Physiological Effects of Subvisible Microspheres Administered Intravenously to Beagle Dogs

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Abstract □ Experiments were performed to determine the acute effects of polystyrene microspheres in four size ranges (3, 8, 15, and 25 μm) upon intravenous administration to dogs. The parameters monitored were arterial pressure, arterial blood pH, blood gases (pO_2 and pCO_2), white blood cell count, heart rate, and single lead ECG. A filtered (particle-free) control volume was administered after establishing background readings for each dog for 30 min, and the monitoring was continued for an additional 30 min before dosing with the suspension of spheres. The animals were followed for up to 3 hr after dosing. Initial experiments with 15-μm spheres at bolus dose levels of 0.1, 1, 5, and 10 million spheres revealed no drastic changes in the clinical parameters monitored; therefore, an incremental dosing regimen was followed in which 0.5, 1, 5, and 40 million spheres/min were administered at sizes of 25, 15, 8, and 3 μm, respectively, for 1 hr (total of 30, 50, 300, and 2400 million spheres). Even at these exaggerated doses, the only appreciable change observed was a slight increase in white cell count. The dogs were further observed for 4 weeks after the experiment and appeared to remain normal. Histological evaluation of representative tissue samples showed no evidence of damage that could be attributed to the presence of microspheres in the organs.

Keyphrases □ Microspheres—intravenous administration to dogs, physiological effects evaluated □ Physiological effects—intravenous administration of microspheres to dogs

Many literature reports described the long-range effects on tissues caused by foreign insoluble materials contained in intravenous fluids (1). Most accounts reported adverse effects caused by particulate matter injected intravenously by accident or through limitations of the technique. One exception is a study (2) that evaluated the long-term biological effects of polystyrene spheres administered intravenously to rats.

In spite of the abundance of reports on long-range effects of foreign particulates on tissues, a sparsity of information exists on the acute effects of particulate matter injected intravenously. For obvious reasons, the acute effects have not been studied directly in humans. The few accounts are a direct consequence of accidental administration of air that resulted in massive embolism (3, 4) and accidental intravenous administration of suspensions not intended for such use (5, 6). One report (7) presents the results of this type of mistake: dizziness, shortness of breath, cyanosis, tachycardia, increased blood pressure, and death. Similar reactions were reported in drug abuse when tranquilizer tablets dispersed in water were injected intravenously (8–10).

Surprisingly, the acute effects of particulate matter injected in experimental animals have not been studied systematically. The only observation recorded in several reports is death, as was the case in early experiments dealing with the "toxicity" of ground glass, silica, and cotton fibers (11–14) and the one study (2) that attributed the death of several rats to unreasonably high levels of particulate matter. A recent study (15) of the distribution of radiolabeled microspheres also implicated the spheres as a major factor in the death of several experimental animals, and the resulting speculations emphasized the need for a systematic study to determine the acute effects of particulate matter injected intravenously.

The present study investigated the acute effects of particulate matter as a function of size and concentration and evaluated the histological damage induced by inert particulate matter of known size 4 weeks after administration.

EXPERIMENTAL

Preparation of Doses—Polystyrene divinylbenzene microspheres[1] were obtained in four nominal size ranges, 3, 8, 15, and 25 μm, as dry powder. The actual size distribution was verified microscopically. Although normal saline solution was a suitable vehicle for suspending the spheres at low doses, low molecular weight dextran was superior at the higher doses. Therefore, dextran was chosen as the suspending vehicle for some studies. Tables I and II summarize the properties of the suspensions used in the actual dosing.

A master suspension of spheres at the maximum possible concentration was prepared; from this suspension, the desired concentrations of spheres were prepared using particle-free diluent. Once the desired count per unit volume was obtained, the suspensions were filled into 50-ml multiple-dose vials, labeled, stoppered, and autoclaved for 20 min at 121°. Following sterilization, the suspensions were again monitored to assure that there were no changes in the size and count of the doses prepared.

All glassware and vials were washed thoroughly and dry heat sterilized at 180° for 4 hr before use. The stoppers were boiled for 30 min in 0.1% sodium pyrophosphate and thoroughly rinsed with filtered water for injection. The vehicles were filtered through a 0.22-μm membrane filter, and all operations were performed under laminar air flow conditions.

