RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Technetium-Labeled Heparin: Preliminary Report of a New Radiopharmaceutical with Potential for Imaging Damaged Coronary Arteries and Myocardium

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Heparin has been labeled with [^{99m}Tc] pertechnetate and its ability to image damaged coronary vessels and myocardium during and following myocardial ischemia has been studied in experimental animals. The data obtained indicate that Tc-99m heparin localizes in damaged myocardium and coronary vessels in canine models of temporary myocardial ischemia and reperfusion and in damaged myocardium during fixed coronary occlusion. Scintigraphic detection of damaged myocardium was possible in both models, but the highest levels of Tc-99m heparin in damaged myocardial tissue were found in those dogs with temporary coronary occlusion and reflow. The data suggest that Tc-99m heparin may be of value as a positive imaging agent when coronary arteries or myocardium are injured and either reperfusion is allowed and/or significant blood flow persists in the damaged area.

J Nucl Med 19: 810–815, 1978

In one of our laboratories there is strong interest in identifying venous thromboembolism in a positive manner with radiolabeled heparin. It is known that damage to vascular endothelium changes the normal negative surface charge to a positive one (*1,2*). We postulated that this phenomenon would provide damaged endothelium with an affinity for highly negatively charged molecules such as heparin. We also wished to determine whether damaged myocardium might be visualized scintigraphically using radiolabeled heparin. Consequently, we have labeled heparin and studied its ability to indicate damaged coronary-artery endothelium and myocardium in scintigrams following temporary and fixed coronary-artery ligation in experimental animals.

MATERIALS AND METHODS

Preparation of Tc-99m heparin. Heparin from hog intestinal mucosa (No. 8013, 20,000 units per ml, sterile solution)* was used for labeling and for subsequent injection into the animals for myocardial imaging studies. The Tc-99m was obtained from generators. Crystalline $SnCl_2 \cdot 2H_2O$ was procured commercially, and other chemicals used in the preparations were standard laboratory reagents.

The pH of 0.1–1.0 ml of heparin was adjusted to 1.5–2.5 with 0.1 *N* HCl. One milligram of $SnCl_2 \cdot 2H_2O$ in 0.5 ml of 0.1 *N* HCl was added to the acidified heparin solution. The desired amount of $^{99m}TcO_4^-$ in saline (0.1–2.0 ml) was added and the reaction mixture allowed to stand for about 15 min. In these studies, 50 mg of heparin was labeled with 50–60 mCi of reduced Tc-99m. Next, the pH was slowly adjusted to approximately 5.5 with 0.1 *N* NaOH. The preparation was then diluted with saline

Received Aug. 30, 1977; revision accepted Nov. 29, 1977.

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(3–7 ml) and passed through a 0.2-μ membrane filter.

The presence or absence of free pertechnetate in these preparations was determined by paper chromatography and by instant thin-layer chromatography, using both methylethylketone and acetone as the solvents. The effect of labeling on the anticoagulant property of heparin was studied with an APTT test kit (*3*). As we have previously reported (*4*), this kit determines the activated partial thromboplastin time (APTT).

Twelve mongrel dogs of both sexes weighing approximately 30 kg were anesthetized with sodium pentobarbital (30 mg/kg). An endotracheal tube was inserted and the animal placed on a Harvard respirator. The chest was opened through a left lateral thoracotomy and the proximal left anterior descending coronary artery (LAD) visualized and gently dissected free. The LAD was then occluded with a purse-string suture passed through a narrow plastic tube so that the artery could be pulled against the tube with the string and held firmly in place. After occlusion for 60–85 min, the artery was gradually released to allow return of blood through the artery. A venous catheter was placed in the jugular vein to permit the withdrawal of blood samples. The dog's chest was closed after releasing the LAD occlusion. Tc-99m-labeled heparin (3–6 mCi) was injected through the venous catheter within 5–10 min after coronary release. After injecting the radioactive heparin, the syringe was flushed with saline. Any residual radioactivity left in the syringe was measured with a radionuclide calibrator. The animal, kept lightly anesthetized with nembutal, was then immediately placed on a table under a scintillation camera with a high-resolution collimator and imaging was begun, accumulating 300,000 counts in each image. Serial imaging was obtained in the anterior, left anterior oblique, right anterior oblique, and left lateral positions. For blood clearance studies, one ml of blood was drawn for radioassay at 5, 10, 15, 20, 30, 40, 50, 60, 90, 150, and 180 min postinjection.

