Renal Imaging in Humans with the Technetium Labeled Polypeptide, Caseidin

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DISCLAIMER

The use of radiomercurials in renal scintigraphy is limited by the high radiation dose associated with their use and the inability to differentiate vascular from non-vascular lesions (1). As a solution to the latter deficiency, ^{99m}Tc-pertechnetate can be used in conjunction with a radiomercurial in dual-isotopic scintiphotography (2). A solution to both these limitations would be the availability of a safe and effective renal-cortical localizing agent like the radiomercurials but carrying ^{99m}Tc or other suitable short-lived nuclides. A ^{99m}Tc-Fe-ascorbate complex introduced by Harper and his associates (3) represents a step toward this goal (4).

Recently, ^{99m}Tc labeled caseidin was shown to be an effective agent for renal cortical imaging in experimental animals (5). The present study was undertaken to determine whether ^{99m}Tc-caseidin would be similarly effective in humans, and such was found to be the case.

Materials and Methods

Crystalline caseidin was obtained from Miles-Yeda Co., Rehovoth, Israel. Stock solutions of the caseidin at pH 7 were made in saline to a concentration of about 100 mg/ml, Millipore-filtered (0.22 μ), and kept frozen until use. To prepare the ^{99m}Tc-caseidin, 2-3 ml of technetium generator eluate was added to 1 μmole of anhydrous $SnCl_2$ dissolved in 1 ml of 0.2 N HCl solution. After 5-min mixing, 1 ml of the stock caseidin solution was added. Mixing was continued for an additional 10 min to promote binding of reduced technetium to the caseidin (6,7). After a Millipore-sterilization (0.22 μ pore size), the mixture (pH 2.4) was used in patients. By assay in rats, the preparation was found to be stable for 10 hr when kept under N_2 at

4°C in a refrigerator. When air oxidation of the Sn(II) present in the preparation was allowed to occur by standing the preparation under air in a bright room, there was a significant regeneration of free pertechnetate in the preparation by 10 hr.

A total of twenty patients received the preparation for renal imaging. All patients except one uremic child were adult out-patients in good general condition. Each of these patients was evaluated clinically prior to the study. Intravenous pyelography was performed in eleven, double-isotopic renal studies with ^{99m}Tc-pertechnetate and ^{203}Hg-chlormerodrin in eight, and selective renal arteriography in one patient. Serum creatinine was 1.7 mg/100 ml or less in all except the uremic child.

The ^{99m}Tc-caseidin preparation was administered by injection into an antecubital vein in all patients except S.W., who received it through a venous catheter placed in her right heart. Each patient received a single dose of 6-18 mCi containing 14-60 mg of the caseidin. No patient experienced any discomfort from the dose. Prior to the intravenous administration, each patient received a test dose of 0.5 μg of the caseidin intracutaneously to produce a 5-mm wheal. None had any reaction to the test dose. In each case, the wheal simply faded over 30-40 min. The skin test was similarly negative when it was repeated with 0.5 and 5 μg doses in each of four patients 2-3 weeks after the intravenous administration of the ^{99m}Tc-caseidin.

Renal imaging was performed using a scintillation camera with a parallel 5800-hole collimator or a 3/16-inch pinhole collimator. The patient lay prone for posterior viewing. When the pinhole collimator was used, the pinhole-to-back distance was 1.5-2.5 inches.

To study ^{99m}Tc disappearance from the blood, the blood concentration of the radioisotope at varying times after the administration was measured, plotted, extrapolated back to zero time, and expressed as percent of the value of the zero time extrapolate. The fraction of administered dose excreted in the urine was determined by comparing the technetium content of collected urine with an appropriate standard. Whole body scanning was performed using a whole body scanner previously described (8).

