

Cardiovascular effects of radioactive microsphere suspensions and Tween 80 solutions

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MILLARD, RONALD W., HANK BAIG, AND STEPHEN F. VATNER. *Cardiovascular effects of radioactive microsphere suspensions and Tween 80 solutions*. Am. J. Physiol. 232(3): H331-H334, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(3): H331-H334, 1977. — The cardiovascular effects of two concentrations of Tween 80 (polyoxyethylene sorbitan mono-oleate), a surface-active agent commonly used to prevent aggregation of radionuclide-labeled microspheres, were examined in conscious dogs. Two types of adverse reactions were noted. The first (*Type A*) consisted of reductions in cardiac dimensions as well as hypotension and tachycardia. The second (*Type B*) was less severe and involved only a decrease in cardiac dimensions with no change in left ventricular systolic pressure or heart rate. A 10% dextran solution with $.05 \pm .02\%$ Tween 80 injected into the left atrium caused systemic and/or cardiac alterations in all four dogs studied. Administration of a lower concentration of Tween 80 ($0.01 \pm 0.005\%$), which was the minimum concentration necessary to prevent aggregation of microspheres, induced adverse reactions in 6 of 41 dogs studied. Subsequent administration of this concentration of Tween 80 on the same day rarely induced adverse reactions. Thus, care must be exercised in application of microsphere techniques to organ blood flow measurements when Tween 80 is used to prevent microsphere aggregation, since this surface-active agent causes profound alterations in cardiac dynamics in concentrations normally employed in experiments involving microsphere techniques.

cardiac volume; radionuclide; myocardial blood flow; cardiac dynamics

RADIONUCLIDE-LABELED microspheres are widely used to assess the distribution of regional blood flows (7, 13) and distribution of blood flow across the myocardial wall (3-6, 9). Many potential sources of error for this technique have been analyzed in detail. For instance, Buckberg et al. (3) have defined requirements for speed of arterial reference blood withdrawal and number and distribution of microspheres in tissue for statistically valid flow determination. Another possible but important source of error is recognized, i.e., formation of large aggregates of microspheres, which would result in both nonuniform distribution of the spheres and might also cause hypo-

tension if major channels to the heart and brain were occluded. For the prevention of aggregation, the microspheres are generally kept in suspension by addition of the surface-active, polyoxyethylene sorbitan mono-oleate (Tween 80; Fisher Scientific Co., Pittsburgh). Since the application of microspheres to the study of the circulation assumes that the microsphere suspension does not alter cardiovascular dynamics, it is surprising that despite previously demonstrated hemodynamic actions of Tween (4, 10), little attention has been focused on the effects of this agent, per se, when microsphere techniques are employed.

The results from preliminary experiments in our laboratory in conscious dogs indicated that significant hemodynamic changes occurred subsequent to left atrial injection of microspheres, which were suspended in 0.05% Tween 80–10% dextran and whose dispersion was verified by microscopic examination. To determine the cause of these adverse reactions we examined responses of conscious dogs to solutions of 10% dextran and Tween 80 with and without microspheres. Since microspheres are frequently used to assess intramyocardial distribution of coronary blood flow, particular attention was paid to the effects of left ventricular pressures and dimensions, i.e., important determinants of myocardial oxygen consumption and consequently coronary blood flow (2).

METHODS

Mongrel dogs of either sex, 20–25 kg, were anesthetized with sodium pentobarbital, 30 mg/kg. The instrumentation was implanted through a left thoracotomy in the fifth intercostal space. Catheters were inserted into the left atrium for injection of microsphere suspensions and Tween solutions. Left ventricular pressure was measured with an implanted miniature solid-state gauge (Konigsberg P 22; Konigsberg Instruments Inc., Pasadena, Calif.). A segment length of the left ventricle was measured with a pair of miniature (1 mm) ultrasonic crystals implanted intramyocardially 1–2 cm apart to assess regional myocardial segment length (40 dogs)

or a pair of crystals implanted across the ventricular wall to measure wall thickness (three dogs) (8, 12).