Preparation of Dogs—Small beagle dogs, 9.1–11.3 kg (20–25 lb), were randomly assigned to groups corresponding to each particle-size range. Following induction of anesthesia with 20 mg of pentobarbital sodium/kg iv, the dog was secured in a supine position to an operating table. With aseptic techniques, the right femoral vein and artery were catheterized, and 5% dextrose in 0.2 *N* saline[2] was administered through a 0.45-μm membrane filter at a rate of 0.1 ml/min. The artery was connected to a pressure transducer using tubing filled with heparinized saline. Stopcocks were placed in the line to allow removal of blood samples and subsequent flushing of the lines, and ECG electrodes were glued to the dog's limbs.

After the instrumentation was connected, the dog was allowed to stabilize for 30 min. Background samples and readings were obtained during this period. Blood pressure and ECG were recorded simultaneously on a two-channel polygraph recorder[3]; the pH and partial pressures of oxygen and carbon dioxide were obtained using a blood gas analyzer[4], and the cell counts were performed on an automatic counter[5].

Before the doses were administered, each dog was given a series of volume control doses of filtered vehicle and allowed to stabilize for an additional 30 min. Thus, each dog served as its own control. In addition, one dog was carried through a 4-hr experiment as a sham, and a second dog was evaluated extensively over 2 hr before receiving the microspheres. Multiple or repetitive bolus administration of microspheres was performed at 5-min intervals, starting after collection of data corresponding to background or volume control doses.

[1] Particle Information Services, Grants Pass, Ore.
[2] Travenol Laboratories, Morton Grove, Ill.
[3] Model 296, Sandborn, Waltham, Mass.
[4] Model 113, Instrumentation Labs, Lexington, Mass.
[5] Coulter counter model S, Coulter Electronics, Hialeah, Fla.

Table I—Characterization of Suspensions Used to Determine Acute Effects

Size, μm			Sphere Counts, 10^6/ml	Administration (Volume and Mode)	Nominal Dose, millions	Dog Number
Nominal	Actual	Vehicle				
Sham	—	Saline	0.000	25-ml single bolus	0.0	833
3	3.7 ± 0.8	Saline	1.120	10-ml single bolus	10.0	676
15	15.9 ± 1.0	Saline	0.014	10-ml single bolus	0.1	696
15	15.9 ± 1.0	Saline	0.097	10-ml single bolus	1.0	832
15	15.9 ± 1.0	Saline	0.405	25-ml single bolus	10.0	831
8	8.4 ± 0.6	Saline	0.987	12 × 10-ml repetitive bolus	120.0	1050
15	15.9 ± 1.0	Saline	0.405	10 × 5-ml repetitive bolus	20.0	847
25	24.7 ± 1.1	Saline	0.0051	12 × 20-ml repetitive bolus	12.0	1046
3	3.7 ± 0.8	Dextran	41.80	12 × 5-ml repetitive bolus	2400.0	1095
8	8.4 ± 0.6	Dextran	4.87	12 × 5-ml repetitive bolus	300.0	1057
15	15.9 ± 1.0	Dextran	1.03	12 × 5-ml repetitive bolus	60.0	1042
25	24.7 ± 1.1	Dextran	0.53	12 × 5-ml repetitive bolus	30.0	1080

Since no other procedures were performed on the dogs selected for the histological evaluation, the doses were administered intravenously *via* a right forelimb vein in unanesthetized animals. In an attempt to correlate information from this study with the findings of the previously reported distribution study (15), corresponding doses were prepared. Since matching of sizes for the 3-μm nominal spheres was expected to be critical due to their wider distribution over the body of the experimental animal, the tissue samples used for this pathological examination were taken from a dog that had received the reduced dose of 3-μm radioactive spheres (15). Four weeks after the administration, these dogs were anesthetized with 20 mg of pentobarbital sodium/kg iv and sacrificed by administering a saturated solution of potassium chloride by cardiac puncture.

The autopsy procedures were performed in the presence of a physician who observed the internal organs for gross changes such as color and size. Representative samples of organs were taken, placed in a 5% formaldehyde solution, and submitted to the pathology laboratory for preparation and evaluation of damage. Tissues selected for submission were based on findings of the previous distribution study (15). For the 25-μm treated dog, only a lung sample was submitted; in all other size ranges, samples included lung, heart, liver, spleen, and kidney. All samples were taken from corresponding locations within the organs; the lung samples were taken from the inferior segment of the lingula, heart samples were taken from the apex of the left ventricle, liver samples originated from the anterior edge of the right lobe, and the spleen and the left kidney specimens were wedged from the superior poles. All tissues were prepared by standard embedding techniques and sliced to a thickness of 10 μm with a microtome.

