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# Preparation and Properties of Chromium-51 Labeled Inulin

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Inulin, a polysaccharide which is not metabolized by the body, is excreted by glomerular filtration without tubular excretion or reabsorption. Determination of the glomerular filtration rate is not only of great value in the study of renal physiology, but is of considerable importance in evaluating patients with renal disease (1).

In an effort to simplify the determination of glomerular filtration rate, many investigators have used radioactive compounds believed to be excreted in the same manner as inulin. These compounds include $^{131}I$ and $^{125}I$ allyl inulin (2,3), $^{14}C$-carboxyl labeled inulin (4), vitamin $B_{12}$ $^{57}Co$(5); $^{131}I$ labeled sodium diprotiozoate (Miokon) (6), sodium diatrizoate (Hypaque) (6,7), meglumine diatrizoate (Renografin) (6,8,9), and sodium iothalamate (Angio-Conray) (10). Most of these compounds are tagged with gamma-emitting isotopes which can be counted quickly and accurately in a scintillation counter.

At the present time, it is impractical to use the beta labeled $^{14}C$ carboxyl-inulin for the determination of renal clearance rates, since few general hospitals have the necessary equipment for these determinations and the preparation and purification of $^{14}C$ carboxyl-inulin is difficult.

For this study, the trivalent chromium-51 cation was chosen as a label for inulin because of its availability and its stability in forming organic acid salts and chelates. It is expected that all heavy metal cations with valences of +3 or greater will form stable complexes with this inulin derivative. Inulin was oxidized by several methods commonly used in carbohydrate chemistry, but only those using dichromate, hydrogen peroxide and oxygen gave suitable results. Atmospheric oxygen at elevated temperatures in the presence of a heavy metal cation rapidly oxidizes the terminal reducing group and then proceeds to rupture the glycositic linkage along the polymer chain with production of metallic salts or complex chelates. In all three methods of oxidation, one product predominates. The simplest procedure is the one with autoxidation at 80°C and only the more soluble lower molecular weight inulin is tagged (16). Therefore, only this preparation is described (Figure 1).

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PREPARATION AND IDENTIFICATION OF A CHROMIUM-51 TAGGED INULIN DERIVATIVE

A supersaturated solution of inulin, prepared by Warner-Chilcott as 10% inulin in a .5% sodium chloride solution, is poured into 7 volumes of ethanol. The heavy precipitate which forms on standing is filtered by suction, washed with 50 ml ethanol, followed by 50 ml acetone. After drying at room temperature, the fluffy white powder of 4.5 to 4.7 gm is suspended in 50 ml of 95%