All animals were allowed to recover from operation for a period of 1–4 wk before study. When the conscious dogs were resting quietly, recordings of left ventricular pressure, segment length, and heart rate were made. Radioactive microspheres (3M Co., St. Paul, Minn.) of either $15 \pm 5 \mu\text{m}$ or $9 \pm 1 \mu\text{m}$ in diameter were suspended in solutions of either $.05 \pm .02\%$ or $.01 \pm .005\%$ Tween 80 in 10% dextran. Aliquots (0.7–2 ml) of these suspensions were injected in the left atrium, followed by 2–5 ml of physiologic saline. To determine if the vehicle, rather than the microspheres, was responsible for the alterations in cardiovascular hemodynamics, the supernatant derived from the suspensions by centrifugation was injected into the left atrium in different dogs. To determine if dextran alone was responsible, dextran was injected into the left atrium.

In a final series of experiments microsphere suspensions that were in $.05\%$ Tween 80 for several weeks were centrifuged. The supernatant was drawn off and replaced with saline. This procedure was repeated and the supernatant derived from the second centrifugation was injected into the left atrium of conscious dogs.

RESULTS

Two types of adverse reactions were observed. The first (*Type A*) was more severe and involved systemic as well as cardiac changes (Fig. 1). Left ventricular (LV) systolic pressure fell from 116 ± 4 to 85 ± 12 mmHg, while heart rate rose from 101 ± 7 to 146 ± 7 beats/min. LV end-diastolic pressure fell from 7 ± 1 to 3 ± 1 mmHg,

while LV end-diastolic dimension fell by $17 \pm 6\%$ from a control of 12.05 ± 1.38 mm and wall thickness rose by 13% . In the seven animals in which this type of reaction was observed, these changes occurred from 0.5 to 2.0 min after the injection, were all significant, $P < 0.01$, and returned to control between 2 and 25 min. More prolonged reactions were observed in animals not included in this study, where more than 2 ml of $.05\%$ Tween was injected in the left atrium.

The second type of reaction (*Type B*) involved only decreases in cardiac dimensions and LV end-diastolic pressure while LV systolic pressure and heart rate did not change significantly. In the six animals in which this reaction was observed LV end-diastolic dimension fell by $5.3 \pm 1.2\%$ from a control of 15.70 ± 0.99 mm and wall thickness rose by 5% while LV end-diastolic pressure fell from 10 ± 2 to 6 ± 1 mmHg. Peak dP/dt did not change significantly, which, in the face of reduced preload, may reflect a slight increase in the inotropic state. Figure 2 shows a typical *Type B* response. These changes occurred from 0.5 to 2.0 min and returned to control within 5 min.

The number of dogs exhibiting each type of reaction is shown in Table 1. Microsphere-free supernatant from centrifuged suspensions produced similar hemodynamic reactions as did the suspensions themselves, indicating that the Tween 80 solutions rather than the microspheres, per se, were responsible for the adverse reactions. This response was also obtained with Tween 80 solutions never in contact with microspheres. However, prolonged contact between Tween solutions and microspheres seemed to increase the probability of adverse reactions, since $.05\%$ Tween 80-microsphere suspen-

