

# Experimental and laboratory reports

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## The use of radio-iodinated toluidine blue for myocardial scintigrams\*

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Although heart disease is the principal cause of death in many countries and its diagnosis is of major importance, methods for direct visualization of the myocardium in intact subjects are few. The roentgenogram of the heart shows the myocardium plus other tissues, especially the blood contained in the cardiac cavities, as a single shadow. Various techniques show cavities and vessels rather than the myocardium itself. On the basis of the important earlier studies which had demonstrated myocardial uptake of potassium and its analogues,<sup>1</sup> it was possible to develop a method for myocardial scintigrams,<sup>2</sup> using salts of cesium-131. This method permitted the demonstration of the left ventricular myocardium through the intact chest wall, without significant contribution to the image from the cardiac blood pool or adjacent non-cardiac tissues. After original development of the technique in dogs, it was extended to humans and the myocardial scintigram has subsequently been shown capable of demonstrating myocar-

dial infarcts,<sup>2-9</sup> aneurysm of the left ventricle,<sup>2,6</sup> cardiomyopathies,<sup>7,8</sup> neoplasm in the ventricular wall,<sup>10</sup> and rejection of the transplanted heart.<sup>11</sup>

However, the long biologic half-life of cesium creates difficulties, not only from the standpoint of radiation dose, but also through interference with performance of serial scintigrams. As skeletal muscles retain cesium much longer than cardiac muscle,<sup>12,13</sup> the uptake by intercostal muscles interferes with serial myocardial scintigrams during the days that follow injection of a single dose of cesium-131. Moreover, its principal emission, a 29.4 KEV x-ray, poses a considerable problem as a result of its low energy and consequent high absorption in tissues. Therefore, although cesium-131 has proved an important radionuclide by permitting the demonstration of the validity of the myocardial scintigram for diagnosis, the general clinical usefulness of the myocardial scintigram would be greatly increased by the availability of a more convenient radioactive compound for this

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purpose. Toluidine blue (tolonium chloride),  $C_{15}H_{16}N_3SCl$ , a phenazathionium dye, was found to concentrate selectively in parathyroid glands of dogs and man after intravenous or intra-arterial injection.<sup>14</sup> Toluidine blue has subsequently been found to concentrate heavily in myocardium of dogs after intravenous injection.<sup>15</sup> Iodotoluidine blue, labeled with iodine-131 or iodine-125, was suggested as a potential radioactive imaging agent for parathyroid scintigrams on the basis of such distribution studies.<sup>16</sup> Moreover, a preliminary study<sup>17</sup> showed that unlabeled toluidine blue (hereafter abbreviated TB) was present in decreased concentration in myocardial infarcts, as compared to normal myocardium, after intravenous injection in dogs. As the possibility of obtaining myocardial scintigrams with a compound of iodine-131 suggested a way of avoiding the problem posed by the weak emission of cesium-131, further investigation of radioactive iodotoluidine blue (hereafter abbreviated as ITB) as a myocardial imaging agent seemed indicated.

But initial studies with ITB were reported as discouraging by other authors, as they did not confirm a significant selective uptake of ITB by parathyroid glands<sup>18-20</sup> or myocardium<sup>18,20</sup> of rats after intravenous injection of the radioactive compound. It is of interest that the use of a preliminary priming or "loading" dose of the stable compound, TB, before injection of the radioactive compound, ITB, was not reported in any of these negative studies.

We have now demonstrated, in both rats and dogs, a significant selective uptake of ITB by myocardium when the administration of this radioactive compound is first preceded by a priming dose of the stable compound TB. This uptake by normal myocardium permits demonstration of myocardial infarcts as "cold" areas of decreased uptake in dogs. We have further demonstrated that the major fraction of administered radioactive iodine given as ITB is excreted with sufficient rapidity to give this compound significant advantage over radioactive cesium as a myocardial imaging agent if serial scintigrams are desired.

## Methods

Toluidine blue (TB) USP, was dissolved in isotonic saline solution in appropriate concentrations to provide the required dose when given at a rate of 0.02 ml. per minute to rats and 1 ml. per minute to dogs. Iodinated toluidine blue (ITB), labeled with either iodine-131 or iodine-125, was obtained\* in specific activities ranging from 0.3 to 1.2 millicuries per milligram. The dose of ITB, given as a bolus intravenously, was 60 microcuries per kilogram of body weight and 20 microcuries per kilogram of body weight for rats and dogs, respectively. Rats used in this study weighed from 153 to 410 grams. Dogs weighed from 8 to 17 kilograms.

*Studies of the effect of priming doses of stable TB on subsequent distribution of radioactive ITB.*

**RATS.** Forty-nine rats were divided into 7 groups of 7 each and were anesthetized with chloral hydrate. To establish the effect of TB, doses ranging from 0 to 10 mg. per kilogram of body weight were administered in isotonic saline through a tail vein at a rate of 0.02 ml. per minute over various time periods. One group of rats served as controls, receiving no TB; this control group received isotonic saline alone prior to the administration of ITB. Immediately following the completion of the infusion of the priming dose, all rats were given ITB. The rats were killed 15 minutes after administration of ITB. Tissue samples from all major thoracic and abdominal organs, including 4 samples of left ventricular myocardium, were taken from each rat. The samples were weighed and counted in a scintillation detector.