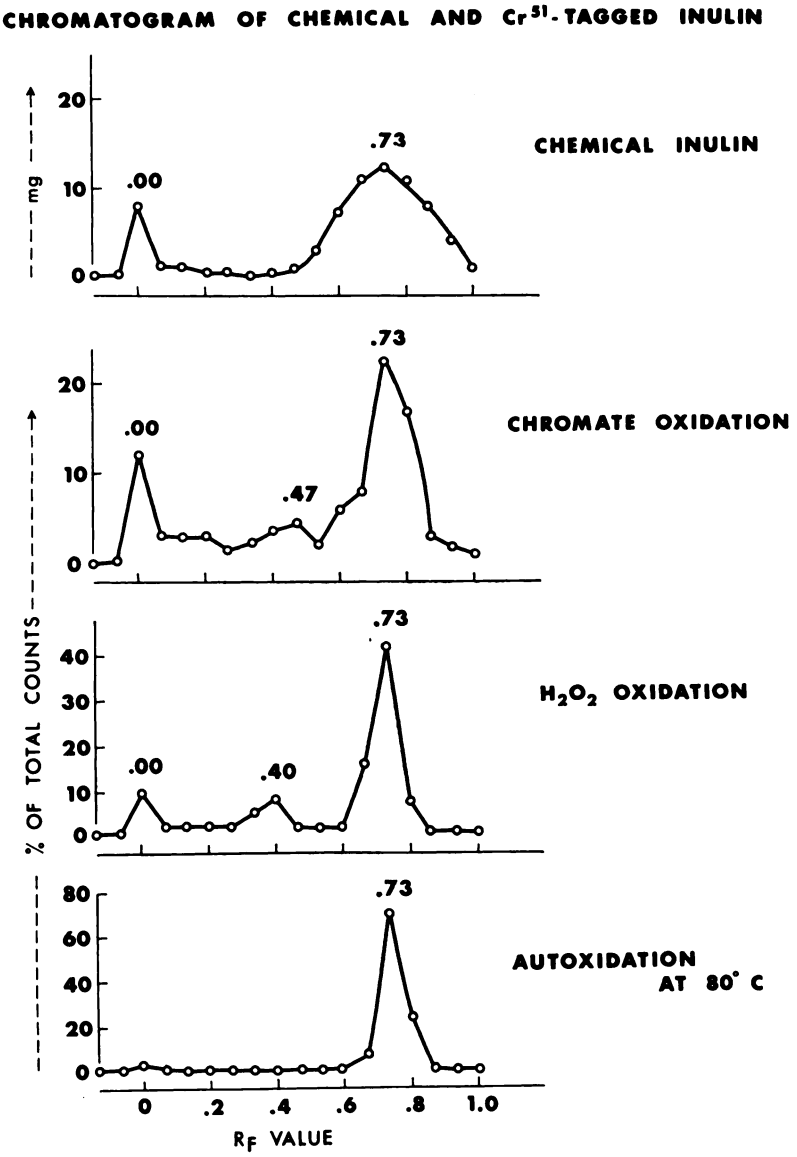


Fig. 1. Ascending chromatograms of stable inulin and of three oxidized radioactive derivatives.

ethanol; radiochromic chloride ($^{51}Cr$) is added to the alcohol-inulin suspension and gently refluxed for 30 minutes at 80°C; it is then cooled to room temperature, filtered by suction and again washed with 50 ml ethanol, followed by 50 ml acetone. After air drying at room temperature, the powder of 4.5 gm contains 97 to 99% of the added radioactivity. A paper chromatogram of this material shows 99.2% of the activity to be tagged to inulin. Further purification is obtained by dissolving this material in 50 cc distilled water at 40°C and precipitating the purified labeled inulin by pouring the aqueous solution into 7 volumes of ethanol, filtering by suction and washing with 50 ml ethanol and 50 ml acetone. After drying at room temperature, this material is dissolved in saline at 40°C, placed in a multi-injection vial and sterilized in an autoclave at 250°F. The clear solution of this material is stable and a repeat chromatogram 73 days after sterilization does not show any changes or loss of the $^{51}Cr$ tag (Figure 2).

The separation and identification of chromium-51 chloride, chromium-51 labeled inulin and stable inulin by filter paper chromatography is illustrated in the following procedure.

Place 20 ul of radioactive solution on the paper (Whatman No. 3MM, about 4 × 30 cm). Insert the filter paper strip into a 500 ml cylinder containing 50 ml of the ethanol water solvent (60:40 v/v). Allow the preparation to proceed for about 7 hours or until the front has ascended about 20 cm. Then, after marking the position of the solvent front, dry the paper at room temperature. Cut the dried filter paper strip into 1 cm-wide sections and determine the radioactivity of each section by counting in a scintillation well counter. The counting rate is plotted as a function of the distance from the starting line on the filter paper strip. Stable inulin is extracted from the individual 1 cm sections with 2 ml boiling distilled water and the inulin is determined by spectrophotometric chemistry (17). The mgs of inulin are plotted as a function of the distance from the starting line on the filter-paper strip.

Although the initial solubility of inulin depends upon the mode of crystallization, once it is in solution, it always converts to the more stable form. Thus, in running the chromatograms of freshly labeled material, we find the $R_F$ value to be .67 for material refluxed in ethanol and .80 for inulin prepared in boiling water; but on standing, both preparations shift to the .73 $R_F$ value and remain there throughout their use. Any free chromium-51 remains at the origin.

Chromium-51 tagged inulin with specific activity of 6 mc/gm has been readily prepared with this autoxidative procedure; however, much higher activities can be obtained if desired. Very high specific activities can be prepared by gel filtration (Figure 3). Columns of a total volume of 16 ml (11 mm × 170 mm) are prepared from a cross-linked dextran preparation, Sephadex-50[1] (18). The radioactivity-lebeled derivative is applied and eluted from the columns with distilled water. Volume cuts of one ml are collected and the radioactivity measured in a scintillation well counter. The counting rate is plotted as a function of the ml eluted. The stable inulin is determined on these fractions by spectrophoto-