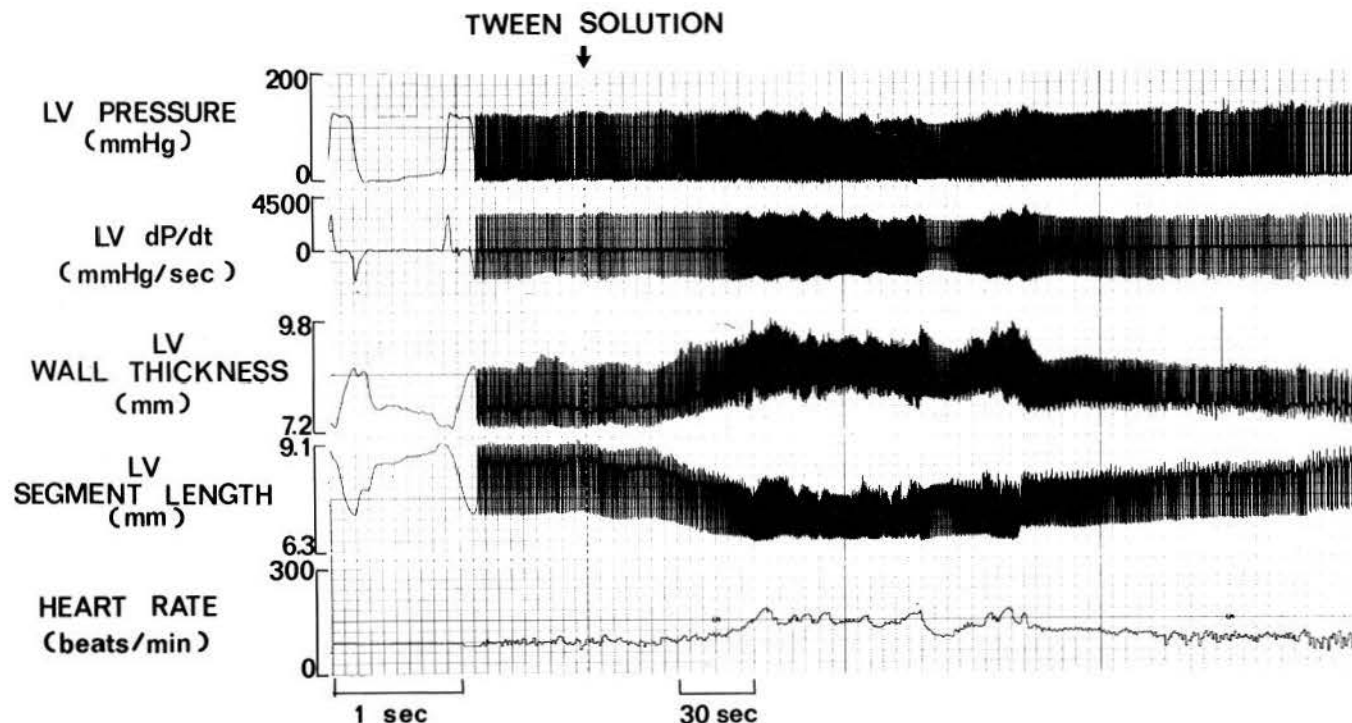


FIG. 1. Typical *Type A* response to injection of 0.05% concentration of Tween 80 in 10% dextran solution through a left atrial catheter is shown to induce hypotension, tachycardia, and a substan-

tial reduction in left ventricular end-diastolic segment length together with a reciprocal increase in wall thickness.