**DOGS.** Eight dogs were divided into 4 groups of 2 each. Priming doses of TB ranging from 1 mg. per kilogram of body weight to 10 mg. per kilogram of body weight were given intravenously at an infusion rate of 1 ml. per minute over a period of 45 minutes. Following completion of the infusion of TB, ITB was given intravenously to all dogs. The dogs were killed 15 minutes after administration of ITB. Tissue samples were taken from all the

\*We are grateful to Gerald Bruno of E. R. Squibb and Sons, who generously supplied the radioiodinated toluidine blue.

major thoracic and abdominal organs, including six samples of left ventricular myocardium from each dog. The samples were weighed and counted in a scintillation detector.

*Studies utilizing a fixed priming dose of stable TB.* Following the experiments described above, a standard priming dose of 10 mg. per kilogram of body weight, given intravenously over a period of 45 minutes, was selected. Except as otherwise specified, all studies described below utilized this priming dose for both rats and dogs. The bolus dose of ITB was given immediately after the conclusion of the infusion of TB.

*Retention of radioactivity by myocardium and whole body of dogs after injection of ITB.* Eight dogs were divided into four groups of two each. Each dog received a priming dose of TB and a subsequent injection of ITB, as described above. These dogs were killed at periods ranging from 15 to 90 minutes after administration of ITB. Tissue samples, including six samples from left ventricular myocardium, were removed from each dog and were weighed and counted in a scintillation detector.

For an estimation of whole body biologic half-life of radioactive iodine when given as ITB, three additional dogs were given TB and ITB. The dogs were housed in metabolic cages; excreta were collected and counted daily. The dogs were killed from 7 to 11 days later. A complete set of over 30 tissues was removed from each dog. Appropriate samples were weighed and counted in a scintillation detector. Wherever possible, whole organs were weighed; otherwise, the weight of whole organs was estimated from a standard reference table.<sup>21</sup>

Two other dogs received TB and ITB. They were studied serially by whole body scintigrams, using an Ohio Nuclear Scanner with 5-inch crystal, 85-hole collimator, and scan minification recording attachment, permitting automatic reduction of the scintigram to one-fifth original size. The serial whole body scintigrams were obtained at various times ranging from one-half hour to 5 days after injection of ITB.

*Myocardial scintigrams.* Eleven normal dogs received TB and ITB. Approximately 15 minutes after injection of ITB, the dogs were scanned under pentobarbital anes-

thesia in the supine position with the legs extended. In some instances they were subsequently scanned in the left lateral position also. For these scintigrams a Baird-Atomic Scanner with 3-inch crystal and 19-hole focusing collimator was used. The scanning speed ranged from 7.5 to 10 inches per minute with the scanner set for sharp contrast ("per cent background" setting at 80). Further details of the techniques employed have been previously described.<sup>2</sup>

Experimental myocardial infarcts were induced in 11 dogs by ligation of the anterior descending branch of the left coronary artery under sodium thiamylal anesthesia. (Except in the two instances noted below, none of these dogs had previously been studied among the 11 normals described in the previous paragraph.) After ligation of the coronary artery, the chest wall incision was closed and each animal was allowed to recover. Twenty-four hours after coronary artery ligation each dog was again anesthetized, using pentobarbital. Each dog received 10 mg. per kilogram of body weight of TB over a period of 45 minutes followed by 20 microcuries per kilogram of body weight of ITB intravenously as a single bolus dose. Fifteen minutes after the administration of the radioactive compound each dog was killed by an excessive intravenous dose of barbiturate. Myocardial scintigrams were then performed, using the same scanning factors employed for the normal dogs that had been scanned alive. After completion of the scintigrams, the heart was removed from each killed dog, emptied of all blood, and scanned again as an isolated organ. Samples of auricles, right ventricle, lung, liver, rib, muscle, skin, and fat were taken for scintillation counting. Three samples of myocardium from areas of anterior left ventricle distal to the site of arterial ligation and three control samples (one from a site proximal to ligation and two from posterior left ventricle areas not dependent upon the ligated artery for their blood supply) were also taken; these were each divided to permit histologic examination as well as scintillation counting. Further details of the techniques employed have been previously described.<sup>22</sup> Three of the 11 normal dogs described above were subjected to coronary artery ligation 4 days

Table 1. Radioactivity in tissues of rats given various priming doses of stable toluidine blue before radio-iodinated toluidine blue\*

Priming dose (mg./kg.) and duration of infusion	Radioactivity $\frac{(\% \text{ administered dose}^\dagger)}{\text{Gm. tissue}}$ in		
	Left ventricle	Liver	Blood
Saline control	0.34‡	0.81	0.14
5 in 15 minutes	0.49	0.43	0.10
5 in 30 minutes	0.32	0.45	0.09
5 in 90 minutes	0.59	0.44	0.08
10 in 45 minutes	1.20§	0.62	0.10
10 in 90 minutes	0.82§	0.36§	0.06§
10 in 180 minutes	0.78	0.32§	0.07§

\*See text for further details.

†In this and subsequent tables the administered dose used in the calculation is the number of microcuries given *per kilogram* of body weight.

‡Each value represents the mean of 7 rats.

§Differs significantly ( $p < 0.05$ ) from control.

after a normal myocardial scintigram had been obtained in the living dogs. Two of these 3 were among the 11 dogs that received a dose of TB and ITB 24 hours after ligation and underwent myocardial scanning after killing. The purpose of sacrificing dogs with myocardial infarcts before scanning was to insure that the subsequent scintillation counting of tissue samples would permit comparable data to be obtained among 11 dogs that had all lived 15 minutes after injection of the radio-nuclide. However, one normal dog that subsequently underwent coronary artery ligation and received TB and ITB 24 hours later was not killed. This permitted a scintigram of the infarct to be obtained in a living dog. Although tissue data from this dog could not, therefore, be compared with data obtained from the 11 dogs killed 15 minutes after ITB injection, this additional dog was killed at the conclusion of the myocardial scanning in order to permit histologic confirmation of infarction.