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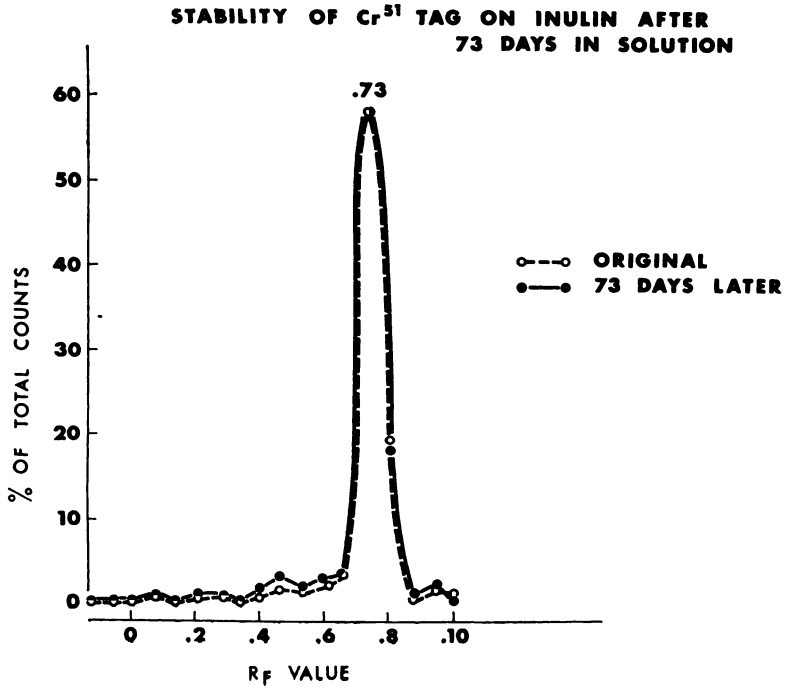


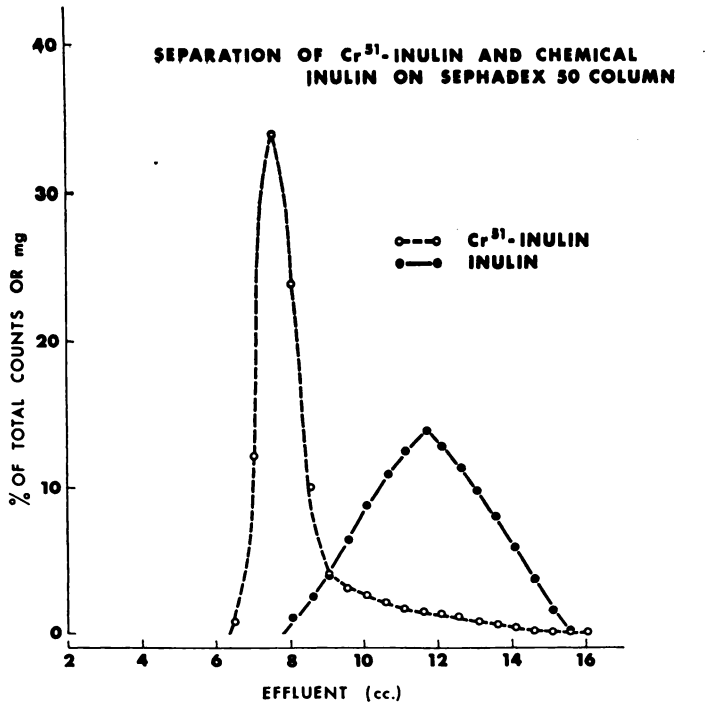




Fig. 2 (upper). Chromatograms of $^{51}$Cr-inulin immediately after sterilization and a repeat chromatogram 73 days later.

Fig. 3 (lower). The separation of $^{51}$Cr-inulin from stable inulin using Sephadex-50 column (11 X 170 mm) with distilled water.

metric chemistry and the mgs of inulin are plotted as a function of ml eluted (17). The $^{51}$Cr-tagged material is eluted ahead of the stable inulin, suggesting that its molecular weight is larger. This behavior on the Sephadex column indicates that the separation of the $^{51}$Cr-tagged inulin from stable inulin is not based purely on molecular size, but may be partially due to charge effects.

The same phenomenon is also seen when $^{14}$C-carboxy inulin is separated on Sephadex-50 columns (11). Any unbound chromium-51 is eluted last on the column.

### RENAL EXCRETION OF CHROMIUM-51 INULIN

To ascertain the stability of tagged $^{51}$Cr-inulin *in vivo*, 100 $\mu$C tagged inulin, representing 50 mg of the material, was injected i.v. into an anesthetized dog. Continuous collection of urine gave an 85% recovery of the injected material in three hours. Plasma and red cells retained no radioactivity at the end of this period. Immediately after the injection when plasma reached the highest count, the washed red cells contained no radioactive material. A chromatogram of the urine showed five per cent of the tag at the origin. Extraction of the paper chromatogram with distilled water and analyses of this extract on a Sephadex-50 column showed that the five per cent at the origin was labeled inulin. The chromatogram of stable inulin in Figure 1 originally contained 10% of this less soluble inulin at the origin (14, 15). Other investigators have found that the solubility and the crystal structure of inulin vary considerably in different media (16). However, the volume of distribution and renal clearance remains the same (19).