Results

Distribution and excretion in patients without renal disease

^{99m}Tc activity disappearance from the blood was measured following intravenous administration of the ^{99m}Tc-caseidin in four patients. Fig. 1 shows the results obtained. The disappearance was initially rapid with a 50% disappearance time of about 14 min, becoming slower after about 30 min with about one fourth of the initial blood activity left in the circulation at 2 hr. The disappearance data could be described with a sum of three exponential terms, each accounting for the disappearance of approximately one third of the initial blood activity with a half time of 3 min, 10 min, and 3.5 hr, respectively. The initial distribution volume in these four patients varied from 7 to 12 liters and was generally greater than the anticipated blood volume in these patients.

In two patients, serial whole body scanning following the administration showed a rapid and progressive renal clearance of the blood activity (Fig. 2). A substantial portion of the administered technetium was retained in the kidneys. Of the dose, an average of 19% (range 15-24%, 7 patients) was found in the urine at 2 hr, and 27%

(range 22-33%, 4 patients) at 4 hr.

Renal Imaging

Renal scintigraphy with the ^{99m}Tc-caseidin was performed in twenty patients. From all available clinical data, sixteen of the twenty patients were judged to have essentially normal kidneys, and four to have renal disease. In one patient, a hypoplastic left kidney incapable of concentrating hypaque could not be visualized with the ^{99m}Tc-caseidin during the first 3 hr after its administration. In a child suffering from subacute renal failure with blood urea nitrogen over 100 mg/100 ml, only a minimal activity over the background was found in the region of the kidneys 3 hr after the administration of the ^{99m}Tc-caseidin. We concluded that grossly malfunctioning kidneys could not be visualized with ^{99m}Tc-caseidin.

Adequately functioning kidneys showed prompt concentration of the ^{99m}Tc-caseidin. Fig. 3 shows abnormal renal scintiphotos in patients S.W. and S.C. S.W. had acromegaly. An avascular (Fig. 3,A) round (Fig. 3,C) lesion in the midportion of her left kidney suggested a solitary cyst. When a "close-up" was obtained with a small pinhole collimator, the lesion appeared as a circumscribed area devoid of outer cortical substance (Fig. 3,D). S.C. had polycythemia. An avascular lesion was noted in the upper pole of his right kidney, and the defect appeared to be in the posterolateral aspect of the upper pole (Fig. 3,F-J. First-passage views not shown). Fig. 3 further shows the following results. First, diagnostic renal images could be obtained with a dose-to-imaging interval as short as 5 min (Fig. 3,B and F). Second, kidney/background contrast increased with time after the first few minutes. Multiple viewing and excellent delineation of the

entire kidney were possible throughout the following hours (Fig. 3, B-J). Third, throughout the first few hours, urinary activity in the renal pelvis was never substantial enough to interfere with delineation of the renal cortex (Fig. 3, G-J), Fourth, with a 10-15 mCi dose, sufficient renal ^{99m}Tc concentration could be attained to allow for practical use of a 3/16-inch pinhole collimator resulting in improved resolution in renal scintiphotography.

When the high resolution pinhole collimator was used, the renal scintigraphic image obtained showed a non-uniform distribution pattern of renal activity in all patients studied. Fig. 4 shows the renal image in three patients felt to have "normal" kidneys. The non-uniform pattern varied between the two kidneys in the same patient and from one patient to another. However, in all cases, the activity was localized primarily in the renal cortex. One source of the variation in the image pattern appeared to be a variable degree of collection of urinary activities in the region of major calyces (Fig. 4, C and H-J). Small major calyces seen on intravenous pyelograms were never found to be associated with visible activity collections at corresponding locations on scintiphotos. Periodic breath holding was not employed in obtaining the images shown in Fig. 4. The pinhole-to-back distance changed slightly with respiration to a varying degree in individual patients. Such a change in the position of the kidney relative to the pinhole was noted to cause a variable degradation of the non-uniform image pattern. In serial images of the same kidney, features of the image pattern tended to remain unchanged with a slight accentuation of the same features with time (Fig. 4, E-G and H-J).