## Results

*Studies of the effect of priming doses of stable TB on subsequent distribution of radioactive ITB.*

RATS. Table I compares the distribution of ITB in rats given various priming doses of TB over various periods of time before injection of ITB. Analysis of variance was

first performed on all uptakes of ITB by left ventricle, to determine whether any significant difference existed among the 7 means—i.e., to determine whether the use of any priming dose significantly increased uptake over control. After this analysis had demonstrated a significant ( $p < 0.05$ ) difference, further analysis was carried out by Duncan's multiple range test to determine which of the means differed significantly from the control. The latter test demonstrated a significantly higher ( $p < 0.05$ ) uptake of radioactive iodine by left ventricle in rats given 10 mg. per kilogram of body weight of TB over a 45 minute period, when compared to controls. The respective uptakes were  $1.20 \pm 0.66$  per cent administered dose per gram of tissue and  $0.34 \pm 0.13$  per cent administered dose per gram. Similarly, the uptake by left ventricle in rats receiving 10 mg. per kilogram of body weight TB over 90 minutes ( $0.82 \pm 0.38$  per cent administered dose per gram) was significantly ( $p < 0.05$ ) higher than uptake by controls. Uptake by left ventricle of rats receiving 10 mg. per kilogram of body weight over 180 minutes, and uptake by left ventricle of rats receiving priming doses of 5 mg. per kilogram of body weight, did not differ significantly from controls.

Similar statistical analysis, using the multiple range test, showed that the uptake of radioactive iodine by liver was signifi-

Table II. Radioactivity in tissues of dogs given various priming doses of stable toluidine blue before radio-iodinated toluidine blue\*

Priming dose (mg./kg.)	Radioactivity $\left(\frac{\% \text{ administered dose}}{\text{Gm. tissue}}\right)$ in			Ratio of radioactivity	
	Left ventricle (LV)	Blood (B)	Liver (L)	$\frac{LV}{B}$	$\frac{LV}{L}$
1	0.78†	0.11	1.28	7.1	0.6
3	1.35	0.10	1.16	13.5	1.2
5	1.61	0.10	1.49	16.1	1.1
10	1.56	0.12	0.98	13.0	1.6

\*See text for further details.

†Each value represents the mean of 2 dogs.

cantly ( $p < 0.05$ ) less in rats receiving a priming dose of TB, 10 mg. per kilogram of body weight over 90 minutes, or 10 mg. per kilogram of body weight over 180 minutes, as compared to controls. No other treatment resulted in a significant difference from control values for hepatic uptake. The radioactivity of blood followed the pattern seen in liver, with significant ( $p < 0.05$ ) decreases in rats receiving priming doses of 10 mg. per kilogram of body weight over 90 minutes, or 10 mg. per kilogram of body weight over 180 minutes, as compared to controls.

Except for liver, uptake by non-cardiac tissues adjacent to the heart was not sufficiently high in rats given priming doses of TB to lead to significant interference with the use of ITB as a myocardial imaging agent (see below).

DOGS. As with rats, the most important uptake by an organ adjacent to the heart, likely to cause interference with the myocardial scintigram, was uptake by liver. Table II shows the radioactivity in left ventricle, liver, and blood of dogs receiving various priming doses of stable TB. The use of a priming dose increases the ratios, radioactivity in left ventricle/radioactivity in blood, and radioactivity in left ventricle/radioactivity in liver, thus increasing the likelihood of good resolution of the left ventricle by scanning techniques. In dogs

this effect of a priming dose is chiefly by way of increased uptake by left ventricle.

*Studies utilizing a fixed priming dose of stable TB.*

RETENTION OF RADIOACTIVITY BY MYOCARDIUM AND WHOLE BODY OF DOGS AFTER INJECTION OF ITB. The concentration of radioactivity in left ventricle of dogs at various times after injection of ITB is shown in Fig. 1. The high uptake of radioactivity obtained when a priming dose of TB is followed by ITB does not lead to prolonged retention of radioactivity in the myocardium.

From the eight actual measurements obtained during the first 90 minutes after injection, the equation shown in Fig. 1 was derived.\* The curve of this equation is also shown in the illustration. The uptake at 24 hours after injection, calculated from this equation, is shown by the dot at the extreme right of Fig. 1. A ninth dog was actually studied at 24 hours and the uptake found in this dog is shown by a bar at the extreme right. The calculated and determined points agree very well. Despite the rapid decay of myocardial radioactivity during the first 90 minutes after injection, the ratio of myocardial radioactivity to radioactivity in the blood (not shown in Fig. 1) remained  $>4$  at all times during the

\*We acknowledge with thanks the assistance of Dr. John G. Wagner.