### RENAL CLEARANCE OF CHROMIUM-51 INULIN IN DOG

In order to compare the glomerular filtration of $^{51}$Cr-labeled inulin with the standard clearance technique, 100 $\mu$C of $^{51}$Cr-labeled inulin in 50 cc of 10% chemical inulin in .5% sodium chloride solution were infused over a 60-minute period into an anesthetized dog (12). The blood and urine concentration of the chromium-51 labeled derivative agreed with the chemically determined inulin (Figures 5, 6). A calculation of the clearance rate gave an average value of 94 ml/min for the $^{51}$Cr-inulin and 94 ml/min for the simultaneous stable inulin determination (Table I).

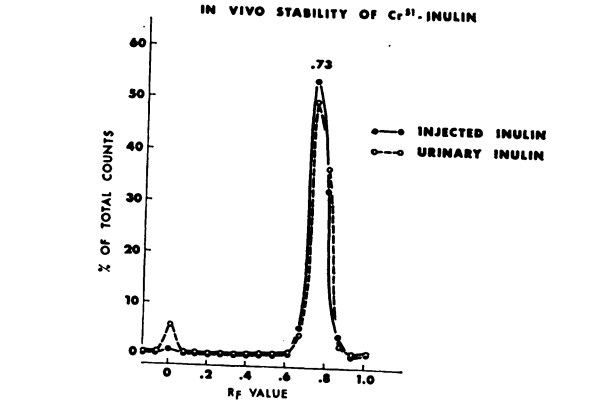
The $^{51}$Cr-inulin space of this dog weighing 25 kg was calculated to be 5585 cc or 22.3% of weight. Normal values of inulin space in dogs are 20.9-23.2% (13).

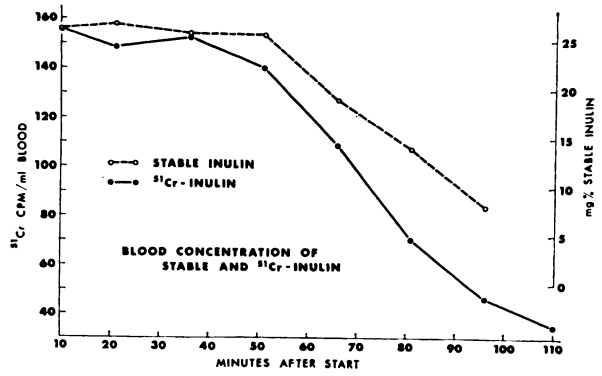
Simultaneous measurements of $^{14}$C-carboxy inulin and the $^{51}$Cr-inulin derivative in the urine over a three-hour period also showed an excellent correlation (Figure 7).

### EXTERNAL MONITORING OF CHROMIUM-51 INULIN

If we substitute external monitoring of the kidney for urine collection by ureter catheterization during an infusion procedure, we find that the monitored curve of renal radioactivity is essentially the same as the rate of change of radioactivity being excreted in the serial urine samples (Figure 8). An anesthe-

tized dog was injected intravenously with a single dose of 100 $\mu$C chromium-51 inulin and the relative count rate of radioactivity from the blood, kidney and urine was continuously monitored by external scintillation detectors. One probe was placed over the heart, another over the exposed left kidney, and the urine