RESULTS

Acute Effects—The clinical data collected from the dogs that received a single dose of spheres are summarized in Table III. There was essentially no change in the blood pressure or pulse after single bolus dosing of microspheres. There was a slight difference between the sham and experimental animals in terms of oxygenation over the data collection period. As can be seen from Table III, the pO_2 of the sham animal increased 59% during the period of observation while the pO_2 of the dosed animals increased only slightly. There was minimal variation in the pH and pCO_2. The white cell counts in the experimental animals rose steadily, as much as 50–60%, after bolus injection of microspheres while the white cell count of the sham animal remained stable near the background value.

Table IV summarizes the clinical data obtained from the dogs that were given repetitive doses at the intermediate sphere count level. Once again, there were no appreciable changes in the heart rate and blood pressure readings; in two dogs, pH and pCO_2 values remained stable. The pO_2 rise noted in Dog 847 was accompanied by a decline in pCO_2, consistent with a change in ventilation over time. The white cell counts per cubic centimeter doubled for the 15- and 25-μm doses, while a substantially smaller increase of about 20% was observed for the 8-μm dose. The ECG's corresponding to the dogs in this group are reproduced in Fig. 1. Slight changes in the PR and QT intervals can be detected. There were also some

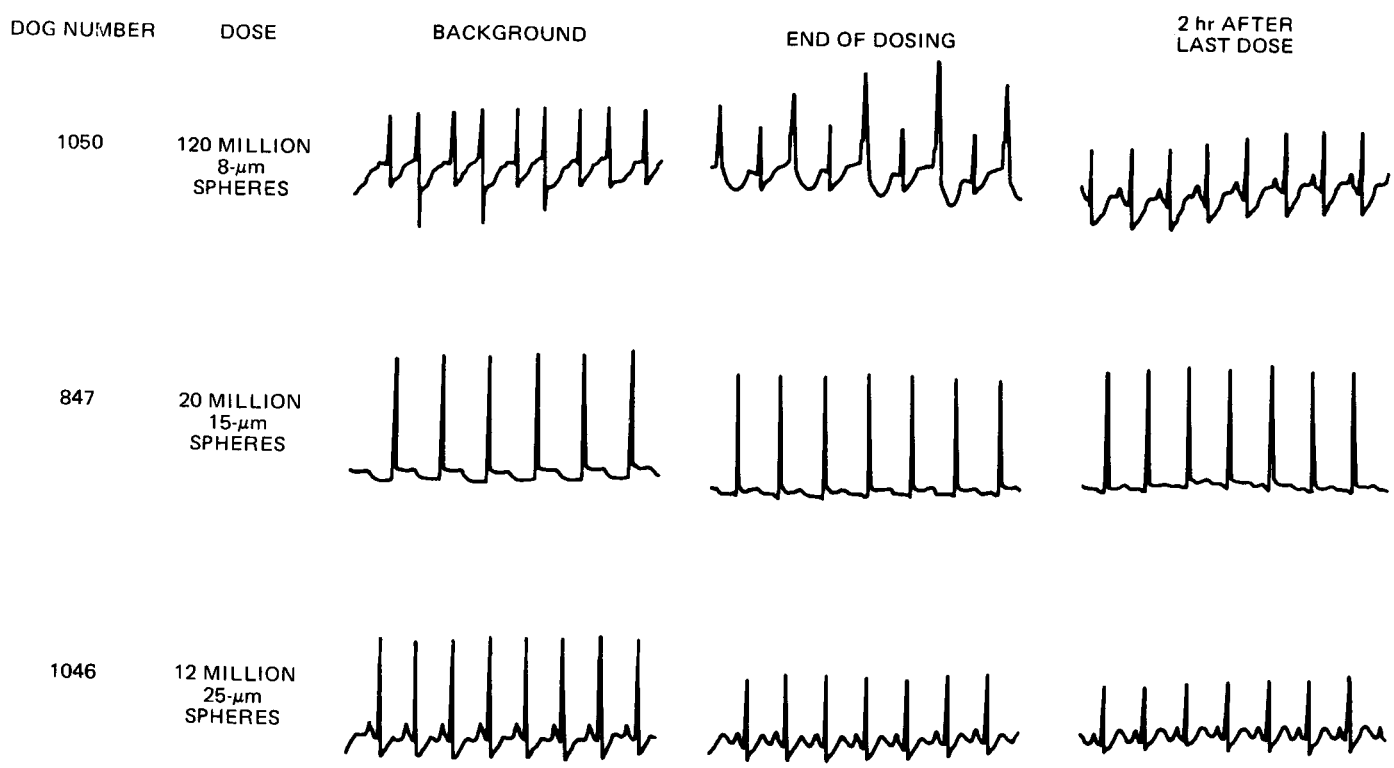

Figure 1—*ECG's of dogs that received repetitive doses of 8-, 15-, and 25-μm microspheres.*

Table II—Doses Administered for Histological Evaluation

Size, μm	Total Dose, millions	Dog Number
3.3 ± 0.6	83.0	995
8.4 ± 0.6	34.0	894
15.9 ± 1.0	9.40	984
24.7 ± 1.1	1.51	985

changes in the configuration of the QRS and ST segments, which can be attributed to the ventricular strain.

