 2 minutes after $DxSO_4$ injection from data described above (7×10^9 platelets), and the number of rabbit platelets constituting 1 mg platelet protein [2.2×10^8 platelets/mg protein (personal observation)], it was possible to estimate that approximately 100 μ g histamine and 217 μ g serotonin could have been released from these platelets. For the reasons discussed

above, the calculated number of platelets trapped in lung may well be an underestimate. On the other hand, not all dense granules were extruded from the trapped platelets. Nevertheless, with these approximate amounts of histamine and serotonin in mind, further studies were performed to determine whether these agents could have caused $DxSO_4$ -induced hypotension.

Effect of Histamine on the Arterial Pressure of the Rabbit

A biphasic arterial pressure response following bolus histamine injection (100–300 μ g, intravenously) was obtained, as has been described previously (Brimblecombe et al., 1974). An initial marked hypertensive phase was followed within 30 seconds of histamine injection by a more prolonged hypotensive phase lasting about 4–5 minutes (data not shown). As has also been reported (Carroll et al., 1974), the initial hypertensive response could be

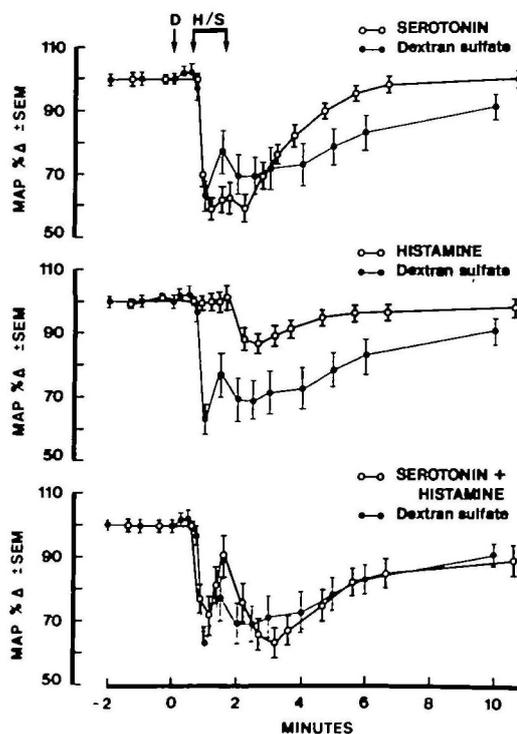


FIGURE 9. In each panel (top, middle, and lower) the blood pressure response following intravenous injection of dextran sulfate (D) in seven rabbits is plotted (●). In the top panel, the intravenous infusion of serotonin (200 μ g over 1 minute) is superimposed upon the $DxSO_4$ response in the same animals. In the middle panel, the intravenous infusion of histamine (100 μ g over 1 minute) is superimposed upon the $DxSO_4$ response in the same animals. In the bottom panel, the intravenous infusion of a mixture of serotonin and histamine (200 μ g and 100 μ g, respectively, infused over 1 minute) is superimposed upon the $DxSO_4$ response in the same animals. These data show that the infusion of serotonin and histamine together more closely mimicked the blood pressure changes following $DxSO_4$ injection than the infusion of either agent alone. Infusion of serotonin caused immediate marked hypotension (together with bradycardia—not shown in Fig. 9), whereas infusion of histamine did not cause hypotension until after the infusion had stopped.

blocked by the H₁ antagonist, chlorpheniramine (20 mg/kg) (data not shown), and the hypotensive response could be blocked by the H₂ antagonist, cimetidine (10 mg/kg) (data not shown). However, this pattern of arterial pressure response following bolus histamine injection bore no resemblance to the DxSO₄-induced hypotensive response. Infusion of 50–200 μg histamine over a 1-minute period instead of as a bolus caused mild hypertension or no change in arterial pressure during the infusion. When the infusion was stopped, mild hypotension occurred (Fig. 9, middle panel). These arterial pressure changes did not resemble the changes seen after DxSO₄ injection. Furthermore, in other studies, infusion or injection into the right ventricle (to bypass the sinus node) also caused hypertension followed by mild hypotension, as described above. Thus it was not possible to reproduce the DxSO₄-induced hypotension and bradycardia by histamine injection over a wide dose range, no matter how the histamine was administered.