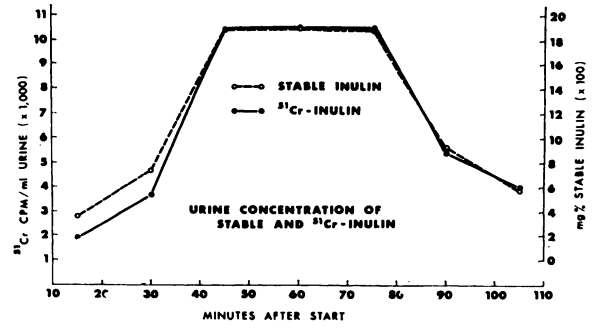




**Fig. 4 (upper). Chromatograms of intravenous and of urinary chromium-51 inulin.**

**Fig. 5 (middle). Blood concentration of stable and chromium-51 inulin during a renal clearance procedure.**

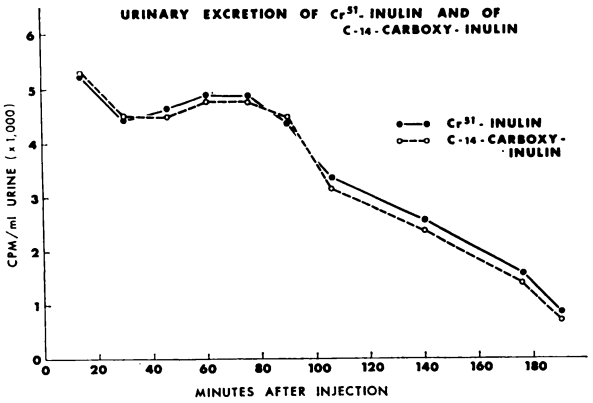
**Fig. 6 (lower). Urine concentration of stable and chromium-51 inulin during a renal clearance procedure.**

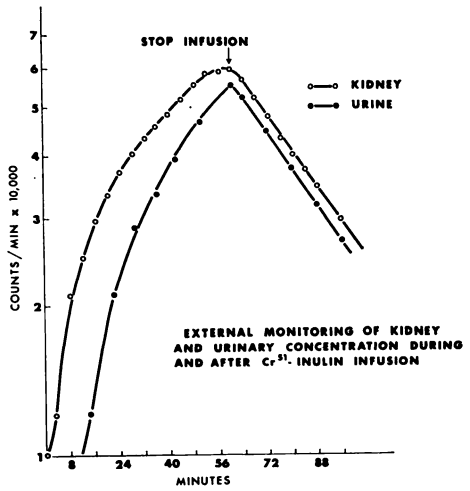
TABLE I

SIMULTANEOUS CLEARANCE OF $^{51}$Cr-INULIN AND STABLE INULIN IN DOG

| *Determination No.* | *V ml/min* | *STABLE INULIN* | | | *$^{51}$CR-INULIN* | | | *STABLE TO $CR^{51}$ INULIN RATIO* |
|---|---|---|---|---|---|---|---|---|
| | | *U mg/ml* | *P mg/ml* | *C ml/min* | *U CPM/ML* | *P CMP/ML* | *C ML/MIN* | |
| 1 | 7.33 | 3.66 | .259 | 104 | 1,907 | 156 | 90 | 1.16 |
| 2 | 3.43 | 7.41 | .265 | 96 | 3,743 | 149 | 86 | 1.12 |
| 3 | 1.30 | 19.0 | .256 | 97 | 10,593 | 153 | 90 | 1.08 |
| 4 | 1.43 | 19.0 | .259 | 105 | 10,368 | 141 | 105 | 1.00 |
| 5 | 0.97 | 18.25 | .190 | 93 | 10,502 | 109 | 94 | .99 |
| 6 | 1.17 | 9.25 | .130 | 83 | 5,403 | 71 | 89 | .93 |
| 7 | 1.20 | 5.80 | .088 | 79 | 3,970 | 46 | 104 | .76 |
| | | | | Average 94 | | | Average 94 | Average 1.01 |
| | | | Standard Deviation: | 10 | | | 8 | |
| | | | S. D. of Mean: | 94 ± 4 | | | 94 ± 3 | |

All clearances were calculated by the formula $C = \frac{uv}{P}$, where C is the plasma clearance in ml/min, V is the minute urine volume in ml/min, U the urine concentration, and P the plasma concentration. The concentrations of stable inulin were expressed in mg/ml, and those of $^{51}$Cr-inulin in net counts per minute per milliliter.



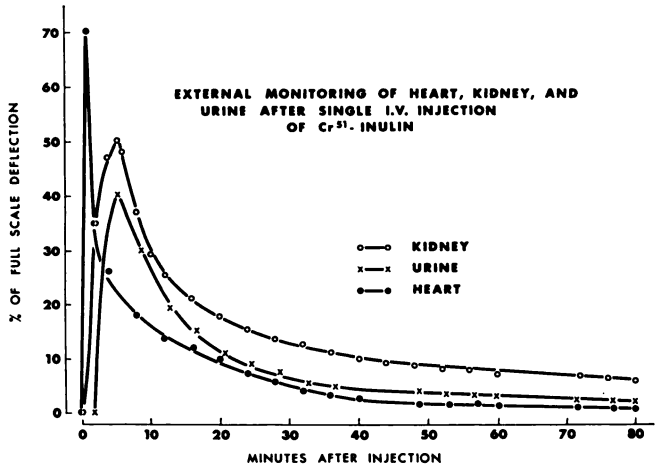




Fig. 7 (upper). Urine concentration of $^{14}$C-carboxy inulin and chromium-51 inulin during a three-hour interval.