Discussion

This study demonstrates that ^{99m}Tc labeled caseidin is promptly concentrated by human kidneys and allows for static scintiphotography of adequately functioning kidneys within several minutes after its administration as well as for evaluation of their vascular perfusion. In this regard, ^{99m}Tc-Fe-ascorbate complex (3) has a similar capability in that it can be used to perform both perfusion and static studies of the kidneys (4). However, renal localization of the ^{99m}Tc-Fe-ascorbate appears to occur more slowly and to a lesser extent as compared to ^{99m}Tc-caseidin (5). In the clinical experience of others with the ^{99m}Tc-Fe-ascorbate, kidney/background ratio reaches a level sufficient for renal imaging in about 1 hr post-injection with optimum results usually obtained by 3 hr (4).

It was found in this study that following administration of the ^{99m}Tc-caseidin, a substantial portion of the technetium was cleared by the kidney and retained in the cortex and that approximately 1/5 and 1/4 of the dose were excreted in the urine in 2 and 4 hr, respectively. This rate of urinary excretion of the technetium is similar to that of a ^{203}Hg-neohydrin determined in humans (9,10). Such cortical retention and slow urinary excretion of the label in the case of the ^{99m}Tc-caseidin stands in contrast to the case of many radioactive chelates (11,12). These other radioactive chelates, which include ^{99m}Tc-DTPA (13), are rapidly excreted in the urine and are not retained to a great degree by the kidneys (12). The rapid excretion of radioactive chelates is associated with a rapid and substantial collection of urinary activity in the renal collecting system (4,11). This urinary activity may introduce "artefacts" (4).

With regard to evaluation of renal cortical integrity, activity in the renal pelvis can interfere with detecting lesions located in the hilar region of the renal parenchyma in posterior and anterior viewing. It can also interfere with visualizing laterally located lesions in oblique viewing. With ^{99m}Tc labeled renal-cortical localizing agents, the problem related to activities in the collecting system is minimal, and ample time is available for multiple viewing (1).

This study further demonstrates the feasibility of using the ^{99m}Tc-caseidin in conjunction with a small pinhole collimator for improved resolution in renal imaging. It seems likely that the improved resolution obtained and the possibility of multiple viewing would provide an improved accuracy in detecting small mass lesions involving outer cortical substance of the kidneys. "Anatomical features" of the image so obtained may be expected to depend on distribution of cortical substance in the renal parenchyma, spatial extent of the renal sinus, and residual activity in the collecting system. Respiratory movement of the kidney with respect to the pinhole during the imaging tends in effect to "average out" the non-uniform activity distribution in the kidneys and can degrade the information content obtainable in the renal image.

Caseidin, a small polypeptide of molecular weight about 2,500 (14), is stated to be nontoxic and nonantigenic in gram quantities in mice and rabbits (15). It is produced by controlled hydrolysis of the milk protein, casein (14). The casein is also the usual base in the manufacture of protein hydrolysate (16), a therapeutic for intravenous use (17). This hydrolysate contains short-chain peptides in addition to individual amino acids (16). Toxicity and antigenicity

of short-chain peptides that may form following degradation of caseidin *in vivo* would seem unlikely in humans. Adverse reactions were not encountered in the twenty patients studied. However, further data are necessary to establish the safety of routine use of this effective radioisotope carrier for renal imaging.

Summary

Trial in twenty patients with a ^{99m}Tc labeled polypeptide, caseidin, has shown it to be an effective agent for renal imaging in humans. It was found to concentrate in the cortex of functioning kidneys following intravenous administration. The concentration was prompt, sustained, and high enough to allow dose-to-imaging intervals as short as 5 min, ample time for multiple imaging, and practical use of a small pinhole collimator for improved resolution in the imaging of kidneys.

No adverse effects from the administration of this material was encountered in this limited trial study. Definitive evaluation of the safety of this effective renal imaging agent requires further study.