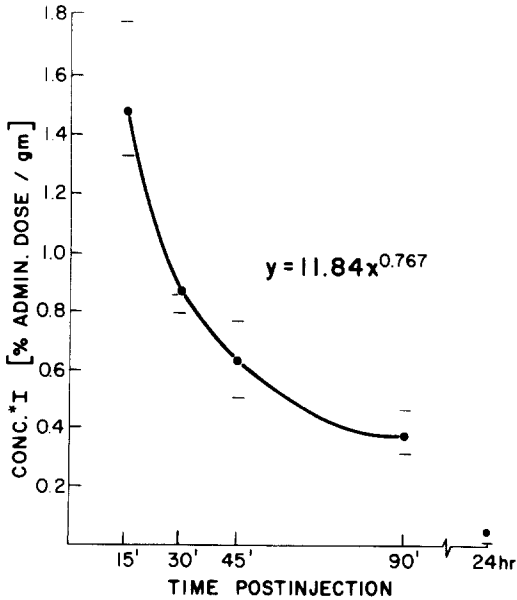


Fig. 1. Concentration of radioactive iodine in the left ventricle of dogs at various times after intravenous injection of ITB. Horizontal lines (—) indicate measured values. The dot at 24 hours represents a calculated value. See text for details.

first 90 minutes. The average of this ratio for the eight points during that time was 10.9.

The retention of radioactivity in dogs, as calculated from daily urine and stool collections, is shown in Fig. 2. At the time of death, the total radioactivity remaining in the carcass of dog A (killed at 7 days), dog B (killed at 10 days), and dog C (killed at 11 days) was 3.28, 6.15, and 3.84 per cent of administered dose, respectively. By the usual curve-stripping method, the retention curve of radioactivity was separable into two components, as shown in Fig. 2. The first component had a half-life of 0.6, 0.6, and 0.7 days, respectively, in the 3 dogs (mean, 0.63 days). The second component had a half-life of 24, 32, and 28 days, respectively, in the 3 dogs (mean, 28 days). Extrapolation of the second component back to the origin indicated that 24 per cent, 31 per cent, and 20 per cent of the total dose was retained in the 3 dogs, respectively, with a mean biologic half-life of 28 days. In estimating whole body radiation dose, we have made the conservative assumption that the residual in the carcass would not have been excreted and would have been lost only through physical decay.

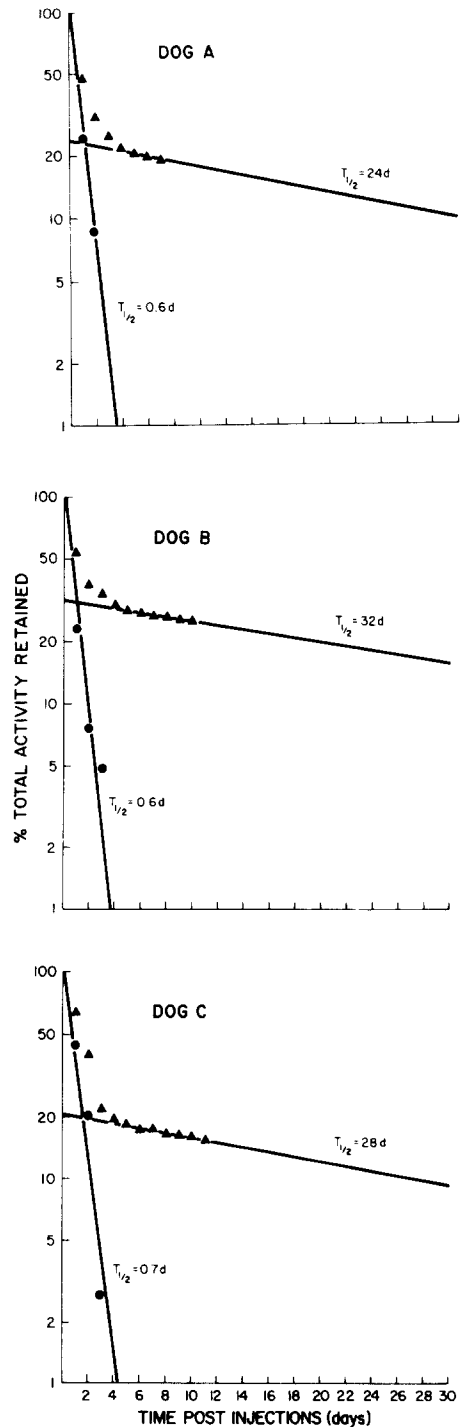


Fig. 2. Whole-body retention of radioactivity by dogs given ITB.

As it was possible to account for a mean of 84 per cent of the administered dose of radioactive iodine through the total collected in urine and stools and the analysis of residual radioactivity in the carcass,

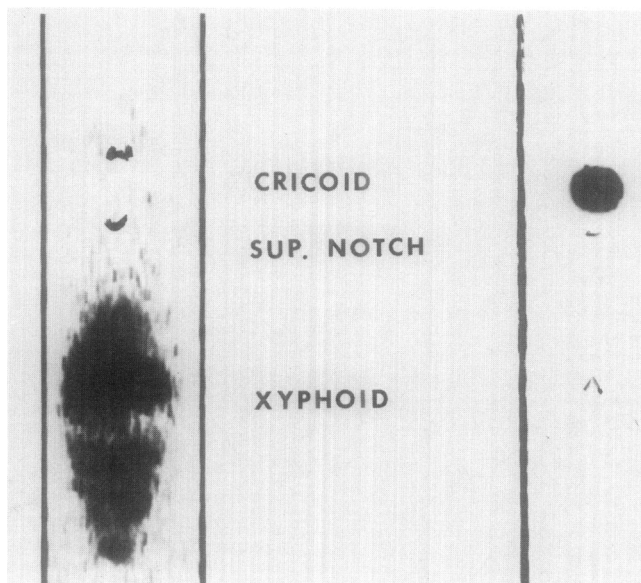


Fig. 3. Whole-body scintigrams of Dog No. 1 given ITB. Thirty minutes after injection of the radioactive compound, scanning started at the head and proceeded caudally. The results are shown on the left. The scintigram on the right shows the same dog, scanned 5 days later.