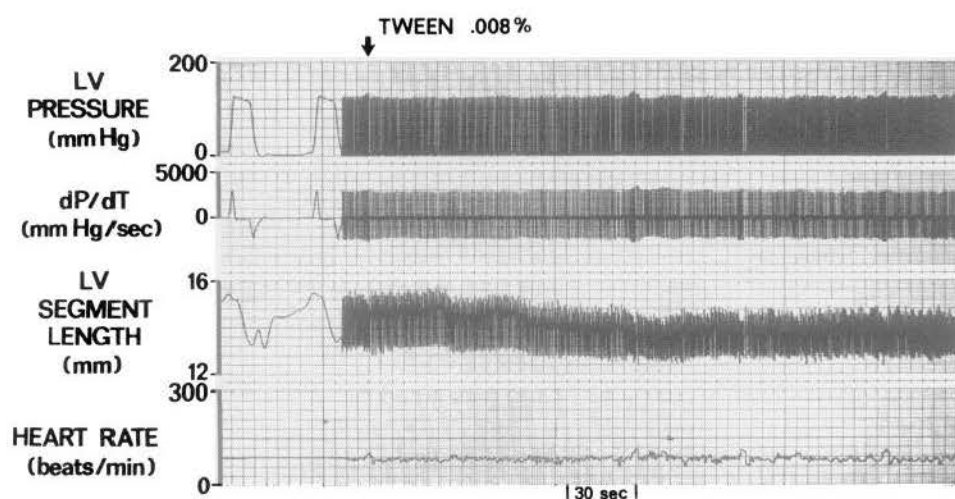


FIG. 2. *Type B* reaction showing a decrease in regional myocardial dimensions without associated changes in heart rate or left ventricular systolic pressure, when 0.01% concentration of Tween 80–10% dextran solution was injected into left atrium in a conscious dog.

TABLE 1. *Adverse reactions to Tween 80*

	Total Number of Animals Studied	Number of Reactions Observed	
		<i>Type A</i>	<i>Type B</i>
1) 0.05% Tween solution	4	2	2
2) Aged microsphere-Tween suspension*	8	2	1
3) 0.01% Tween solution with microspheres	25	3	2
4) 0.01% Tween solution	16	0	1

* Microspheres were suspended in a .05% Tween and held for 2–4 wk. Prior to injection, the microsphere suspension was centrifuged twice and replaced with saline (no Tween) each time. After the second saline replacement, the suspension was centrifuged and the supernatant was injected.

sions, which had been in contact for several weeks, but prior to injection had been centrifuged and washed twice with saline, still frequently produced adverse reactions (Table 1). Dextran alone did not produce detectable hemodynamic changes. When dry microspheres were freshly suspended in .01% Tween 80 solution, adverse reactions were rarely noted.

The occurrence of adverse reactions subsequent to .05% Tween 80 solution injections could not be repeated in the same animal on subsequent injections in the same day, despite return of cardiac and hemodynamic parameters to preinjection control values. However, 24–48 h after the initial reaction a second adverse reaction could be evoked with injection of Tween 80 solution.

DISCUSSION

Tween has been reported to have extremely low toxicity; subcutaneous injections in rats have failed to significantly increase mortality until a dose of 0.25 ml of 12% Tween 80 solution was injected each week for 27 wk (11). However, a related polyoxylated fatty acid derivative (Tween 20) has been reported to release endogenous stores of histamine, increase capillary permeability, and result in an anaphylactoidlike syndrome (4, 10). The similarity between these findings and those reported in this paper are noteworthy, especially considering the

Type A reaction, which involved hypotension. Hypotensive reactions subsequent to microsphere injections have been noted by others as well (5), but have not been attributed to the surfactant. Since Tween 80 solutions, both with and without microspheres, produced similar hemodynamic changes in the present study, it was concluded that the surfactant, rather than the microspheres, were responsible for the adverse reactions.

In addition to the reaction involving systemic hemodynamic changes (*Type A*), we observed milder adverse reactions involving a reduction in cardiac dimensions without detectable systemic effects (*Type B*). The effect of the surfactant would have been overlooked in these cases if cardiac dimensions were not measured (Fig. 2). Moreover, the effect would have been more difficult to observe in the open-chest anesthetized dog, where cardiac size is diminished (14). The observation in conscious dogs that cardiac size fell while left ventricular systolic pressure and heart rate did not change implies that wall tension fell, which would lower myocardial oxygen requirements and consequently alter myocardial blood flow. This latter aspect gains importance in view of the fact that suspensions of radioactive microspheres and Tween 80 are widely used to determine the distribution of intramyocardial blood flow (1,5,6,9).

The onset of the observed cardiac size changes or systemic hypotension occurred from 30 s to 2 min after the beginning of microsphere injections. Potential errors in regional myocardial flow determinations should be anticipated in cases in which the adverse effect had an early onset (30 s), but should be minimal in cases in which cardiovascular effects occurred later (2 min) since the microspheres should have been distributed prior to the change in hemodynamics. However, in both instances, subsequent flow determinations would be seriously compromised if an injection were undertaken during recovery from the adverse response. Subsequent injections of microspheres should be carried out only after cardiac size, arterial pressure, heart rate, and indices of contractility have returned to control, which in these animals occurred within 0.5 h, but in other animals occurred much later where more than 2 ml of .05% Tween was injected.