Effect of Serotonin upon the Systemic Arterial Pressure of the Rabbit

Serotonin injection (22 μg, intravenously) caused immediate bradycardia and hypotension with a fall in arterial pressure of about 30% (Fig. 10, panels B and C). This pattern of hypotension and bradycardia was very similar to that caused by DxSO₄ injection. This effect could be inhibited by prior injections of cimetidine (60 mg/kg) (Fig. 10, panels D and E). Intraarterial injection of serotonin (22 μg) reproducibly produced only a small rise in arterial pressure (Fig. 10, panel A). Furthermore, injection of serotonin (200 μg) alone as a 1-minute infusion immediately induced hypotension and bradycardia similar to that seen after injection of DxSO₄ (Fig. 9, top panel), although the duration of the hypotension was slightly less than that observed after DxSO₄. However, mixing serotonin (200 μg) together with histamine (100 μg) produced a more prolonged hypotensive effect, which was almost identical to that observed after DxSO₄ injection (Fig. 9, bottom panel). These findings implied that the major agent responsible for hypotension and bradycardia following DxSO₄ injection was serotonin, although histamine probably reduced the immediate hypotension and enhanced the second phase of hypotension. We therefore performed further studies to determine whether serotonin could be the major agent responsible for DxSO₄-induced hypotension and bradycardia.

Plasma Serotonin Concentration after DxSO₄ Injection

Blood was removed from an intraarterial catheter at 30-second intervals after DxSO₄ injection (Methods). The plasma was immediately separated and frozen for serotonin assay (Methods). The results are shown in Figure 11. Immediately after DxSO₄ injection, the level of plasma serotonin rose 5-fold

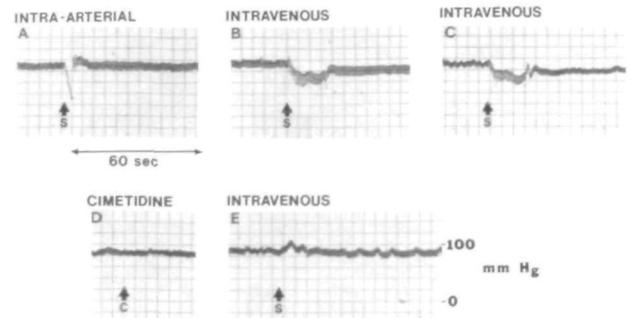


FIGURE 10. Effect of serotonin (S) injection (22 μg) on the cardiovascular system of a rabbit. Intraarterial injection (panel A) caused a small rise in arterial pressure (the downspike is an injection artifact caused by injecting through the catheter used to monitor arterial pressure). Intravenous injection of serotonin caused bradycardia and hypotension (panel B) which was reproducible (panel C). Cimetidine (C) injection (60 mg/kg) intravenously had no effect on heart rate or blood pressure (panel D), but it changed the cardiovascular effects normally seen after intravenous injection of serotonin (hypotension and bradycardia) to a small rise in pressure similar to that seen after intraarterial injection (panel E).

and then returned rapidly to baseline values within 3 minutes. Injection of serotonin (22 μg) doubled the plasma serotonin concentration (Fig. 11). The mean baseline serotonin concentration was 552 ± 51 ng/ml. From these data it can be estimated that approximately 100–300 μg of serotonin (equivalent to the content of $3-9 \times 10^9$ platelets) were released after DxSO₄ injection (assuming a blood volume of 180 ml in a 2.5-kg rabbit). This is in good agreement with the value of 217 μg of serotonin potentially released from trapped platelets in the lung (see above).

Since the method used for measuring plasma serotonin employed fluorescence of OPT in acidified

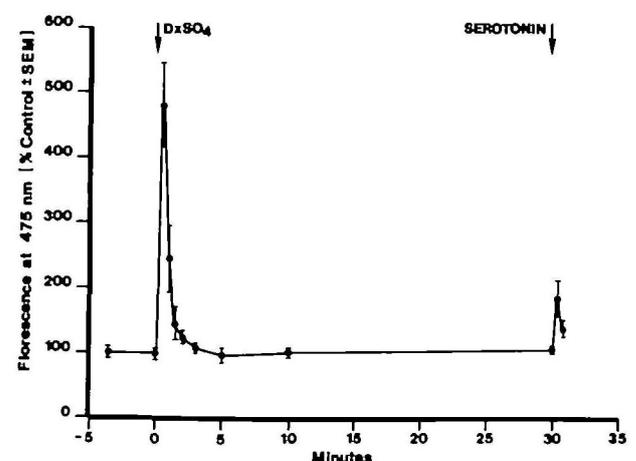


FIGURE 11. Plasma serotonin measured by fluorescence at 475 nm (see Methods) increased nearly 5-fold in arterial blood 1 minute after DxSO₄ injection ($n = 5$). Within 5 minutes, the excess serotonin had disappeared from plasma. Injection of serotonin intravenously (22 μg, the amount required to produce hypotension and bradycardia) caused a doubling of the measured fluorescence at 475 nm.

plasma, it was not entirely specific for serotonin. To confirm that serotonin had been released into plasma after DxSO_4 injection, we analyzed samples (in the absence of OPT) by high pressure liquid chromatography (see Methods). Under these conditions, a peak of fluorescence at 210 nm was observed in plasma removed immediately after, but not before, DxSO_4 injection. It corresponded exactly to the peak observed when serotonin alone was analyzed on the same system with respect to both peak size and retention time (data not shown). These data confirm that serotonin appeared in blood immediately after DxSO_4 injections and that this assay was quantitative.