calculation of whole body radiation dose, using the formulas of Quimby and colleagues<sup>23</sup> requires an assumption regarding the fate of the 16 per cent which was not found in collected urine, stools, and residual carcass. In the "best possible case," one assumes that this radioactivity was lost very early after administration of the ITB, when small errors in collection of the highly radioactive excreta would result in significant losses. In the "worst possible case" one assumes that the entire 16 per cent was actually in the carcass, in addition to the approximately 4 per cent found by analysis. Thus, in the "best possible case" a mean of 71 per cent of total radioactivity was excreted as a short half-life component, with a biologic half-life of 0.63 days, 25 per cent was excreted with a biologic half-life of 28 days, and 4 per cent remained to be eliminated by physical decay. The respective figures for the "worst possible case" are 55 per cent, 25 per cent, and 20 per cent.

These data in dogs are useful for estimating expected radiation dose in man following the use of ITB. Although species differences obviously may exist and cannot be resolved until studies are conducted in man, this preliminary estimation may be of interest. The whole-body combined beta

and gamma radiation dose, for a 70 kilogram man receiving 1 microcurie per kilogram of body weight of ITB, labeled with iodine-131, after an appropriate priming dose of stable TB, is estimated, on the basis of the above assumptions, as 53 millirads in the "best possible case" and 73 millirads in the "worst possible case."

Serial whole-body scintigrams of a dog given 10 mg. per kilogram of body weight of TB followed by ITB are shown in Fig. 3. The scintigrams suggest disappearance of most of the radioactivity from the body within a few days. The results obtained in a second dog subjected to serial scintigrams were very similar.

*Myocardial scintigrams.* Figs. 4 and 5 show anterior views of normal and infarcted myocardium, respectively. Although our tissue studies have repeatedly shown that uptake by normal right ventricle and atria is comparable to uptake by normal left ventricle, the myocardial scintigram results almost exclusively from radiation emanating from the left ventricle, as a consequence of the preponderant mass of the latter. Figs. 6 and 7 show lateral views of normal and infarcted left ventricle, respectively. These four scintigrams were obtained in 4 different dogs. Fig. 8, however,

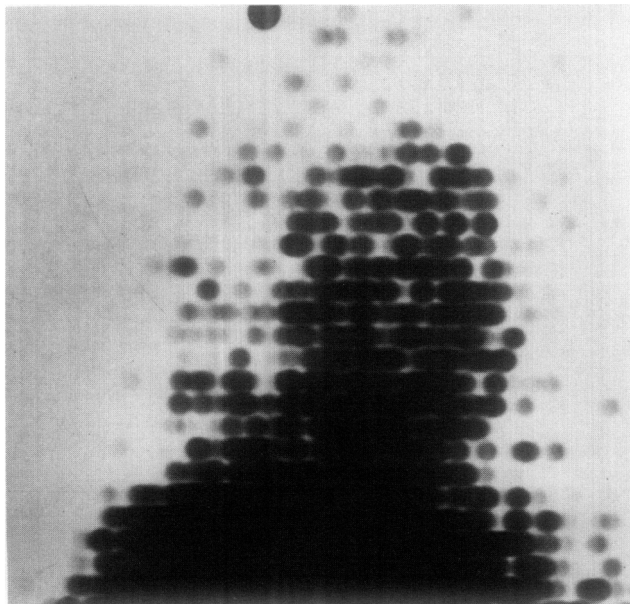


Fig. 4. Myocardial scintigram of a normal dog heart (Dog No. 2). Anterior view. For this and all subsequent figures a priming dose of stable TB was given intravenously over a 45 minute period, followed by a bolus injection of radioactive ITB: scintigrams began 15 minutes after the latter injection. Except where otherwise specified, the dose of TB was always 10 mg. per kilogram of body weight and each dog was killed at the beginning of the scintigram.

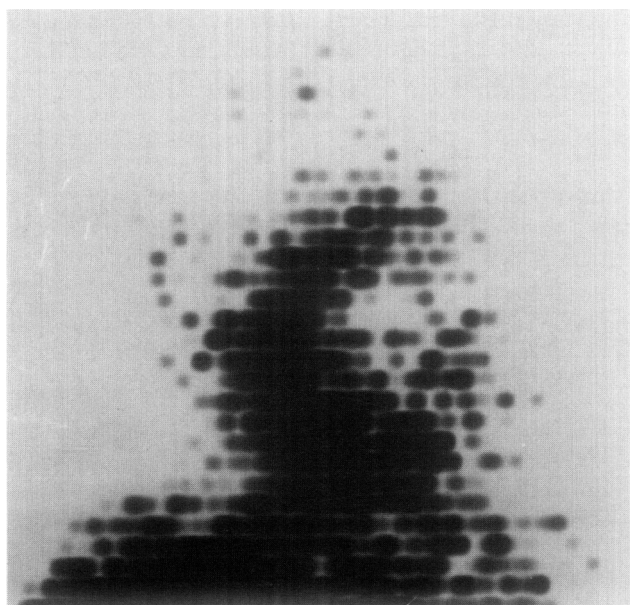


Fig. 5. Scintigram of Dog No. 3, showing a myocardial infarct. Anterior view. Infarcts shown in this and all subsequent figures were confirmed by histologic examination.