Because of the relatively small changes observed in the clinical parameters (except the white cell counts) and the ECG, the doses of spheres were increased substantially as indicated in Table I. The clinical data reported in Table V correspond to the dogs that received repetitive bolus injections at these high sphere concentrations in dextran. Even at these high concentrations, the heart rate, blood pressure, blood pH, and gases remained close to the background values. The highest increase in white blood cell count was observed for the 8-μm dose, from 7000 to 12,000 cells/cm^3. For the remaining size ranges, the changes were smaller than would be anticipated considering the increases observed at the intermediate dose level. The ECG's for the dogs that received spheres at the high levels are reproduced in Figs. 2–6. Although the changes observed were somewhat more pronounced, they were consistent with the changes observed at the intermediate dose levels.

Following the collection of clinical data, the dog's vein and artery were tied off and the animals were kept under observation for 4 weeks. None of the animals showed any abnormality in overall health, appearance, and behavior.

Histological Evaluation—The autopsies of the animals selected for this part of the study revealed no gross abnormalities in appearance, color, and size of the internal organs. Histological examination of the tissues of the dog that received the 3-μm spheres was relatively difficult because of the small size of the microspheres and the lack of a fluorescent label. The samples originating from the liver and the spleen showed a high concentration of spheres because of phagocytosis into an intracellular position. Similar observations were made on samples originating from the lungs, but no spheres could be found in the heart or kidney samples. Although spheres were found in several organs, there was no evidence of tissue damage in the form of inflammation, necrosis, or infarction associated with the lodgment of the microspheres.

The 8- and 15-μm spheres were found without difficulty in the tissue samples since the larger size and the fluorescent label made localization and observation easier. However, spheres were found only in the alveolar septal vessels of the pulmonary sections and, as observed for 3-μm spheres, no damage could be associated with the lodging of the 8- and 15-μm microspheres in the lung. The heart, liver, spleen, and kidney samples showed neither traces of spheres nor tissue damage.

The lung sample corresponding to the 25-μm particles revealed no evidence of spheres because the slices taken for evaluation were 10 μm thick and the microtome blade evidently dislodged the spheres from their sites rather than slicing them. Although a large number of the 15-μm spheres were observed in the corresponding sample, it is suspected that some spheres in that size range also were dislodged. An attempt to prepare thicker slices was not practical since the inherent tissue fluorescence of these thicker slices produced a diffuse bright fluorescent field in which individual spheres could not be distinguished. Since there was no evidence of tissue damage, the search for the spheres was not pursued further.

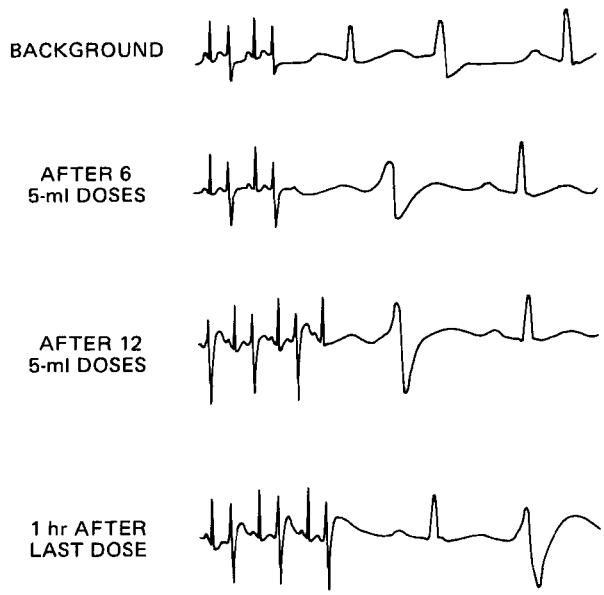

Figure 2—*ECG's of Dog 1095 that received 12 repetitive doses of filtered dextran solution.*