The animals were killed, 3½ hr after the injection of Tc-99m heparin, by overdose of sodium pentobarbital (1.1 g/kg). The heart was removed, washed with saline, and imaged by placing it in a tray under the scintillation camera, where 100,000 counts were collected.

Four mongrel dogs were anesthetized and otherwise prepared in a fashion similar to the temporarily occluded animals described above. In the new group, however, the LAD artery was permanently suture-ligated proximally; the animals were allowed to recover for 48 hr, at which time they were injected with the Tc-99m heparin. The same blood withdrawal and imaging sequence was performed as in the temporary-occlusion animals.

Assays for radioactivity. Each heart was divided into transverse slices, and multiple transmural blocks were obtained from three left-ventricular regions—namely, grossly normal posterior myocardium, anterior myocardium containing foci of gross damage, and grossly normal myocardium immediately adjacent to the damaged areas (border samples). Sections for histologic examination were removed from selected blocks and fixed in phosphate-buffered 10% formalin. Tissue from each block was divided into epicardial and endocardial halves and counted for radioactivity. Samples were also obtained from the LAD artery, above, at, and below the site of ligation, as well as others from the uninvolved circumflex artery. All samples of arteries were washed with saline, weighed, and subsequently counted for radioactivity. Samples for assay were obtained from other tissues, including bone, skeletal muscle from the thoracotomy site, normal skeletal muscle, liver, lung, kidney, pancreas, and bile.

All samples were placed into previously weighed counting tubes and weights determined. Technetium-99m emissions were quantitated with a gamma spectrometer. A standard was prepared by diluting a known amount of Tc-99m solution (0.1–1.0 mCi) to 500 ml with distilled water; 1.0 ml of the solution was counted along with the tissue and blood samples. Counts per minute per gram of sample were derived. The data for organ distribution were tabulated as gram percentage of administered dose (per gram of wet tissue weight) from standards of the administered Tc-99m. The data were also tabulated in terms of the ratio of the cpm per gram of sample to the cpm per gram of normal myocardium. The rate of clearance of Tc-99m heparin from blood was calculated as the gram percentage of injected dose in the blood at the various time intervals from 5 to 180 min postinjection.

RESULTS

Test for radiochemical purity. Tests for radiochemical purity of the Tc-99m-labeled heparin preparations showed that less than 5% of free pertechnetate was found in these preparations, and that the labeled heparin stayed at the point of application on the chromatography preparations. The absence of any colloidal material was ascertained by the quantitative passage of the labeled material through 0.1- or 0.2-μ membrane filters. No noticeable changes were observed in the anticoagulation property of heparin due to the labeling process as measured by the APTT test system.