Fig. 8 (middle). External monitoring of exposed kidney with relative concentration of radioactivity in the urine at various time intervals.

Fig. 9 (lower). External monitoring of blood, kidney and urine after a single i.v. injection of $^{51}$Cr-inulin.

from the catheterized bladder was monitored with a flow-through detector. Continuous recording during an 80-minute period showed the initial $^{51}$Cr-inulin renogram and the resultant count rate of radioactivity from blood, kidney and urine. After 40 minutes, the rate of decrease of radioactivity monitored in the blood, kidney and urine was constant (Figure 9).

Instead of external monitoring of the heart, one can also use external monitoring of the limb. After a single i.v. injection of $^{51}$Cr-inulin, the external moni-

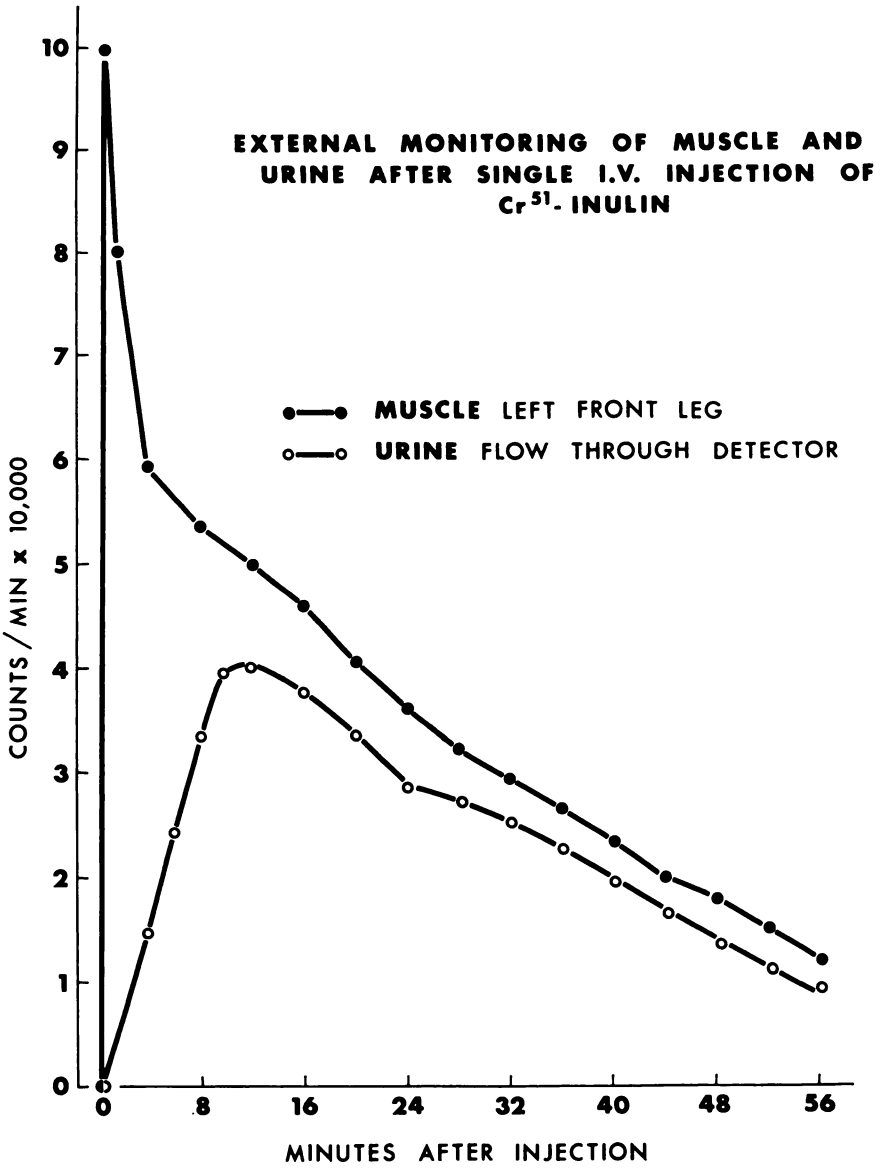



Fig. 10. External monitoring of leg and urine.