References

1. Gottschalk A: Renal scanning. JAMA 202:221-223, 1967
2. Rosenthall L: Radionuclide diagnosis of malignant tumors of the kidney. Ame J Roentgenol 101:662-668, 1967
3. Harper PV, Lathrop KA, Hinn GM, et al: Technetium-99m-iron complex. In Radioactive Pharmaceuticals, AEC Symposium Series No. 6:347, 1966
4. Winston MA, Halpern SE, Weiss ER, et al: A critical evaluation of ^{99m}Tc-Fe-ascorbic acid complex as a renal scanning agent. J Nucl Med 12:171-175, 1971
5. Winchell HS, Lin MS, Shipley BA, et al: Localization of a polypeptide, caseidin, in the renal cortex: A new radioisotope carrier for renal studies. J Nucl Med 12:678-682, 1971
6. Lin MS, Winchell HS, Shipley BA: Use of Fe(II) or Sn(II) alone for technetium labeling of albumin. J Nucl Med 12:204-211, 1971
7. Eckelman W, Meinken G, Richards P: Chemical state of ^{99m}Tc in biomedical products. J Nucl Med 12:596-600, 1971
8. Anger HO: Whole-body scanner, Mark II. J Nucl Med 7:331, 1966
9. McAfee JG, Wagner HN: Visualization of renal parenchyma by scintiscanning with ^{203}Hg-neohydrin. Radiology 75:820-821, 1960
10. Blau M, Bender MA: Radiomercury (Hg^{203}) labeled neohydrin: A new agent for brain tumor localization. J Nucl Med 3:83-93, 1962
11. Hosain F, Reba RC, Wagner HN: Visualization of renal structure and function with chelated radionuclides. Radiology 93:1135-1138, 1969
12. Hauser W, Atkins HL, Nelson KG, et al: Technetium-99m DTPA: A new radiopharmaceutical for brain and kidney scanning. Radiology 94:679-684, 1970