Plasma Histamine Concentration after DxSO_4 Injection

Blood was removed at intervals after DxSO_4 injection, and the plasma was separated and assayed for histamine, as described in Methods. The normal histamine level in rabbit plasma was 8.1 ± 2.9 ng/ml ($n = 7$). Within 0.5–1 minute after injection of DxSO_4 the level had risen 7-fold (Fig. 12). The histamine level returned to normal within 5 minutes of DxSO_4 injection. Injection of histamine (100 μg), intravenously, in two rabbits yielded values of 333.6 and 342.3 ng/ml at 20 seconds and 6.5 and 24.0 ng/ml at 60 seconds after histamine injection.

These results confirm that histamine was released after DxSO_4 injection. They emphasize how transient was the rise of measurable histamine in blood. However, the amount of histamine required to produce hemodynamic effects of the magnitude seen after DxSO_4 injection, produced a peak of histamine approximately 6-fold higher than that seen after DxSO_4 injection. This is in contrast to the results with serotonin, described above (Fig. 11), where the amount of serotonin required to produce hemodynamic effects comparable to those seen after DxSO_4 injection gave a peak amount of serotonin approximately 6-fold lower than that seen after DxSO_4

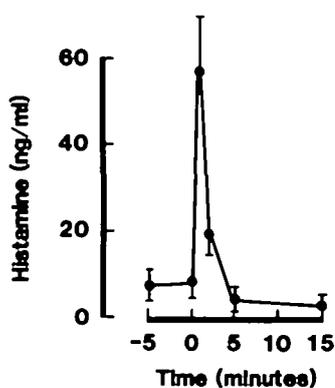


FIGURE 12. Plasma histamine measured by formation of [^3H -methyl] histamine by histamine *N*-methyltransferase (see Methods). The histamine concentration in blood increased 7-fold within 1 minute after DxSO_4 injection ($n = 4$). Within 5 minutes, the histamine concentration had returned to normal.

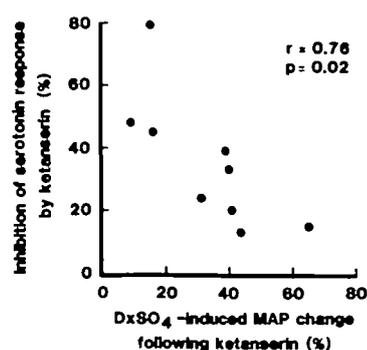


FIGURE 13. Nine rabbits were pretreated with ketanserin (4 mg/kg, intravenously, before injection of serotonin, histamine, and DxSO_4). Those rabbits in which ketanserin inhibited the serotonin response had a small hypotensive response to DxSO_4 . Those rabbits in which ketanserin did not inhibit serotonin-induced hypotension had a large response to DxSO_4 ($r = 0.76$, $P = 0.02$).

injection. These findings support the concept that the major agent responsible for immediate hypotension following DxSO_4 injection was serotonin, not histamine.

Effect of Inhibitors on Serotonin and DxSO_4 -Induced Hypotension

Ketanserin

The serotonin antagonist ketanserin was tested for its ability to block the hemodynamic effects seen after both DxSO_4 and serotonin injection. The capacity of ketanserin to block serotonin-induced hypotension was variable in different animals. However, in those animals in which ketanserin did inhibit serotonin-induced hypotension, the DxSO_4 -induced response also was prevented. In those animals in which ketanserin did not inhibit serotonin-induced hypotension, the DxSO_4 response was not prevented (Fig. 13). No inhibition of the histamine response by ketanserin was seen (data not shown). These results are consistent with mediation of DxSO_4 -induced hypotension by serotonin.

Methysergide

Methysergide, a serotonin antagonist, was also tested as a potential tool for use in these studies. However, methysergide infusion, even at high doses (up to 20 mg/min), failed to prevent serotonin-induced hypotension and bradycardia (data not shown). Furthermore, histamine-induced hypotension was enhanced by methysergide infusion (data not shown). Therefore, methysergide could not be used as a serotonin antagonist for these studies.