The observed adverse reactions to Tween-microsphere suspensions were determined to be due both to the amount of Tween injected and to the length of time that the Tween solution remained in contact with the microspheres. Accordingly, to avoid these adverse reactions, we use a minimal level of Tween 80 (.01%) to suspend the microspheres. The solution is then agitated for 30 min by ultrasonic probe and placed in an ultrasonic bath until the moment of injection. Dispersion of the microspheres is verified by microscopic examination. Since the length of time during which microspheres and su-

pernatant are in contact may affect the probability that an adverse reaction will occur, fresh microsphere suspensions are prepared every 3 days to minimize undesirable hemodynamic responses.

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REFERENCES

1. BECKER, L. C., R. FERREIRA, AND M. THOMAS. Effect of propranolol and isoprenaline on regional left ventricular blood flow in experimental myocardial ischemia. *Cardiovascular Res.* 9: 178-186, 1975.
2. BRAUNWALD, E., J. ROSS, JR., AND E. H. SONNENBLICK. *Mechanisms of Contraction of the Normal and Failing Heart*. Boston: Little, Brown, 1967.
3. BUCKBERG, G. D., J. C. LUCK, D. B. PAYNE, J. I. E. HOFFMAN, J. P. ARCHIE, AND D. E. PYLER. Some sources of error in measuring regional blood flow with radioactive microspheres. *J. Appl. Physiol.* 31: 598-604, 1971.
4. BURNELL, R. H., AND G. M. MAXWELL. General and coronary hemodynamic effects of Tween 20. *Australian J. Exptl. Biol. Med. Sci.* 52: 151-156, 1974.
5. COBB, F. R., R. J. BACHE, AND J. C. GREENFIELD, JR. Regional myocardial blood flow in awake dogs. *J. Clin. Invest.* 53: 1618-1625, 1974.
6. DOMENECH, R. J., J. I. E. HOFFMAN, M. I. M. NOBLE, K. B. SAUNDERS, J. R. HENSON, AND S. SUBIJANTO. Total and regional blood flow measured by radioactive microspheres in conscious and anesthetized dogs. *Circulation Res.* 25: 581-594, 1969.
7. FORSYTH, R. P., B. I. HOFFBRAND, AND K. L. MELMON. Redistribution of cardiac output during hemorrhage in the unanesthetized monkey. *Circulation Res.* 27: 311-320, 1970.
8. HEYNDRIKX, G. R., R. W. MILLARD, R. J. MCRITCHIE, P. R. MAROKO AND S. F. VATNER. Regional myocardial functional and electrophysiological alterations following brief coronary artery occlusion in conscious dogs. *J. Clin. Invest.* 56: 978-985, 1975.
9. KJEKSHUS, J. K. Mechanism for flow distribution in normal and ischemic myocardium during increased ventricular preload in the dog. *Circulation Res.* 33: 489-499, 1973.
10. MARKS, L. S., AND S. N. KOLMEN. Tween 20 shock in dogs and related fibrinogen changes. *Am. J. Physiol.* 220: 218-221, 1971.
11. MATSUYAMA, M., AND K. SUZUMORI. Effects of repeated subcutaneous injection of Tween 80 in rats. *Nagoya Med. J.* 12: 1-6, 1966.
12. PATRICK, T. A., S. F. VATNER, W. S. KEMPER, AND D. FRANKLIN. Telemetry of left ventricular diameter and pressure measurements from unrestrained animals. *J. Appl. Physiol.* 37: 276-281, 1974.
13. RUDOLPH, A. M., AND M. A. HEYMANN. The circulation of the fetus in utero. *Circulation Res.* 21: 163-184, 1967.
14. RUSHMER, R. F. Shrinkage of the heart in anesthetized, thoracotomized dogs. *Circulation Res.* 2: 22-27, 1954.