DISCUSSION

Choice of Model—Criteria for the selection of the beagle dog and polystyrene divinylbenzene microspheres were discussed previously (15). Additionally, in the manufacturing process for the microspheres, a fluorescent dye with an excitation wavelength of 485 nm and an emission maximum of 515 nm was incorporated into the spheres. The advantage of such spheres is that, in microscopy, the light can be admitted through a filter corresponding to the excitation wavelength (blue) while the observations are performed by placing a filter in the ocular corresponding to the emission wavelength (yellow). Thus, objects that possess these fluorescing properties are detected without interference from other materials present in the preparation. The risk of overlooking spheres is thus reduced, and spheres in a given preparation can be detected more

Table III—Clinical Data Obtained after Single Bolus Administration of Dose

Dog Number	Size, μm	Dose, 10^6	Event of Data Collection	Heart Rate per Minute	Blood Pressure, mm Hg	pH	pO_2	pCO_2	White Blood Cells, 10^3/ml
676	3	10	Background	88	205/110	7.52	99	18	20.0
			15 min after dose	86	200/110	7.50	105	16	26.5
			120 min after dose	86	200/110	7.50	100	21	27.7
696	15	0.1	Background	81	190/115	7.37	92	24	15.2
			75 min after dose	80	185/115	7.45	96	20	22.4
832	15	1	Background	88	205/100	7.33	88	38	11.6
			Volume control	84	200/105	7.31	89	40	12.2
			30 min after dose	86	205/105	7.27	81	44	13.0
			60 min after dose	84	205/100	7.28	87	40	13.6
			90 min after dose	84	210/105	7.30	92	36	14.4
			120 min after dose	84	190/100	7.30	92	38	14.3
831	15	10	Background	96	220/130	7.19	68	58	11.6
			Volume control	96	225/135	7.21	82	50	13.6
			30 min after dose	96	215/135	7.23	85	49	16.3
			75 min after dose	92	220/135	7.25	87	44	17.6
833	Sham		Background	76	210/125	7.26	63	52	14.7
			Volume control	74	205/130	7.23	72	57	14.4
			30 min after blank dose	76	205/125	7.22	74	59	12.8
			60 min after blank dose	72	205/130	7.24	72	56	12.6
			90 min after blank dose	74	205/130	7.23	70	59	13.7
			120 min after blank dose	74	205/130	7.22	79	55	14.2
			180 min after blank dose	74	200/125	7.26	100	54	15.9

Table IV—Clinical Data Obtained after Repetitive Bolus Administration

Dog Number	Size, μm	Dose, 10^6	Event at Data Collection	Heart Rate per Minute	Blood Pressure, mm Hg	pH	pO_2	pCO_2	White Blood Cells, 10^3/ml
1050	8	120	Background	125	200/120	7.36	96	45	11.8
			60 million	121	200/110	7.38	91	42	10.5
			120 million	116	195/115	7.38	86	44	10.2
			1 hr after last dose	116	205/115	7.40	89	36	12.5
			2 hr after last dose	108	200/125	7.38	87	41	13.6
847	15	20	Background	94	180/115	7.21	66	39	7.1
			10 million	95	180/115	7.22	70	42	8.9
			20 million	96	180/115	7.27	85	39	13.9
			1 hr after last dose	100	185/115	7.32	91	29	14.8
			2 hr after last dose	97	180/115				
1046	25	12	Background	114	200/130	7.34	91	36	8.1
			6 million	106	210/125	7.35	92	34	10.8
			12 million	104	205/130	7.34	96	35	12.1
			1 hr after last dose	104	195/125	7.37	83	38	15.0
			2 hr after last dose	100	200/115	7.37	81	37	15.7

rapidly. Once the spheres are located, the selective filters are removed for a more detailed analysis of that particular field of view in the preparation.

Choice of Clinical Parameters Monitored—Since pulmonary function should be compromised by the lodging of particles in the lung, possible effects were evaluated by monitoring the pH and the partial pressures of oxygen and carbon dioxide in the arterial blood. Since the heart would be pumping against an obstructed capillary bed, one could expect changes in the heart rate, blood pressure, and ECG. The potential inflammatory response due to microsphere embolization was monitored by determination of the white blood cell count.

The results of short-term histological evaluation appear to indicate that most particles above 8 μm were filtered out by the lung and localized there indefinitely. The information obtained using the 3-μm spheres seems to indicate retention by the lungs and that the major mechanism of removal from the bloodstream occurred by phagocytosis in the liver and the spleen, where these spheres appeared to locate. Gross examination upon autopsy and the histological evaluation indicated no evidence of tissue damage, in agreement with a similar study in which rats were used as the model (2). Although these observations apparently conflict with numerous accounts of tissue damage, it should be emphasized that samples were taken only 4 weeks after administration, a time that may be too short for permanent damage to be present or discernible. In addition, the microspheres used were relatively inert (compared to residues from antibiotics for example).