Cimetidine

Cimetidine also was tested as a specific histamine H_2 antagonist for use in these studies, and was used at three doses. In each case, the hypotensive response (measured as the mean arterial pressure) to serotonin (22 μg) and histamine (100 μg) was com-

pared before and after cimetidine injection and was expressed as percent of the preinjection value. Finally, in each animal ($n = 4$), DxSO_4 and cimetidine were injected within a 10-minute period. At a cimetidine dose of 2 mg/kg, the serotonin-hypotensive response was diminished from $47.5 \pm 4.7\%$ to $35.3 \pm 7.1\%$, the histamine-hypotensive response was diminished from $14.3 \pm 6.3\%$ to $8.5 \pm 2.8\%$, and the DxSO_4 response was $32.5 \pm 5.1\%$ (compared to $33.0 \pm 2.5\%$, $n = 7$, for the control group shown in Fig. 9). At a cimetidine dose of 12 mg/kg, the serotonin response was diminished from $42.5 \pm 3.2\%$ to $20.5 \pm 5.3\%$, $P = 0.03$, the histamine-hypotensive response was diminished from $12.2 \pm 5.0\%$ to $3.0 \pm 2.3\%$, $P = 0.05$, and the DxSO_4 response was $21.5 \pm 5.9\%$ (compared with $33.0 \pm 2.5\%$, $n = 7$, for the control group of rabbits shown in Fig. 9). At high doses of cimetidine (60 mg/kg), the hypotensive response to serotonin injection was completely inhibited in five rabbits, as has been reported previously (Wiggins and Campbell, 1983), and as illustrated in Figure 10. This dose of cimetidine also largely prevented the DxSO_4 -induced hypotension (Fig. 14). From these studies, we conclude that cimetidine in the rabbit inhibits serotonin-induced hemodynamic changes even at moderate doses (12 mg/kg). The effect of 2 mg/kg of cimetidine was not statistically significant. However, at this lower dose, which should have been sufficient to inhibit H_2 effects, the DxSO_4 response was not inhibited. At the intermediate dose of cimetidine (12 mg/kg), the DxSO_4 response was somewhat depressed in parallel with the serotonin response. This result does not rigorously discriminate between serotonin and histamine as the mediator of the response, but is compatible with the concept that serotonin is the major mediator, and strongly supports the concept that serotonin and/or histamine caused the DxSO_4 -induced hypotension.

Role of Autonomic Nerves in Mediating Serotonin-Induced Hypotension and Bradycardia

Serotonin has been shown to cause bradycardia and hypotension in rabbits primarily via autonomic

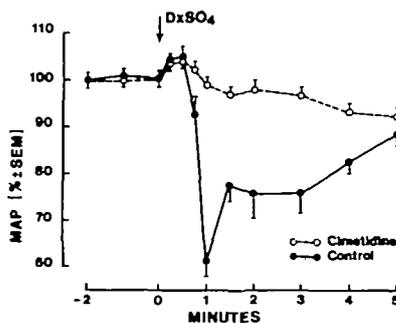


FIGURE 14. Effect of cimetidine (60 mg/kg, intravenously) upon the hemodynamic changes induced by DxSO_4 injection. Both immediate hypotension and bradycardia were markedly inhibited by cimetidine ($n = 3$ for both groups).

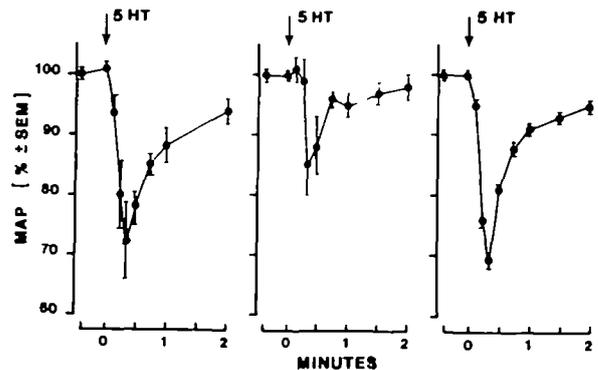


FIGURE 15. Serotonin injection (22 μg , intravenously) caused a 30% fall in mean arterial pressure (MAP) beginning immediately after injection ($n = 3$). Section of vagus and depressor nerves delayed and diminished the serotonin-induced fall in MAP ($P < 0.05$ at 10 and 15 seconds after injection). In control animals, sham section resulted in a fall in MAP that was similar to that seen in the experimental animals prior to nerve section ($n = 3$).

reflexes (Erspamer, 1966). A study was therefore performed to determine whether these reflexes were responsible for DxSO_4 -induced hypotension. Both the vagus and depressor nerves were identified in the neck of rabbits (see Methods). In one group of animals, the nerves were cut. In the other group (sham-operated), the nerves were left intact. The response of these two groups of animals to intravenous serotonin injection is shown in Figure 15. Before section, both groups of animals showed a similar hypotensive response to intravenous serotonin (22 μg). After section, this hypotensive response was both delayed and blunted. However, a definite response still was present after nerve section, which might have been caused by a reflex initiated by receptors in the carotid sinus that was left intact in this experiment. Thus, after serotonin injection into a central vein, the time taken to reach the carotid sinus would be longer, thus causing the delayed response. (In other experiments, injection of serotonin directly into the common carotid artery reproducibly caused hypotension and bradycardia).