Scintigraphic findings. Scintigrams (Fig. 1) showed

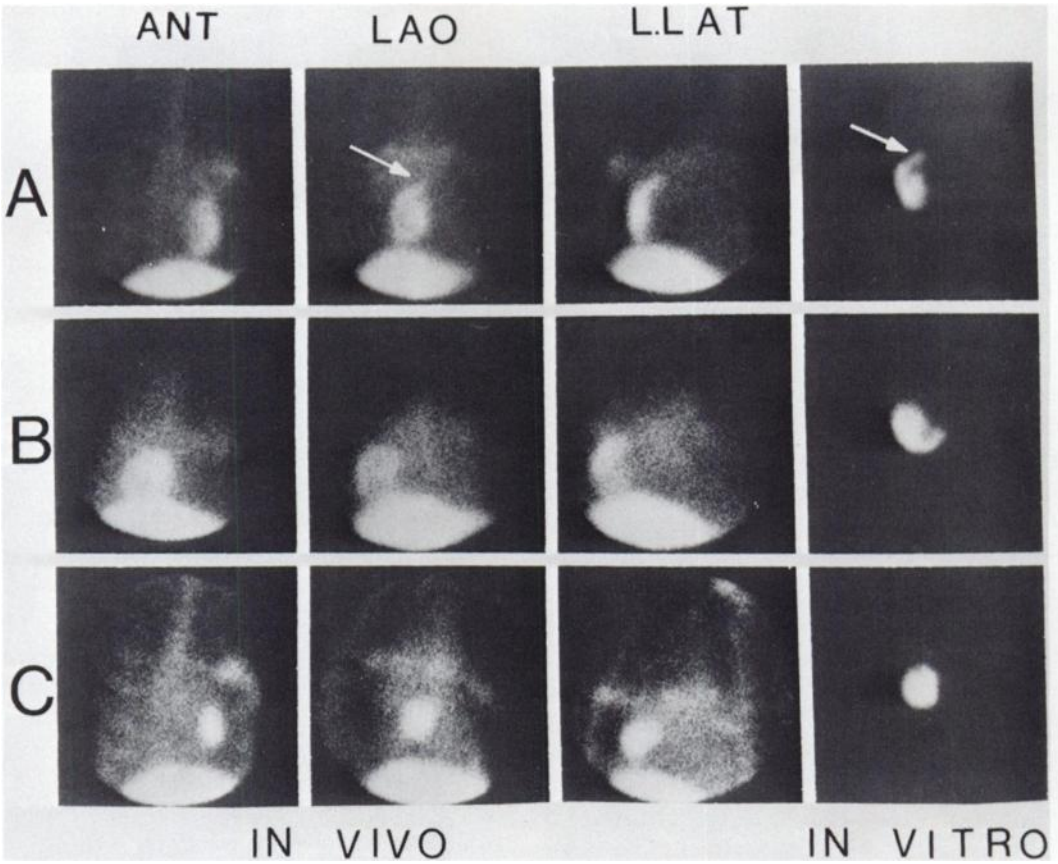

FIG. 1. Anterior (ANT), left anterior oblique (LAO), and left lateral (L LAT) in vivo scintigrams of three dogs (A, B, C) who had 75 min of temporary occlusion of the left anterior descending coronary artery followed by 5 min of release. Myocardial imaging was begun immediately after injection of labeled heparin. Increased myocardial or coronary-vessel uptake could be seen about 45 min after tracer injection. Panels on far right are in vitro scintigrams obtained in left anterior oblique projections. Radioactivity is primarily in damaged myocardium and liver, with some also in the bones. Note obvious LAD labeling in Dog A (arrows).

increased radioactivity in the following regions: liver, kidneys, bladder, and the anterior myocardium corresponding to sites of prolonged ischemic insult. Weak uptake was seen in bony skeleton and at the site of the left lateral thoracotomy.

Radioassay data. The blood clearance of Tc-99m heparin in the dogs is shown in Fig. 2. The gram percentage injected dose in the blood at 5 min postinjection is taken as 100. The data reveal a biphasic clearance curve, showing slow and fast components with half-times of 305 and 16 min.

Reperfusion studies. In the dogs with temporary LAD occlusion and release, the rate of blood clearance of the tracer was rapid enough to permit in vivo imaging of the tracer in damaged myocardium by 25–30 min postinjection (Fig. 1, Tables 1 and 2). The images were easily visible 1 hr after injection of Tc-99m heparin, and they remained visible throughout the entire 3½ hr of the study.

In six dogs subjected to temporary LAD occlusion, maximal damaged-to-normal ratios were high (9.5–36), whereas in five similar dogs the maximal ratios were more modest (4.3–7.5); low levels of radioactivity were found in the one dog without grossly visible damage (Table 2). Typical ratios for damaged myocardium to blood were in the range of 3–10. Grossly normal border zones had uniformly low activity in all dogs.

Damaged anterior myocardium in the region of the temporarily occluded LAD showed gross changes of swelling, pallor, and focal hemorrhage, the alterations being most extensive in the subendocardium. Histologic examination revealed foci of vascular congestion, early infiltration of neutrophils, and severe muscle-cell injury characterized by cytoplasmic disruption and contraction-band formation (Fig. 3). Selective sampling of pale and hemorrhagic areas in one dog revealed no differences in the levels of Tc-99m heparin uptake in the two types of lesion.

Fixed LAD occlusion. The four dogs with permanent LAD occlusion showed less marked uptake of the Tc-99m heparin in the damaged myocardium, but the in vivo and in vitro images still showed increased uptake of the tracer in this area (Fig. 4).