13. Eckelman WC, Richards P, Hauser W, et al: ^{99m}Tc-DTPA preparations. J Nucl Med 12:699, 1971

14. Katchalsky-Katzir A: Novel polypeptide antibiotic substances derived from casein. U.S. patent SN 608.747

15. Katchalsky-Katzir A: Personal communication

16. Sollman T: A Manual of Pharmacology. 8th ed., W.B. Saunders Company, Philadelphia, 1957, p. 55-56

17. Protein Hydrolysate Injection. In Pharmacopeia of the United States, 16th revision, 1960, p. 601-602

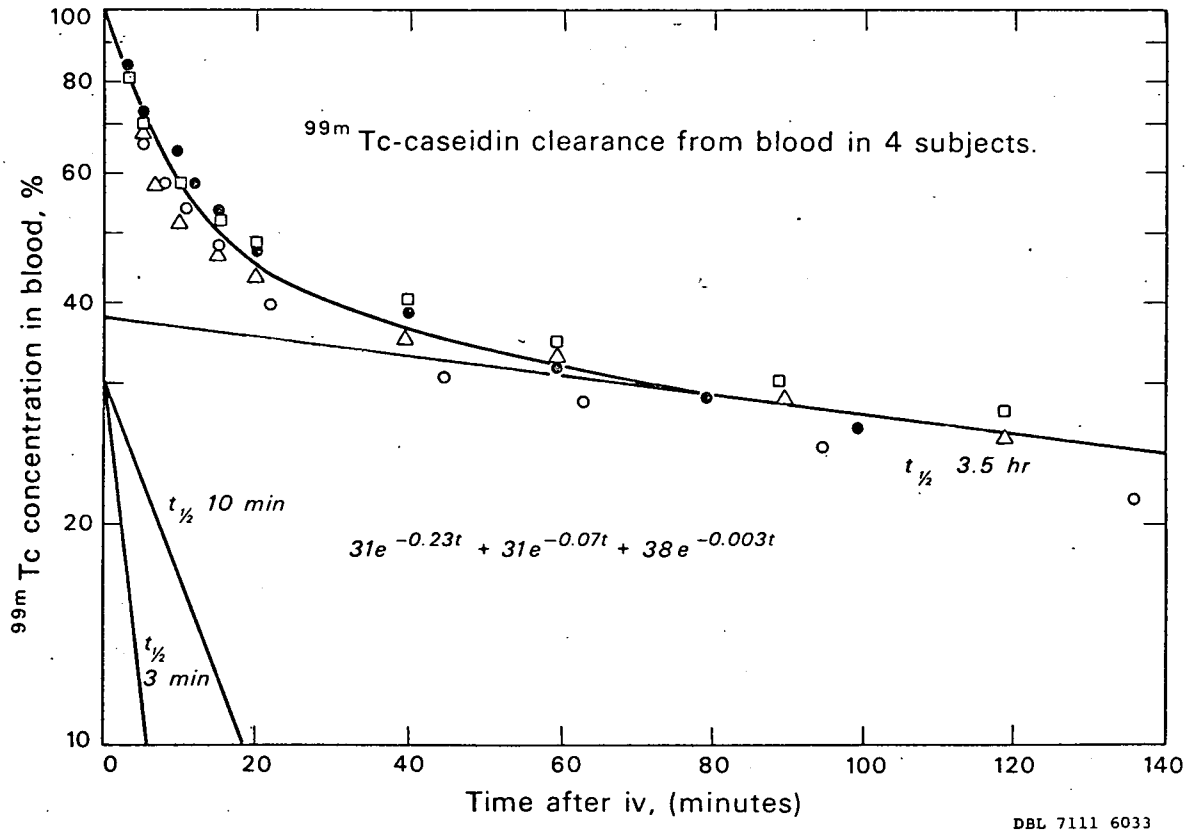
Figure Legends

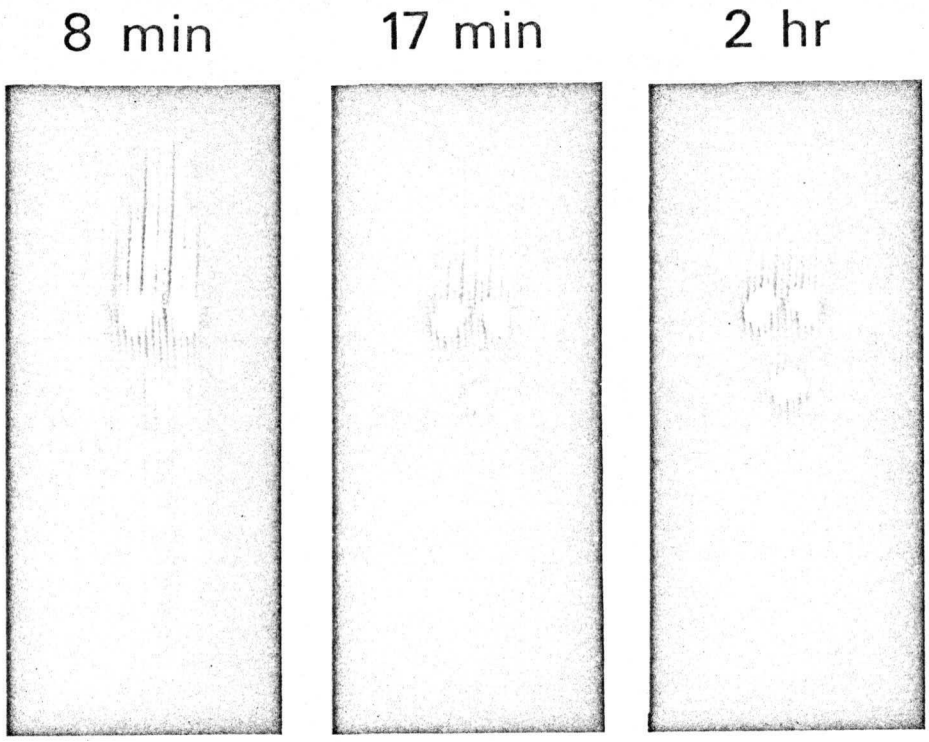
Fig. 1. Technetium disappearance from the blood following intravenous administration of ^{99m}Tc-caseidin in four patients without renal and cardiac disorders. A fitting function and its three exponential components are shown.