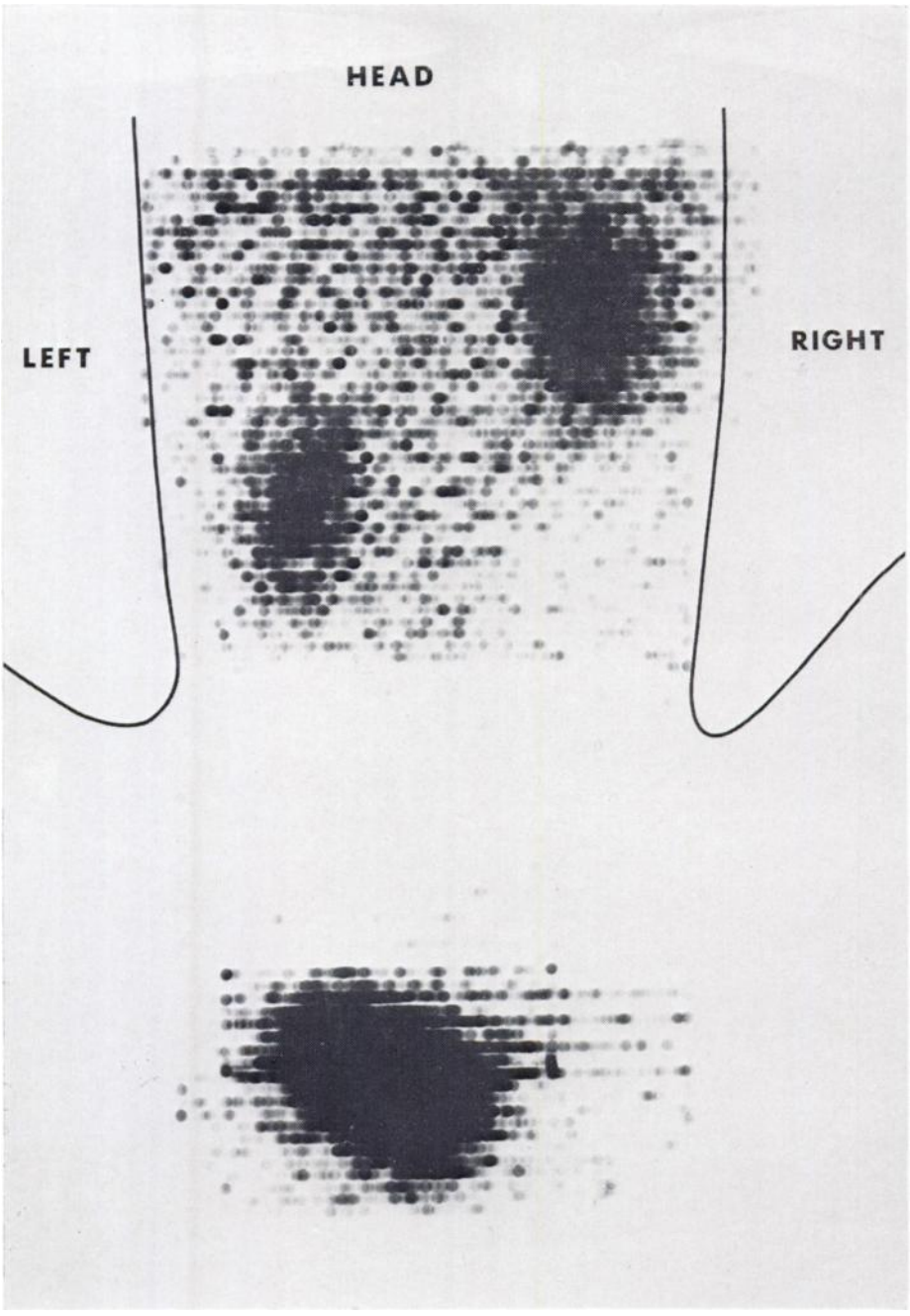



Fig. 11. Renal scan 30 minutes after a single i.v. injection of $^{51}$Cr-inulin in a dog.

toring of the decrease in relative rate of radioactivity in the leg of a dog mirrors exactly the urine excretion of the radioactive inulin (Figure 10).

Since our studies have shown that 30 minutes after the intravenous injection of $^{51}$Cr-inulin, the urine concentration of radioactive material is 100 to 200 times greater than in blood, a rapid scan at that time offers intriguing possibilities. The renal excretion of $^{51}$Cr-inulin is too rapid for kidney scanning using the slow-speed mechanical linear equipment (Figure 11). However, the newer large crystal fast-scanning techniques may give good renal scans with minimal radiation to the patient.

Renograms in an anesthetized well-hydrated dog, using 50 $\mu$C $^{51}$Cr-inulin do not show the fast simple spike seen after sodium orthoiodohippurate, but the slow descent may represent glomerular filtration rate (Figure 12).