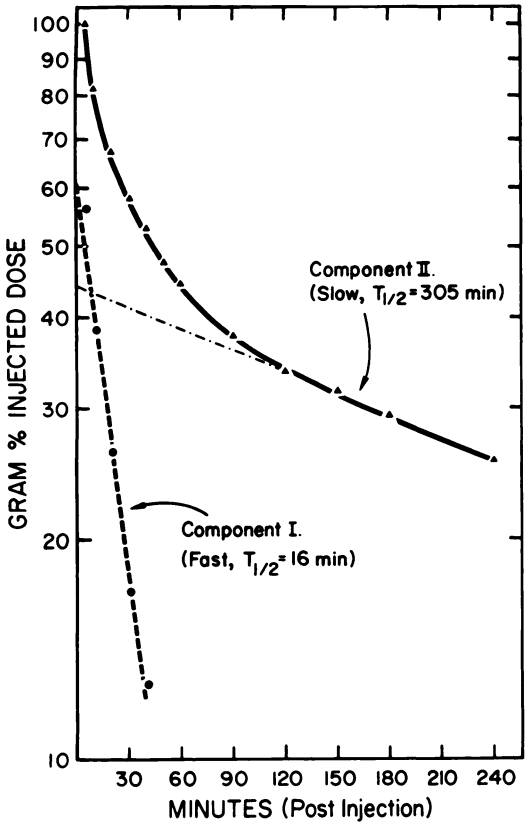

FIG. 2. Blood clearance of Tc-99m heparin in 11 dogs.

In these dogs, uptake in the infarcted areas ranged from 2 to 5 times that found in normal myocardium (Table 2). Each of these animals had homogeneous areas of transmural necrosis.

Damaged LAD vessel. In the animals with LAD occlusion, both temporary and permanent, increased Tc-99m heparin activity was found at or distal to the site of occlusion (Table 2, Figs. 1 and 5). In four temporarily occluded animals and one with permanent LAD occlusion, the LAD was divided into three parts for scintillation counting: the site of occlusion and sites proximal and distal to it. In the temporarily occluded dogs the accumulation of Tc-99m heparin was highest at the site of occlusion; the samples distal to occlusion also had slightly higher accumulations of Tc-99m heparin than the proximal sites (Table 2).

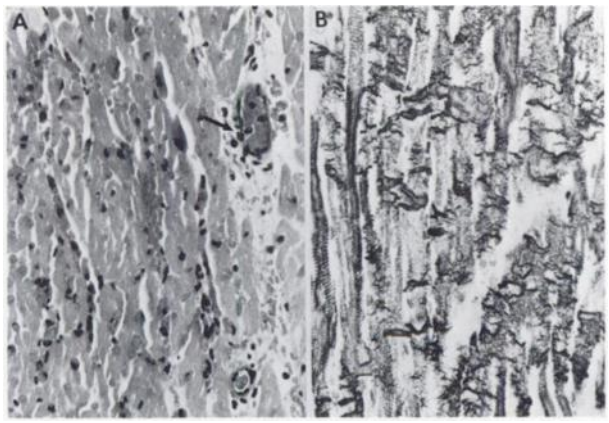

FIG. 3. Histologic changes in grossly damaged anterior myocardium following temporary occlusion of the LAD for 85 min, then 180 min of reflow. (A) Section stained with hematoxylin and eosin shows prominent vascular congestion, mild hemorrhage, and migration of neutrophils across arteriolar endothelium (arrow). (B) Section stained with Masson's trichrome shows severe muscle injury with myofibrillar disruption and contraction bands.

TABLE 1. ORGAN DISTRIBUTION OF Tc-99m HEPARIN 3.5 HR AFTER I.V. INJECTION IN DOGS (n = 16)

Organ	% ID/g of organ × (10^3) Mean ± s.d.	Ratio to normal myocardium* Mean
Blood	6.9 ± 2.10	2.5
Bone	4.8 ± 2.10	1.8
Liver	36.1 ± 12.4	13.3
Lung	6.2 ± 2.70	2.3
Kidney	70.3 ± 21.53	25.8
Pancreas	3.1 ± 1.43	1.1
Bile	16.5 ± 8.79	6.0
Skeletal muscle	1.7 ± 0.66	0.6
Surgical incision muscle	10.3 ± 2.99	3.8
Normal myocardium	2.7 ± 0.91	1.0

* Radio of % injected dose per gram divided by % injected dose per gram of normal myocardium.