shows anterior scintigrams obtained in the same dog before and one day after experimental myocardial infarction. Fig. 9 shows lateral scintigrams obtained in still another dog, before and one day after experimental

infarction. Although the ratio, concentration of radioactivity in normal left ventricle/concentration of radioactivity in whole blood, varies among individual dogs and varies to some degree with time after



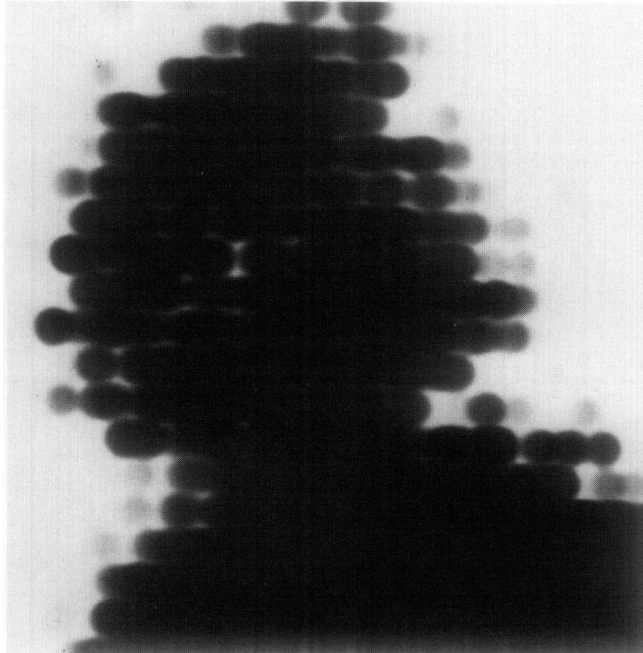


Fig. 6. Scintigram of a normal dog heart (Dog No. 4). Lateral view. The priming dose of TB was 5 mg. per kilogram of body weight in this dog.

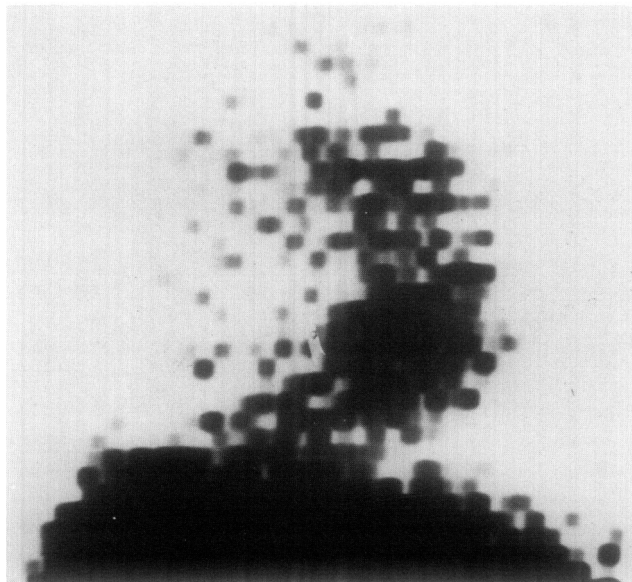


Fig. 7. Scintigram of Dog No. 5, showing a myocardial infarct. Lateral view.

injection of ITB, the mean ratio at time of death in these studies was approximately 12 to 1. The scintigram after administration of ITB in these experiments represents primarily radioactivity from the myocardium, with little contribution from the low level of activity contained in the blood

in the heart cavities. This was repeatedly confirmed by scintigrams of the isolated hearts, removed from the dog and emptied of all blood. The emptied hearts gave scintigrams essentially the same as hearts which contained blood in their cavities, showing normal left ventricle in the normal

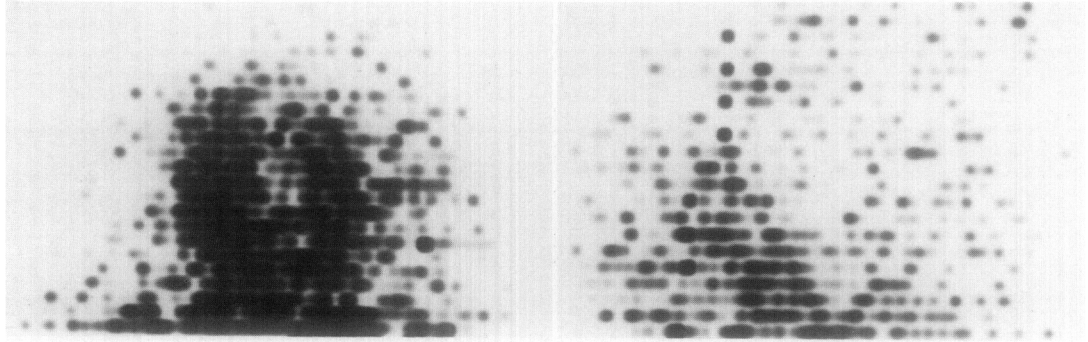


Fig. 8. Myocardial scintigrams of Dog No. 6 before and after myocardial infarction. Anterior view. The scan on the left was obtained one day before infarction. The scan on the right was obtained 4 days after infarction. Both scintigrams were performed in the living dog.