These two groups of rabbits were then injected with DxSO_4 (20 mg/kg). As shown in Figure 16, the initial marked hypotensive response was prevented by vagus and depressor nerve section. Delayed hypotension did occur 2–3 minutes after DxSO_4 injection. We conclude that vagus and depressor nerve section did partially prevent DxSO_4 -induced hypotension. However, a further experiment was performed to determine whether inhibition of the carotid sinus receptors, in addition to the heart and lung receptors, would completely prevent DxSO_4 -induced hypotension.

Effect of Carotid Ligation Plus Vagus and Depressor Nerve Section

Two groups of three rabbits were treated as in the previous experiment, except that, in addition to va-

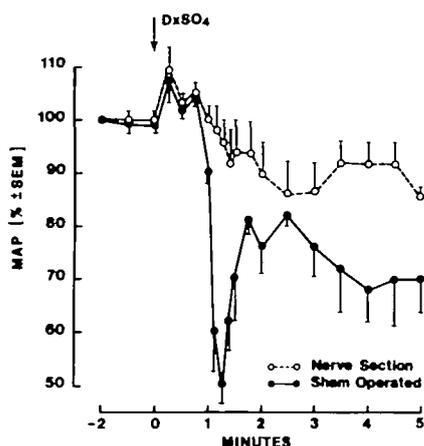


FIGURE 16. Rabbits in which both vagus and depressor nerves were sectioned showed partial inhibition of the response to intravenous $DxSO_4$ compared with control ($P < 0.05$ at 45, 60, 75, 90, and 105 seconds) ($n = 3$ for both groups).

gus and depressor nerve section, the carotid arteries on both sides were ligated (see Methods). Since the baroreceptors were left intact, this procedure itself caused an initial 30% rise in mean arterial pressure. However, if the rabbits were left for 60 minutes following the procedure, the systemic arterial pressure fell to within 10% of precarotid ligation values and was stable. Rabbits treated in this way showed an immediate hypotensive response to serotonin injected into the carotid artery above the point of ligation, but they showed a markedly blunted response to intravenous serotonin injection (Fig. 17). However, the hypotensive response was not prevented completely, and a small response was noted in all animals. This may reflect the effects of other accessible serotonin receptors not excluded by the procedure (see Discussion). However, this maneuver did largely prevent $DxSO_4$ -induced hypotension (Fig. 18). A further study was performed to confirm that nerve section and carotid ligation did not

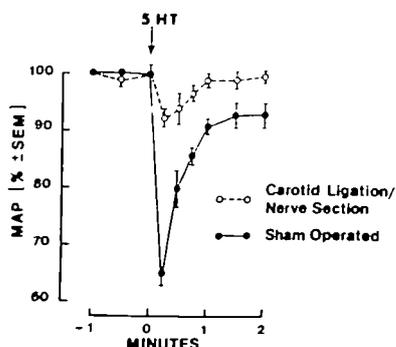


FIGURE 17. Effect of serotonin injection (arrow) upon mean arterial pressure (MAP) in rabbits with both nerve section and bilateral carotid ligation ($n = 3$) compared with control rabbits ($n = 3$). Although serotonin-induced hypotension and bradycardia were not completely inhibited, they were markedly diminished in the carotid-ligated/nerve-sectioned animals.

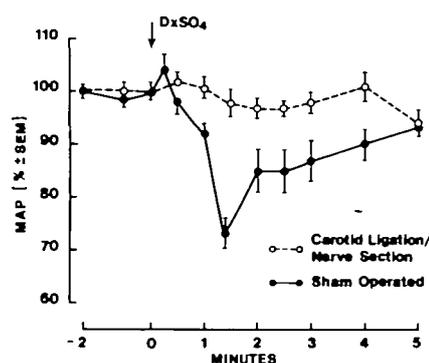


FIGURE 18. Injection of $DxSO_4$ intravenously caused almost no hypotension or bradycardia in rabbits previously treated by section of vagus and depressor nerves together with bilateral carotid ligation ($n = 3$). Control rabbits exhibited the normal hypotensive response together with bradycardia seen after intravenous $DxSO_4$ injection ($n = 3$).

prevent vasodilator responses nonspecifically. As shown in Fig. 19, nerve section diminished the response to serotonin as described above, but did not reduce the response to bradykinin ($1 \mu\text{g}$) or sodium nitroprusside ($50 \mu\text{g}$). Similarly, carotid ligation in addition to nerve section markedly reduced the serotonin response while leaving the response to bradykinin and sodium nitroprusside intact. The values for presection, post-nerve section, and post-nerve section plus carotid ligation, were, for serotonin, 51.3 ± 14.8 , 29.0 ± 3.2 , and $14.3 \pm 3.2\%$, for bradykinin were 61.3 ± 10.3 , 62.3 ± 10.2 , and $57.0 \pm 12.7\%$, and for nitroprusside were 38.3 ± 13.2 , 44.3 ± 9.7 , and $47.3 \pm 15.9\%$, respectively ($n = 4$).