The clinical data obtained from this series of animals show minimal clinical changes due to the intravenous administration of microspheres. Two of three animals subjected to the high repetitive dosing with microspheres showed a drop in pO_2 over time. The one animal showing a rise in pO_2 had a concomitant decline in pCO_2, consistent with changes in ventilation outside the experimental conditions, and should thus be ignored. The fact that the decline in pO_2 can be discerned suggests that major intrapulmonary capillary obstruction is occurring. Additionally, the lack of change seen with the dextran-suspended microspheres in Table V may be related to the dextran vehicle, which is known to influence blood viscosity and platelet aggregation and thereby may have minimized the physiological impact of embolization due to the microspheres.

A consistent finding in all animals subjected to microsphere dosing is a steady rise in white blood cell count, presumably due to an inflammatory response secondary to pulmonary embolization with microspheres. This result correlates well with a previous observation in humans that there was a statistically significant difference in white blood counts in patients who received unfiltered intravenous fluids (particulate containing) compared to those who received filtered intravenous fluids (particulate free) (16). In the present study, there was no apparent correlation between the increase in white cell count and the size or dose level of the microspheres.

The ECG's obtained in this experiment leave no doubt that changes take place during and after intravenous administration of suspensions of microspheres. Although the response appears to increase with increasing dose, the fact that some changes occur during the administration of filtered control volumes points out the need for further investigation. The ventricular strain observed can be attributed to the doses of spheres, but the necessary procedures and experimental steps in the study cannot be overlooked as possible contributors to the changes observed.

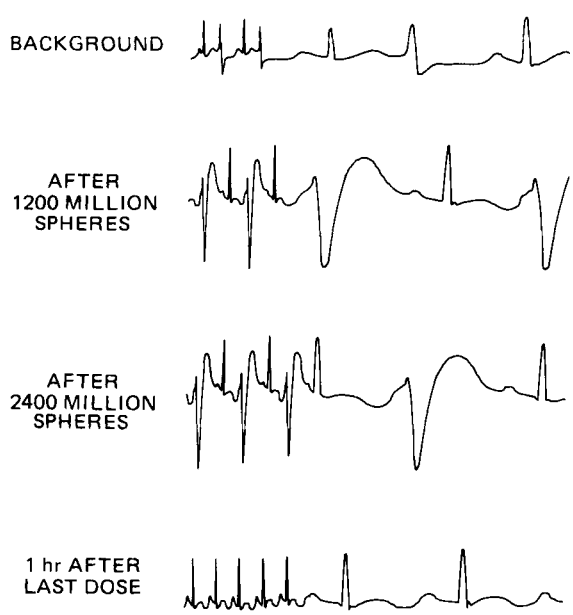

Figure 3—*ECG's of Dog 1095 that received repetitive doses of 3-μm spheres suspended in dextran solution.*

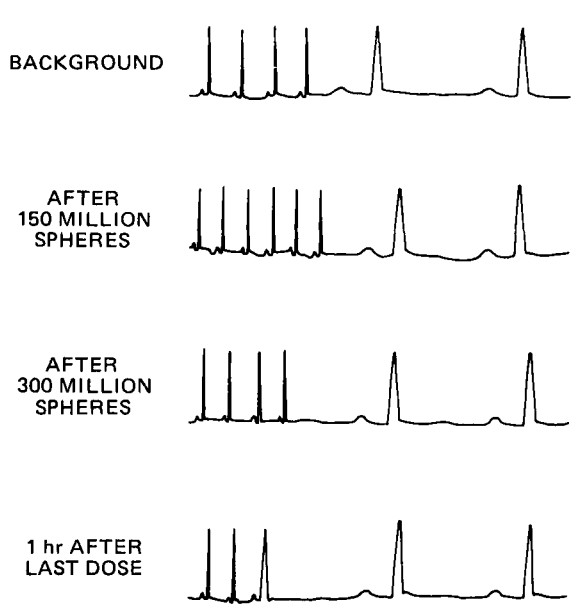

Figure 4—*ECG's of Dog 1057 that received repetitive doses of 8-μm spheres suspended in dextran solution.*

Table V—Clinical Data Obtained after Repetitive Bolus Administration of High Doses of Microspheres in Dextran Solution