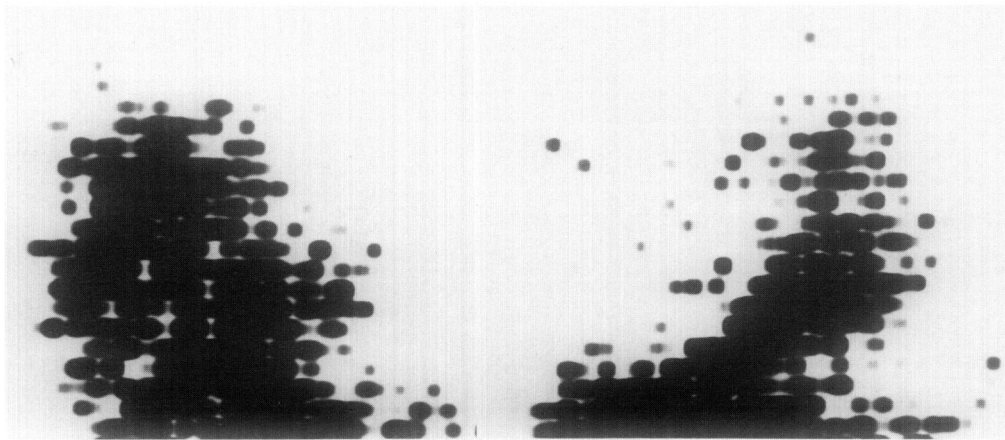


Fig. 9. Myocardial scintigrams of Dog No. 7 before and after myocardial infarction. Lateral view. This dog was studied in the same manner as the dog shown in Fig. 8. This dog was alive during the performance of the scan on the left and was killed just before performance of the scan on the right.

dogs and "cold" areas of decreased uptake in dogs with myocardial infarcts (Fig. 10).

Table III summarizes the data obtained in 11 dogs with myocardial infarctions. Actual necrosis of tissue was histologically confirmed in every instance. The mean ratio, concentration of ITB in infarcts/concentration in normal left ventricle, was  $0.18 \pm 0.31$ . The difference in uptake by normal and infarcted tissue was significant ( $p < 0.005$ ) by the "t" test.

### Discussion

Both iodine-131 and iodine-125 were used as labels in these experiments in animals. The availability of both labels for ITB offers in itself the possibility of serial

scintigrams within a short interval of time, as one may easily detect iodine-131 while discriminating against iodine-125. The usefulness of both of these isotopes in laboratory animals should not obscure the fact that iodine-131, with its more penetrating emission, is likely to be the better isotope in man. The emission of iodine-125 would not represent a significant advantage over that of cesium-131, while iodine-131 has a significant advantage in this respect. The rapid disappearance of radioactivity through excretion is an additional advantage, permitting serial scintigrams without excessive radiation dose. The rapid disappearance of radioactivity from the myocardium strongly suggests that serial

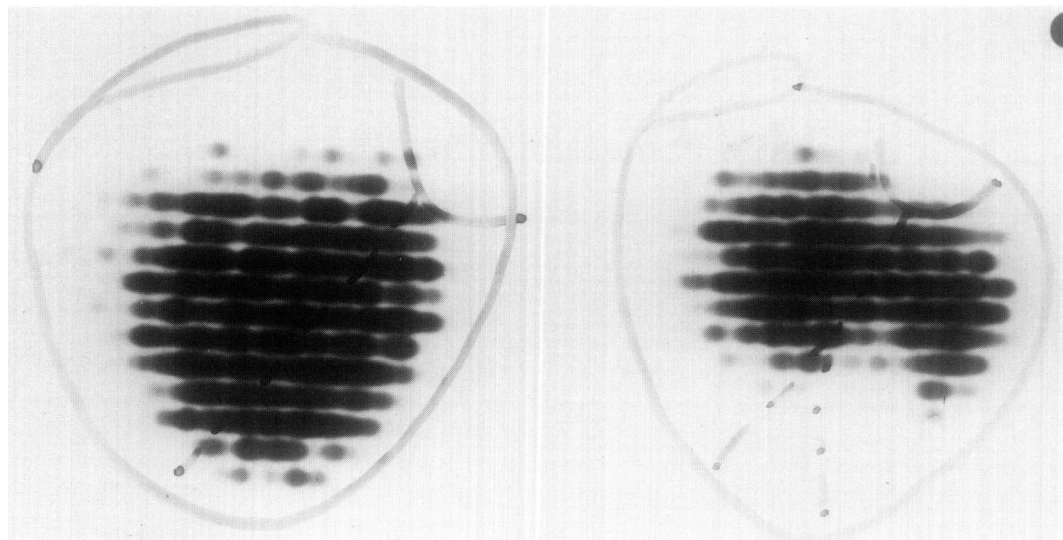


Fig. 10. Myocardial scintigrams of isolated hearts. *Left*: normal heart. *Right*: heart showing myocardial infarct at the apex. A normal dog and a dog with a myocardial infarct, respectively, each received TB and ITB. The respective TB doses were 1 mg. per kilogram of body weight and 10 mg. per kilogram of body weight. Fifteen minutes after injection of ITB the dogs were killed and their hearts were removed. After all blood had been emptied from the heart cavities, as previously described,<sup>22</sup> the hearts were scanned. The actual outlines of the heart are projected on the scan. The density represents left ventricular muscle.

scintigrams may be obtained by repeated doses of ITB, even if labeled with iodine-131.

Although the rapid disappearance of radioactivity from the myocardium after administration of ITB requires that scintigrams be performed promptly after administration of ITB, this has not caused significant problems. The radioactivity of the normal left ventricle remains much higher than the radioactivity of infarcted areas or of blood, even several hours after administration of ITB—i.e., there is a comparable decline of radioactivity in all three types of tissue. Therefore, the ratio of radioactivity in target tissue to that in non-target tissue, the most important factor in permitting a good scintigram, remains very favorable for a considerable period of time.