From these studies on the effect of nerve section and carotid ligation, we conclude that an intact autonomic nervous system was necessary for the hypotension and bradycardia observed following $DxSO_4$ injection.

Discussion

In the experiments described, an anaphylactoid response was induced by intravenous injection into rabbits of the polyanion dextran sulfate ($DxSO_4$). This "anaphylactoid" response included the following features: hypotension, bradycardia followed by tachycardia, irregular respiration, wheezing, bowel peristalsis with occasional defecation and micturition, and a bleeding tendency. The original purpose of the experiment was to observe the consequences of activation of Hageman factor *in vivo* by a substance known to activate the HF system, namely dextran sulfate (Kluft, 1978; Ulevitch and Johnston, 1980). Hageman factor activation caused by $DxSO_4$ injection would be expected to result in kinin generation with consequent hypotension (Cochrane and Griffin, 1982). In fact, although the Hageman factor system was activated in these experiments (as judged by proteolysis of circulating radiolabeled rabbit Hageman factor, prekallikrein and high molecu-

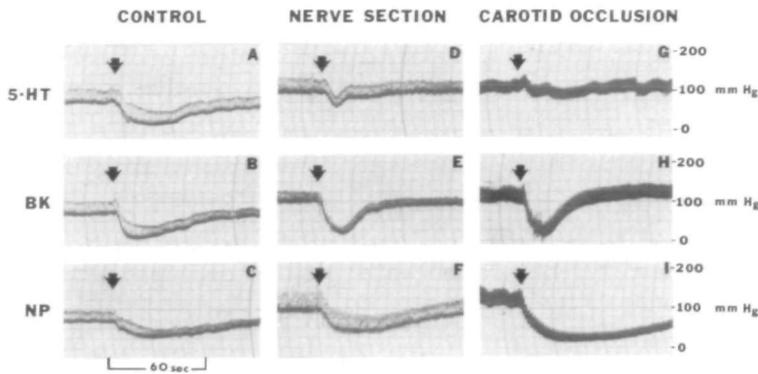


FIGURE 19. Intravenous injection of serotonin ("5-HT," 22 μ g), bradykinin ("BK," 1 μ g) or sodium nitroprusside ("NP," 50 μ g) into intact animal ("control"), animal with vagus and depressor nerves cut ("nerve section"), or animal with nerve section plus carotid ligation ("carotid occlusion"). Although the hypotensive response to serotonin was diminished by nerve section and markedly inhibited by carotid ligation in addition to nerve section, the response to bradykinin and sodium nitroprusside remained intact.

lar weight kininogen (data not shown), HF system activation was not responsible for the observed changes in systemic arterial pressure and heart rate. This was demonstrated by finding that Hageman factor depletion did not prevent the response, and inhibition of kininase II (by SQ20881) did not enhance the hypotension. Therefore, a series of studies was performed to determine the mediators involved in DxSO_4 -induced hypotension.

Dextran sulfate has been reported to activate the alternative pathway of complement (Burger et al., 1975). In addition, in rabbits, cobra factor injection has been reported to cause immediate hypotension similar to that seen after injection of DxSO_4 (Ulevitch and Cochrane, 1977). Furthermore, in the studies described here, DxSO_4 injection caused a 10% fall in antigenic C3 levels in blood, and this was accompanied by blood neutropenia (Fig. 2) in association with neutrophil margination in lung capillaries (Fig. 7). This sequence of events is well described as a consequence of C5a production or injection *in vivo* (Jacob, 1981). Further evidence that complement activation did indeed occur after DxSO_4 injection is provided by the finding that, when rabbits were depleted of C3 using cobra factor, neutropenia was prevented, as was the characteristic rebound neutrophilia that followed DxSO_4 injection (Fig. 2). However, in these same experiments, in spite of the above evidence for complement activation, C3 depletion did not prevent DxSO_4 -induced hypotension and bradycardia (Fig. 3). A complement-independent mechanism must therefore have been responsible.

Platelet depletion has been shown to prevent the hypotension caused by IgE-induced anaphylaxis in the rabbit (Benveniste et al., 1972; Pinckard et al., 1977; McManus et al., 1979). In the studies described here, platelet depletion similarly prevented DxSO_4 -induced hypotension and bradycardia (Fig. 6). Furthermore, following DxSO_4 injection, platelets were shown to disappear from blood (Fig. 2) and appear in lung (Table 1), where ultrastructural studies 2 minutes after DxSO_4 injection showed them to be aggregated and in the process of degranulating (Figs. 7 and 8).