Dog Number	Size, μm	Dose, 10^6	Event at Data Collection	Heart Rate per Minute	Blood Pressure, mm Hg	pH	pO_2	pCO_2	White Blood Cells, 10^3/ml
1095	3	2400	Background	110	210/105	7.30	96	35	15.4
			Volume control (six doses)	100	215/100	7.24	89	40	15.9
			Volume control (12 doses)	102	215/105	7.27	93	37	15.2
			1 hr after volume control	96	195/95	7.25	75	43	17.4
			1200 spheres administered	94	195/95	7.32	86	36	16.7
			2400 spheres administered	94	200/95	7.35	86	38	16.2
			1 hr after last dose	96	185/105	7.39	89	33	19.2
1057	8	300	Background	84	185/110	7.36	96	42	7.1
			150 spheres administered	100	210/105	7.37	71	42	7.9
			300 spheres administered	94	190/105	7.39	97	40	9.7
			1 hr after last dose	92	190/110	7.38	92	38	12.5
1042	15	60	Background	118	220/120	7.29	108	41	5.1
			30 spheres administered	110	220/115	7.31	107	34	5.5
			60 spheres administered	108	215/110	7.26	109	39	6.1
			1 hr after last dose	108	210/105	7.26	109	39	7.1
1080	25	30	Background	106	220/125	7.31	99	42	10.0
			15 spheres administered	98	215/100	7.27	94	57	9.6
			30 spheres administered	96	195/110	7.28	105	49	11.3
			1 hr after last dose	92	200/115	7.27	101	48	11.4

Although not observed in the present study, the deaths of experimental animals reported in other studies cannot be overlooked as acute effects; the particulate matter injected could have contributed. The literature over the past 15 years has cited the many negative aspects of particulate matter in parenterals, and it can be expected that there is a great potential for some long-term damage when particulate matter is introduced into the bloodstream. However, results of this study indicate that rather large numbers[6] of intravenously administered particles can be tolerated without any major immediate physiological or short-term histological effects. The risk of the damage must be evaluated against the recognized and proven benefit of intravenous therapy. In spite of the death of several experimental animals in other studies, the ability to compensate for the particulate load as seen in these animals is an important observation.

Although the main objectives of the study were accomplished in gathering information regarding acute physiological and short-term histological effects, much work lies ahead before the behavior and effects of injected particulate matter can be understood thoroughly. Extrapolation of the results of an animal study to humans can be misleading, but further experiments may be planned to approach the ultimate goal in a more meaningful manner. Repeating the present experiments using primates would be a step in that direction. As pointed out previously, more thorough monitoring in the form of intracardiac pressures, pulmonary artery and wedge pressures, lung compliance, more sophisticated ECG, *etc.*, will have to be performed using a sufficient number of experimental animals.

The results of this study lead to some interesting speculations regarding the use of particulate matter in the form of carefully prepared and controlled suspensions for intravenous and intraarterial therapy. The feasibility of such an approach has been documented to a certain extent by both the successful application of biodegradable spheres in diagnosis (17–19) and indications that poorly water-soluble drugs administered in nonaqueous solvents may precipitate in the bloodstream upon intravenous administration (20, 21). The present study suggests that the administration of suspensions into the bloodstream should be further evaluated.

Very potent insoluble drugs that require low doses (or deliberately prepared, poorly soluble prodrugs of soluble therapeutic agents) could be formulated in the appropriate size to lodge in the target organ, from which they could slowly release locally. One could speculate on achieving a sustained-release effect in addition to enhancing, by virtue of higher localized concentrations, the therapeutic effects while possibly minimizing undesirable side effects. It is hoped that this report will stimulate

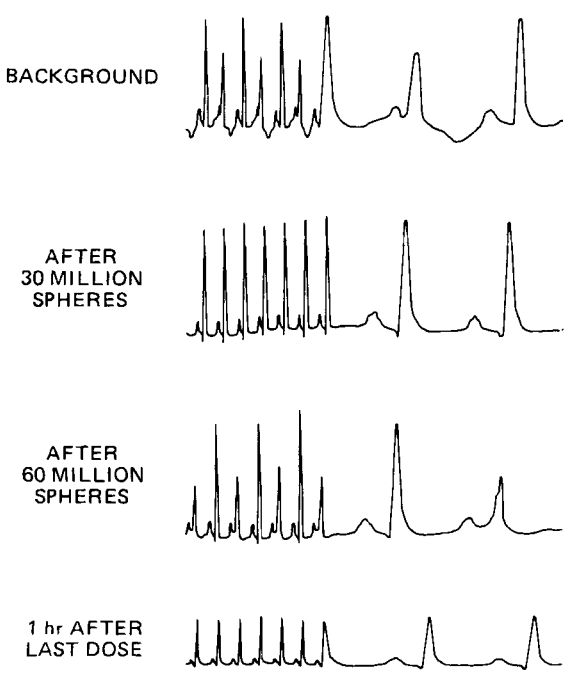

Figure 5—*ECG's of Dog 1042 that received repetitive doses of 15-μm spheres suspended in dextran solution.*