Thus the use of ITB offers the possibility of myocardial scintigrams that do not suffer from either of the two disadvantages of cesium-131, weak emission and long biologic half-life. Cesium-131 has already been shown to be effective in man in demonstrating myocardial infarcts and other myocardial lesions; the possibility of obtaining myocardial scintigrams with the more advantageous radioactive compound may significantly improve diagnosis of

myocardial infarction in the future and may make it possible to evaluate more precisely therapeutic regimens in myocardial infarction. It is of interest that the mean uptake of infarcted myocardium is about 15 per cent the uptake by normal myocardium, whether one uses labeled toluidine blue or radioactive cesium.

To our knowledge, the closest previous approach to demonstration of myocardial infarcts using a compound labeled with radioactive iodine was that reported by Gunton and associates,<sup>24</sup> who reported that oleic acid labeled with iodine-131 could be complexed to human serum albumin and utilized in a technique which, according to the authors' report, just approached the limits of definition of infarction when used for scintigrams in acute cases. As noted above, iodine-131 is likely to be a very satisfactory label for ITB. We have not thus far attempted to use iodine-123. However, despite the short physical half-life of iodine-123, its emission has a particularly favorable energy (159 KEV) for use with a radiation camera. Therefore, the eventual possibility of using iodine-123 should not be entirely discounted, especially if one wishes to use a radiation camera rather than a scanner.

Table III. Radioactivity in hearts of dogs with myocardial infarcts\*

Dog	Uptake† by		Uptake by normal LV Uptake by infarcted LV	Radioactivity in normal LV radioactivity in blood
	Normal LV tissue	Infarcted LV tissue		
A§	0.88	0.11	0.13	13.62
B§	0.98	0.10	0.10	13.07
C§	0.30	0.015	0.05	10.24
D	0.28	0.31	1.11	9.83
E	1.48	0.07	0.05	9.16
F	1.22	0.22	0.18	19.62
G	0.87	0.14	0.16	9.34
H	0.93	0.075	0.08	7.15
I	1.36	0.06	0.04	8.80
J	0.51	0.006	0.01	18.92
K	0.29	0.02	0.07	8.29
Mean ± S.D.	0.83 ± 0.43	0.10 ± 0.09‡	0.18 ± 0.31	11.64 ± 4.23

\*Each dog received 10 mg. per kilogram of body weight stable toluidine blue over 45 minutes preceding the bolus dose of radio-iodinated toluidine blue. Each dog was killed 15 minutes after the latter dose. See text for further details.

†Per cent administered dose/Gm.

‡Differs significantly ( $p < 0.005$ ) from normal left ventricular tissue.

§Not the same dogs as dogs A, B, and C of Fig. 2.

The mechanism whereby a priming dose of TB, first suggested by one of the authors (W. DiG.), influences the distribution of ITB is at present unknown. Both the rate of administration and the total dose of stable TB are significant, as suggested by the studies in rats. The use of a priming dose of TB does not increase the concentration of radionuclide in important non-target tissues (liver and blood). Indeed, the data in rats suggest lower radioactivity in these non-target tissues as a result of a priming dose of TB. It should be noted that the use of a priming dose does not increase the uptake of radionuclide by various other non-cardiac tissues after administration of ITB (e.g., muscle, lung, etc.) but data on liver and blood have been presented here, as they represent particularly important non-target tissues in the myocardial scintigram.

One may postulate two types of binding site for the phenazathionium nucleus, such that one of the sites has a lower capacity but higher affinity than the other. Saturation of the first site—e.g., in liver and blood—by a priming dose of TB might then make more ITB available for the second site, in the myocardium. Such a mechanism is at present entirely speculative although the

reported electrocardiographic changes after an intravenous injection of TB (see below) may be pertinent here.

Toluidine blue has been used intravenously in man<sup>14,15,25,26</sup> in the past and has not proved to be a highly toxic compound. Kiese and associates<sup>25</sup> gave 4 mg. per kilogram of body weight of TB intravenously to humans without ill effect. Doses of 6 mg. per kilogram of body weight have been given safely to humans intravenously<sup>26</sup>; these doses were given over a period of 2 hours. Nevertheless, rapid infusion of a sufficiently high dose may result in electrocardiographic changes. Whereas eight patients studied by DiGiulio and Lindenauer<sup>15</sup> received 5 mg. per kilogram of body weight of TB intravenously over periods ranging from 45 to 135 minutes without reported electrocardiographic abnormalities, one patient who received 3.5 mg. per kilogram of body weight in 30 minutes did show transient depression of the S-T segment.

The development of cardiac arrhythmias after diagnostic procedures is by no means unknown, and even the barium enema is not innocuous in this regard,<sup>27</sup> but particular caution is clearly needed to avoid precipitating cardiac arrhythmias in patients already suffering from myocardial infarcts.

Therefore, these data are recorded only to suggest that the requirement of a priming dose of stable toluidine blue need not pose an insuperable barrier to the use of ITB as a myocardial scanning agent. Although the animal studies reported here are promising, the authors do not, of course, advocate that any initial trial in humans utilize 10 mg. per kilogram of body weight as a starting dose, regardless of the rate of infusion. The same general rules that govern early human trials of therapeutic compounds must govern trials of diagnostic agents—the initial approach must be cautious, utilizing low doses. Moreover, studies in normal subjects should precede studies in patients.

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