DISCUSSION

Blood clearance of Tc-99m heparin in dogs showed the typical biphasic curve of many radiopharmaceuticals. There is significant uptake of the agent in the liver and kidneys. Our data suggest, however, that scintigraphy of the myocardium and cardiovascular system can be carried out 20–30 min after injection and continued for several hours.

Results show that successful imaging of ischemically damaged myocardium can be achieved with Tc-99m heparin, especially with only temporary occlusion. Histologic examination revealed that this model experienced probably irreversible injury of varying severity. Variation in maximal concentration of Tc-99m heparin may have been related to variation in the extent of irreversible injury in the grossly damaged myocardium following temporary coronary occlusion. The modest level of increased Tc-99m heparin uptake in the permanently occluded model might have been related to reduced delivery of Tc-99m heparin via collaterals, and/or to differences in the affinity of Tc-99m heparin for damaged myocardium at later stages of infarction.

TABLE 2. RATIO (% INJECTED DOSE PER GRAM) OF INJURED MYOCARDIAL TISSUE TO NORMAL MYOCARDIUM

Temporary Coronary Occlusion, then release				48-hr permanent ligation
Gross pathology	No visible damage (N = 1)	Subendocardial damage (N = 1)	Transmural damage (N = 10)	Transmural damage (N = 4)
Tissue			Mean ± s.d. or range	Mean and range
Normal LV	1.0	1.0	1.0	1.0
Border epi	NC	1.0	1.4 ± 0.47	1.8 (1.3–2.0)
Border endo	NC	0.9	1.8 ± 0.48 (n = 7)	2.0 (1.5–2.9)
Damaged epi	1.4	1.8	10.1 ± 9.19	3.5 (2.9–318)
Damaged endo	2.6	4.3	14.6 ± 9.61	4.5 (3.4–6.0)
LAD artery proximal to the occlusion	NC	NC	3.2 (2.4–4.2) (n = 4)	5.1 (n = 1)
Site of occlusion	NC	NC	12.8 (9.8–14.1) (n = 4)	2.0 (n = 1)
Distal to LAD occlusion	5.6	2.7	4.9 ± 1.03 (n = 8)	3.2 (1.8–5.5)
Normal circumflex coronary artery	3.7	1.8	2.4 ± 0.43 (n = 8)	2.0 (1.6–2.6)

NC = not obtained for counting.

Note: Out of ten dogs with transmural damage, 1—transmural hemorrhagic; 1—small transmural damage; 1—transmural damage with subendocardial hemorrhage; 1—patchy transmural damage.

The affinity of Tc-99m heparin for damaged myocardium and blood vessels may be related to an attraction and binding of the highly negatively charged heparin molecules to positively charged sites. It has been shown that vascular injury results in a reversal of the electrical charge of the intima from negative to positive and that this may play a role in thrombosis (*1,2*). Our scintigraphic results could be explained by binding of Tc-99m heparin to the positively charged intima of the surgically manipulated segments of arteries as well as to positively charged intima of damaged small blood vessels in ischemically injured myocardium. Other mechanisms, however, could be operative, especially in the labeling of damaged myocardium. A generalized electrochemical reaction seems unlikely, since the pH of acutely necrotic myocardial tissue becomes slightly alkaline (pH 7.4) upon reperfusion, whereas central necrotic regions with minimal perfusion are relatively acidic (*5,6*). Alternatively, the Tc-99m heparin could have affinity for specific constituents of damaged myocardium, including calcium deposits (*7–10*), denatured macromolecules (*11*) or plasma proteins, or other constituents that accumulate in the injured cells (*7–9,12*).