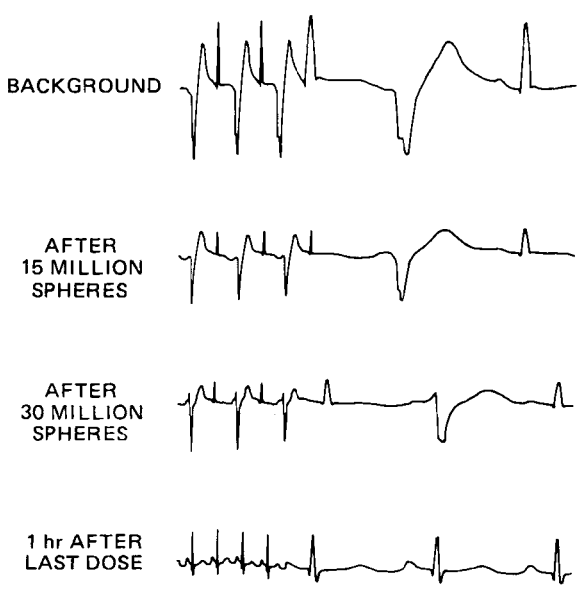

Figure 6—*ECG's of Dog 1080 that received repetitive doses of 25-μm spheres suspended in dextran solution.*

[6] The number of spheres administered in this experiment would be contained in several thousand liters of large-volume parenterals that meet the current USP standards.

further investigations of the behavior of particulate matter administered into the bloodstream.

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Correlation of Log *P* with Molecular Connectivity in Hydroxyureas: Influence of Conformational System on Log *P*

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Abstract □ The correlation of log *P* (in octanol–water) with the nonempirical, topologically dependent, calculated molecular connectivity index ($^1\chi^v$) delineates substituted hydroxyureas into two families of linearly related groups of compounds. The first group, composed of the 3-substituted ethyl, *n*-propyl, and *n*-butyl analogs, is more hydrophilic than the 1-substituted methyl and ethyl and the 3-substituted isopropyl and *tert*-butyl analogs. The unsubstituted model compound hydroxyurea appears between the two groups in equal volumes of octanol. In octanol–water ratios of 5:1, log *P* approaches the range of the more hydrophilic group in high concentrations and becomes more lipophilic (similar to the other group) in lower concentrations. The differences in the relative hydrophilicities–lipophilicities of the two groups are rationalized in terms of the equilibria of internally hydrogen-bonded conformers to those that allow optimal interactions with solvent, water, or other hydroxyurea molecules. The concentration dependency observed with hydroxyurea appears to be due to the ease of interconversion of intermolecularly bonded conformers to those interacting with water, whereas the involvement of internally bonded conformers, which are apparently present to a greater degree in lower concentrations, increases the relative lipophilicity.

Keyphrases □ Hydroxyureas, various—log *P* in octanol–water correlated with molecular connectivity indexes □ Log *P*—various hydroxyureas in octanol–water, correlated with molecular connectivity indexes □ Molecular connectivity indexes—various hydroxyureas, correlated with log *P* in octanol–water □ Topological indexes—molecular connectivity, various hydroxyureas, correlated with log *P* in octanol–water

Many physicochemical properties are presently used in relating molecular structure to biological activities (structure–activity relationships) (1). The term physicochemical activity relationships has been suggested (2) to differentiate these methods from strictly structural approaches, *e.g.*, the Free–Wilson method, or from approaches that derive parameters from theoretical calculations on molecular structures, *e.g.*, quantum mechanical methods. The most extensively applied techniques are those that relate biological activity to free energy changes associated with drug transport through different environmental phases. Of the various methods of physicochemical or quantitative structure–activity relationships developed, Hansch analysis (3) has made significant contributions in the realm of quantitative structure–activity relationships and drug design, primarily because of the important role of transport and absorption in drug activity.

BACKGROUND

One primary function of Hansch analysis and other physicochemical and structure–activity methods is to predict optimal molecular or structural characteristics for the design of chemical agents to improve or optimize biological activity. Another utility of these methods might be to study the dynamics of drugs that have been synthesized, biologically tested, and evaluated for medicinal activity but for which there is not an accepted explanation for the activity or lack of biological activity of members of the class.