Fig. 2. Whole body scan (posterior view at 8 min, 17 min, and 2 hr) with ^{99m}Tc-caseidin in a patient without renal and circulatory disorders showing rapid and progressive renal clearance of the technetium and renal retention of a substantial portion of the administered technetium. The bladder was not emptied following the administration until after the scan was obtained.

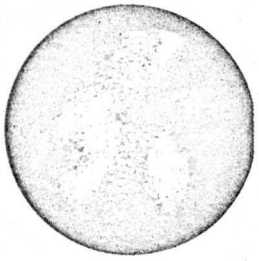
Fig. 3. Two positive renal studies in patients S.W. and S.C. with 12 (S.W.) and 6 (S.C.) mCi of ^{99m}Tc-caseidin illustrating capabilities of ^{99m}Tc-caseidin as a renal imaging agent. Scintiphotos C, I, and J are oblique views as indicated, all others posterior. Scintiphotos D and E were obtained with a 3/16-inch pinhole collimator, all others with a parallel 5800-hole collimator. Time indicated is dose-to-imaging interval. Exposure time/image was 3 sec for A and 5 min for D and E. Accumulated counts/image was 600,000 for all except A.

Fig. 4. Scintiphotos of essentially normal kidneys obtained with ^{99m}Tc-caseidin and a 3/16-inch pinhole collimator in three patients showing cortical localization of the technetium and various patterns of the renal image obtained. All scintiphotos are posterior views. Indicated time is dose-to-imaging interval. The dose was 9 (E.M.), 6 (L.C.), and 12 (B.L.) mCi. Exposure time/image was 5 min for A-D, and 3 min for E-J. Accumulated counts/image was 250,000 for A and B, and 550,000 for C-J.

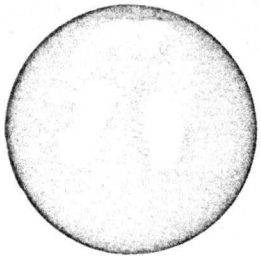

99m Tc-caseidin clearance from blood in 4 subjects.
99m Tc concentration in blood, %
100
80
60
40
20
10
0
20
40
60
80
100
120
140
Time after iv, (minutes)
t½ 10 min
t½ 3 min
t½ 3.5 hr
31e −0.23t + 31e −0.07t + 38e −0.003t
DBL 7111 6033


8 min
17 min
2 hr

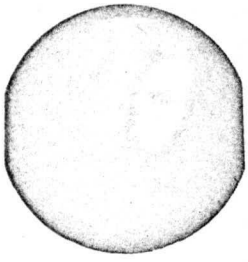
Mrs. S.W.



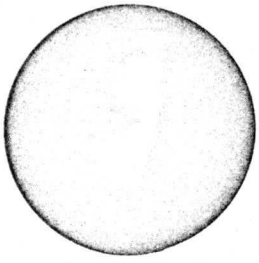
A 20 sec



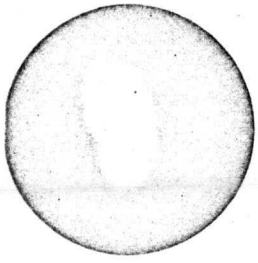
B 4 min



C 16 min (LAO)

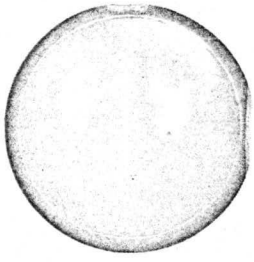


D 40 min (pinhole)