## DISCUSSION

The results of this study suggest that the labeling of an oxidized inulin derivative with chromium-51 does not significantly alter the properties of inulin since the radioactivity and the chemically determined stable inulin measurements are similar. Simultaneous determinations of stable inulin or $^{14}$C-carboxy inulin

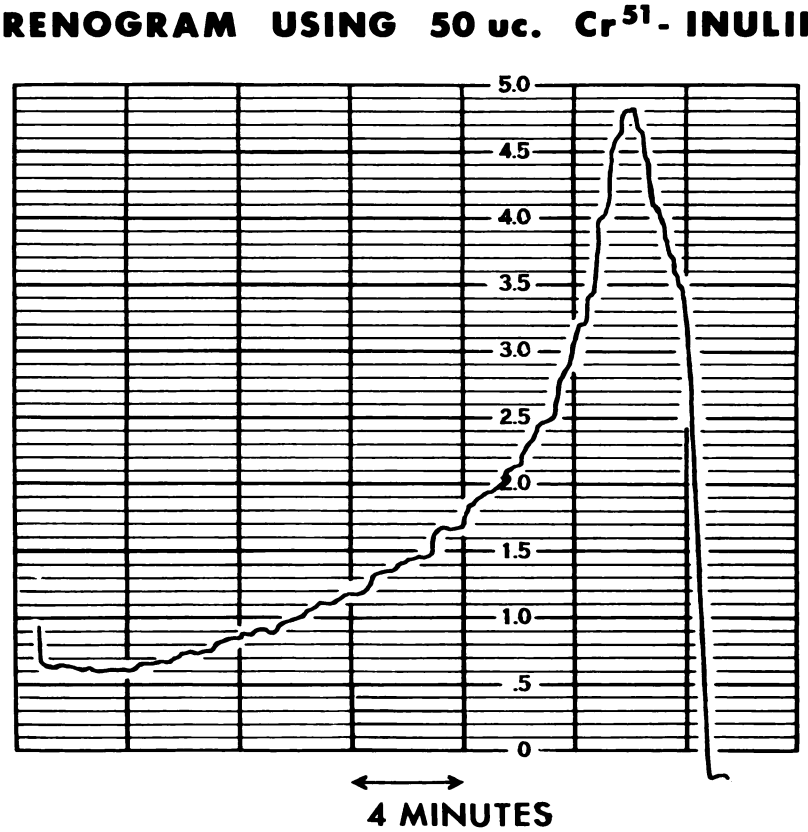



Fig. 12. Renogram in a dog using 50 $\mu$c $^{51}$Cr-inulin.

and the $^{51}$Cr-inulin derivatives in blood and urine showed that the renal excretion of all three compounds is similar.

The substitution of the chromium-51 inulin method for the determination of glomerular filtration rate and inulin space offers advantages in studying renal clearance. The time-consuming chemical analysis of inulin in blood and urine can be reduced to simple counting of radioactivity.

The substitution of external monitoring of the kidney for catheterized urine samples and of external monitoring of the heart or forearm for blood sampling, may possibly offer advantages that are unobtainable with the stable inulin clearance procedures.

The $^{51}$Cr-inulin derivative is stable in the laboratory and urinary $^{51}$Cr-inulin is chemically identical with the injected material.

## SUMMARY

An oxidized inulin derivative has been labeled with chromium-51. This product can be heat sterilized and is stable *in vitro* as well as *in vivo*. Simultaneous glomerular filtration rate determination in one dog, using stable inulin and $^{51}$Cr-labeled inulin, gave similar results. External monitoring of $^{51}$Cr-labeled inulin from the kidneys, other organs and tissues showed a constant rate of disappearance after 40 minutes. Chromium-51 labeled inulin may lend itself also for renograms and renal scans.

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## ADDENDUM

With regard to an article by Stephen A. Landaw and H. Saul Winchell, entitled "Endogeneous Production of Carbon-14 Labeled Carbon Monoxide: An *In Vivo* Technique for the Study of Heme Catabolism," published in the September 1966 issue of the JOURNAL OF NUCLEAR MEDICINE, the authors wish to report that:

More accurate results are now available on the oxidation efficiency of Hopcalite for $^{14}CO$ than those presented in Table II for gases with CO concentrations of 25 and 5 ppm. Using an *undiluted* standard $^{14}CO$ gas with a CO concentration of less than 2 ppm, it was found that the oxidation efficiency was 99.6% at a flow rate of 0.30 l/min, and 98.0% at a flow rate of 0.37 l/min. Thus, the high efficiency of oxidation shown in Table II for gases with a high concentration of CO (5000 ppm), is also found for gases with CO concentrations approximating that found in the expired air of mammals.