But how might platelet activation in the lung cause

hypotension? Rabbit platelets contain both histamine and serotonin in their dense granules (Da Prada et al., 1981). The fact that cimetidine infusion at high doses prevented DxSO_4 -induced hypotension pointed toward histamine as the important mediator. However, when histamine itself was infused or injected intravenously, or into the right ventricle, the predominant result was either no change in blood pressure or immediate hypertension with tachycardia followed by a mild hypotensive phase (Fig. 9), as has been previously described in the rabbit (Brimblecombe et al., 1974; Carroll et al., 1974). It therefore was not possible to account for the DxSO_4 -induced hypotension and bradycardia simply in terms of histamine release from platelets (or from the basophils which disappeared from the blood and appeared in degranulated form in the lung following DxSO_4 injection). In contrast, serotonin injection reproduced the immediate hypotension and bradycardia seen following DxSO_4 injection (Fig. 10), although the hypotension was less prolonged than that seen after DxSO_4 injection, even when high doses of serotonin were injected (300 μ g). That histamine did probably contribute to the DxSO_4 -induced hemodynamic changes was suggested by the finding that a combination of histamine (100 μ g) and serotonin (200 μ g) infused over 1 minute appeared to reproduce more closely both the extent and duration of the events seen after DxSO_4 injection (Fig. 9).

In quantitative terms, enough platelets accumulated in lung following DxSO_4 injection to release about 100 μ g of histamine and 200 μ g of serotonin. Direct measurement of serotonin in blood following DxSO_4 injection confirmed that 5–10 times more serotonin could be detected after DxSO_4 injection than was detected after intravenous injection of 22 μ g serotonin (sufficient to cause hypotension and bradycardia). In contrast, direct measurement of histamine in blood following DxSO_4 injection showed that 5–10 times less histamine could be detected after DxSO_4 injection than was detected after injection of 100 μ g of histamine (sufficient to cause only mild hypotension). These independent measurements confirmed that the amount of serotonin released was adequate to cause the observed hypoten-

sion and bradycardia. In contrast, the amount of histamine measured in blood probably was insufficient to cause the observed hemodynamic effects.

Ketanserin, a serotonin antagonist, did inhibit the $DxSO_4$ -induced hypotension, but only in those rabbits in which it inhibited the serotonin-induced response. These findings are compatible with the concept that the major cause of $DxSO_4$ -induced hypotension was serotonin. This effect of a specific serotonin antagonist in preventing dextran-induced hypotension is similar to that reported in rats by Berstad (1981). However, the effect of serotonin antagonists, such as ketanserin, might occur by inhibiting platelet aggregation in blood vessels rather than by inhibiting the effect of released serotonin on tissue receptors. This concept recently has been emphasized by Meuleman et al. (1983) and Bergqvist et al. (1983).

The finding that cimetidine inhibited $DxSO_4$ -induced hypotension initially pointed toward histamine as the important mediator. However, at the doses used, cimetidine also prevented the serotonin-induced cardiovascular changes (Fig. 10), as has been reported elsewhere (Wiggins and Campbell, 1983). This inhibition of hypotension by cimetidine therefore did not discriminate between histamine and serotonin in these experiments, although it did support the concept that serotonin and/or histamine was the mediator.

Further evidence that serotonin was the major factor operative in causing $DxSO_4$ -induced hypotension was the finding that $DxSO_4$ -induced hypotension could be diminished by section of the vagus and depressor nerves, and could be prevented by carotid ligation in addition to nerve section. That this was not a nonspecific response to this maneuver was shown by demonstrating that the responses to bradykinin and sodium nitroprusside remained intact while the response to serotonin was lost. This again supported the concept that serotonin-mediated autonomic reflexes were largely responsible for the observed hemodynamic changes. Taken together, these results all point toward a serotonin-mediated chemoreflex as the major cause of $DxSO_4$ -induced hypotension. Histamine release undoubtedly occurred after $DxSO_4$ injection, and certainly contributed to the prolongation of hypotension. However, it was not the major factor.