The major pharmacologic effects of heparin are almost entirely confined to the blood. Even large doses given intravenously have no significant effects except on blood coagulation and blood lipids (*13*). The chief danger from heparin is hemorrhage. In

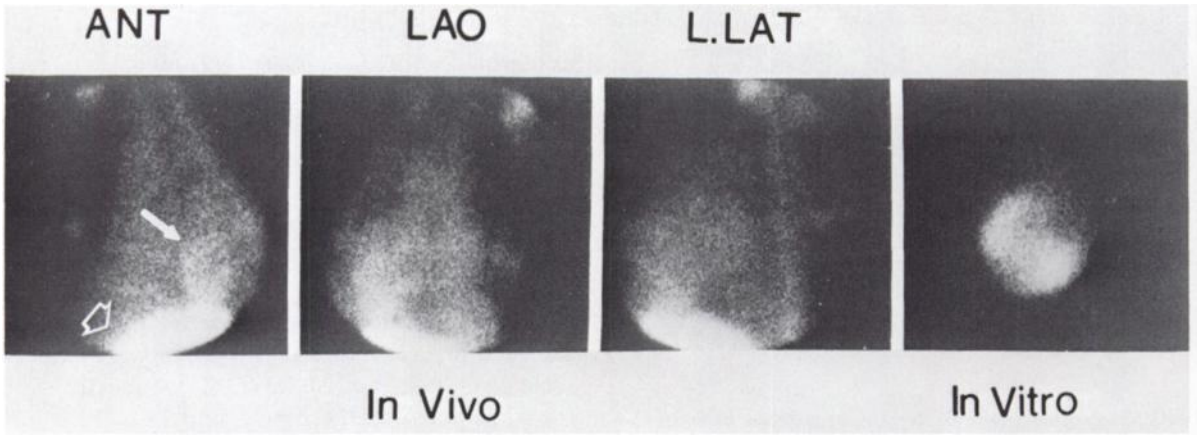

FIG. 4. Anterior (ANT), left anterior oblique (LAO), and left lateral (L LAT) scintigrams of a dog 48 hr after permanent ligation of left anterior descending coronary artery. Small arrow shows radioactive uptake in damaged myocardium. Open arrow shows liver uptake. Far right panel shows the in vitro scintigram in the left anterior oblique projection.

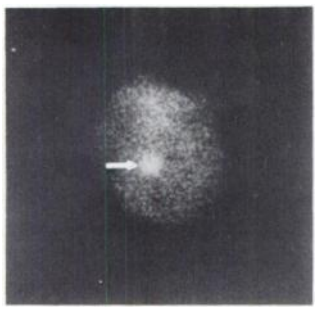

FIG. 5. Anterior in vitro scintigram of dog's heart with increased Tc-99m heparin uptake and histologic damage at site of temporary LAD occlusion (arrow), but no uptake or histologic damage in elsewhere in the heart.

the present study there were no noticeable changes in bleeding from surgical wounds in the animals after administration of the small amounts of labeled heparin. We arbitrarily chose to keep the total amount of heparin injected to less than 1,000 units per dog. It is reported that heparin disappears exponentially from the circulation at a rate dependent upon the dose (*14*). In this study, the half-times of 100, 200, and 400 units/kg of heparin injected intravenously were 56, 96, and 152 min, respectively. Heparin is metabolized by the liver, and a partially degraded, weakly active form of heparin is excreted in the urine.

Heparin is a strong chelating agent and readily forms complexes with many cations. Thus it is not surprising that it forms a stable complex with reduced technetium. It is also likely to form suitable stable complexes with other nuclides, such as In-111 or In-113m. The agent is readily amenable to one-step "kit" preparation.

The results of our study suggest that Tc-99m heparin may have value as a positive imaging agent when coronary arteries or myocardium are injured and significant blood flow persists to the damaged myocardium or coronary vasculature. Another possible use that needs study is its ability to identify damaged peripheral vessels associated with ischemia, trauma, or thromboembolism. The precise mechanism(s) responsible for heparin binding to reperfused and damaged myocardium and coronary blood vessels are still to be determined.

FOOTNOTE

* Supplied by Upjohn Co., Kalamazoo, Mich.

ACKNOWLEDGMENTS

The authors thank Dorothy Gutekunst, Katie Wolf, Saundra Huntzinger, and Rose Sullivan for expert technical assistance, and Mss. Gayle Blust, Mary Rich, and Belinda Lambert for secretarial assistance.

This work was supported in part by the Ischemic Heart Disease Specialized Center of Research (SCOR) Grant HL-17669, NIH Grant HL-17777, the Southwestern Medical Foundation, Harry S. Moss Heart Fund, and Pulmonary Specialized Center of Research (SCOR) NIH Grant HL-1487.

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