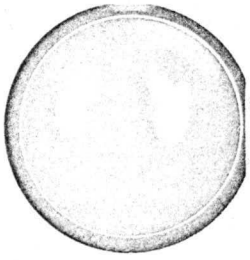


E 2 hr (pinhole)

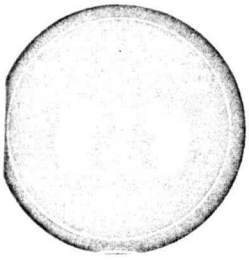
Mr. S.C.



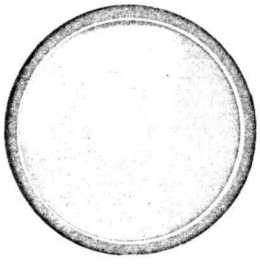
F 4 min



G 10 min



H 1 hr



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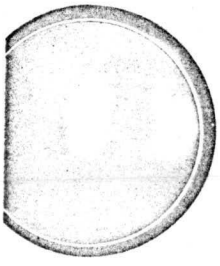
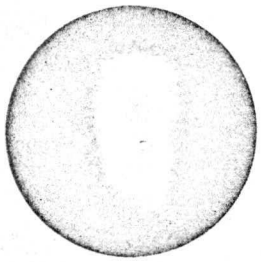
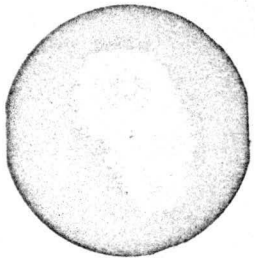


J 2 hr (LPO)

Mr. E.M.

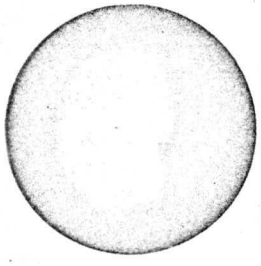


A 1.5 hr

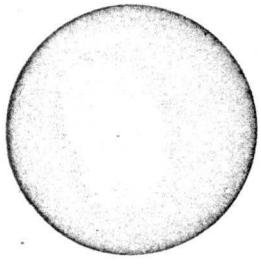


B 1.5 hr

Mrs. L.C.



C 2 hr



D 0.5 hr

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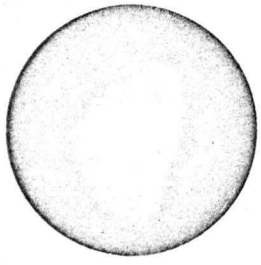

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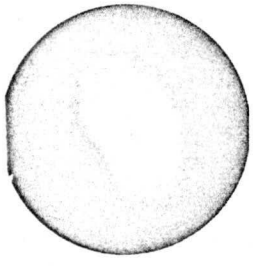
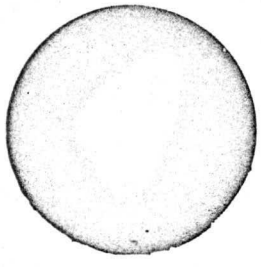


H 0.5 hr



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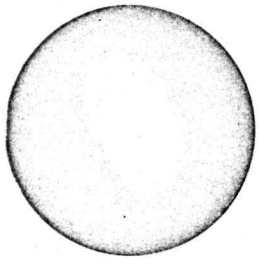

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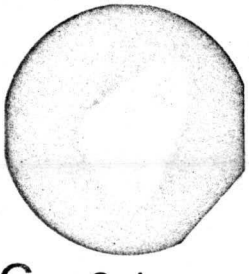

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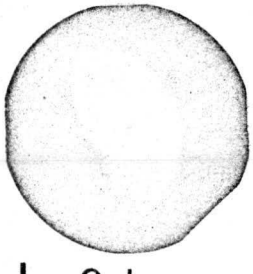

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