The chemoreflexes by which serotonin induces hypotension and bradycardia were originally identified by Bezold and Hirt in 1867 and were further analyzed by Jarish and Richter (1939) using veratrum alkaloids. Dawson and Comroe (1954) defined three separate chemoreflexes in the heart and lungs. These were (1) a coronary chemoreflex, (2) a pulmonary depressor chemoreflex, and (3) a pulmonary respiratory chemoreflex. The afferent limb of these reflexes begins with chemoreceptors in the lungs, heart, carotid sinus, and probably also the nodose ganglion (Jacobs and Comroe, 1971). Signals travel in the vagus nerve, and depressor nerve in the rabbit

(Winton and Bayliss, 1955), to the autonomic nerve centers in the brain. The efferent limb appears to take the form of a general increase in vagal tone and a decrease in sympathetic tone, but there are also more localized organ effects (e.g., on lungs or cardiovascular system) depending upon the site of receptor stimulation (Erspamer, 1966). The results of this change in autonomic climate almost certainly affect every organ. In this study, heart rate and blood pressure were measured. Both decreased markedly. Similar responses to serotonin injection in rabbits and cats have previously been documented (Erspamer, 1966). In other studies, reflex vasodilation due to decreased sympathetic activity has been observed in the isolated hind limb, liver, kidney, intestine, spleen, and retina (Erspamer, 1966). A recent review by Mecca and Webb (1984) suggests that the vasodilator response to serotonin may be the result of a combination of neural and direct vascular events. However, the results from this study suggest that the neural events predominate, at least with respect to systemic blood pressure changes induced by intravenous injection of serotonin in the rabbit.

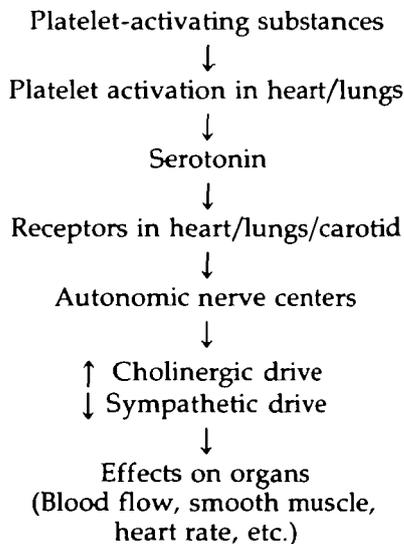
Increased parasympathetic activity can cause smooth muscle contraction in the gut, bladder, and in bronchioles, and might well have accounted for or contributed to the noncardiovascular changes observed in rabbits following $DxSO_4$ injection (wheezing, micturition, defecation), although anaphylatoxins (C3a, C5a), together with histamine release, bradykinin generation, or direct effects of serotonin, might also have contributed to these events. It is possible that the observed irregular respiration was also due to the chemoreflex, since this is a regular feature of the serotonin-induced reflex (Erspamer, 1966).

The mechanism by which $DxSO_4$ caused platelet activation in the lung has not been worked out, although $DxSO_4$ has previously been observed to cause platelet aggregation (Walton, 1953; Brossmer and Pfeleiderer, 1966; Tiffany and Penner, 1981). In preliminary studies, [3H]serotonin was not released from platelet-rich plasma; neither was it released from washed plasma-free platelets when incubated in the presence of $DxSO_4$. These findings imply that some mechanism other than a direct interaction between $DxSO_4$ and platelets was taking place. We observed that blood basophil counts diminished immediately after $DxSO_4$ injection, and that cells which were probably degranulated basophils appeared in lung at the same time. It is therefore possible that $DxSO_4$ injection resulted in basophil activation with consequent release of platelet-activating factor (AGEPC). If so, the sequence of events would be similar, but not as profound, as those observed to occur in the model of IgE anaphylaxis in the rabbit (Benveniste et al., 1972; Pinckard et al., 1977; McManus et al., 1979). No evidence is provided to show that $DxSO_4$ does interact directly with basophils, although such an interaction might occur

either via a charge effect or, as has recently been suggested, by a simple osmotic effect (Findlay et al., 1981). An alternative explanation is that rabbits could have natural antibodies against dextrans (Palosvo and Milgrom, 1981), or that $DxSO_4$ -immunoglobulin interactions might trigger platelet activation (Levy et al., 1981). Activation of complement by $DxSO_4$ may also have contributed to platelet activation and thrombocytopenia as well as neutropenia (Ulevitch and Cochrane, 1977). However, this mechanism cannot totally account for the platelet-induced hypotension, since C3 depletion did not prevent $DxSO_4$ -induced hypotension.

Dextran sulfates have been studied as synthetic heparin-like molecules (Walton, 1954), and the severe bleeding tendency induced by $DxSO_4$ in these studies probably was due to this heparin-like effect.

As has been emphasized by this discussion, $DxSO_4$ injection results in activation of many mediator systems. The question addressed in this study was which system caused the observed cardiovascular changes? Evidence is provided that platelet activation in the lungs caused bradycardia and hypotension by means of powerful autonomic reflexes activated by serotonin as outlined below.



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INDEX TERMS: Serotonin • Platelet • Chemoreflex • Hypotension