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Preparation, Distribution and Utilization of Technetium-99m-Sulfur Colloid[2,3]

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INTRODUCTION

Harper, *et al,* recently reported on the preparation of a technetium-99m-sulfur colloid utilizable for liver, spleen, and possibly bone marrow scanning (1). The colloid was made by passing H_2S through an acidified pertechnetate solution in the presence of a gelatin stabilizing agent. Unreacted pertechnetate was removed by passage through an anion exchange resin and the column eluate (Tc-S colloid) was sterilized by filtration through a Millipore filter.

The necessity of purification by column chromatography coupled with sterilization by filtration, led us to investigate the preparation of the colloid by alternative reactions.

A method developed in our laboratory, which gave a compound comparable to Harper's with similar distribution in animals and clinical usefulness, consists of a simple reduction of pertechnetate with acid and sodium thiosulfate in the presence of either gelatin or carboxymethylcellulose[4] as the stabilizing agent. Thiosulfate is rapidly converted to thiosulfuric acid which immediately decomposes to liberate free sulfur and sulfur dioxide. This method is fairly rapid (less than one hour), requires no column purification, and can be terminally sterilized by autoclaving, when CMC is used, or made under sterile conditions, when gelatin is employed as the stabilizer.

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[4]The abbreviation, CMC, will be used throughout to designate carboxymethylcellulose.

EXPERIMENTAL

MATERIALS AND METHODS

All solutions are made with sterile and pyrogen-free water, placed into serum vials, and sterilized by autoclaving.

(a) ^{99m}Tc pertechnetate (TcO_4^-) as eluted from a ^{99}Mo-^{99m}Tc generator.
(b) 3.0 N sulfuric acid
(c) 1.0 N sodium hydroxide
(d) 0.8 per cent sodium thiosulfate, anhydrous ($Na_2S_2O_3$)
(e) Carboxymethylcellulose (CMC) medium viscosity, pharmaceutical grade, powdered form
(f) Gelatin, U.S.P., granular form
(g) Teflon-coated bar magnet, ½ x ⅜ inches
(h) Combination hot plate-magnetic stirrer
(i) Chromatographic analysis identical to that reported earlier for ^{99m}Tc serum albumin (2).

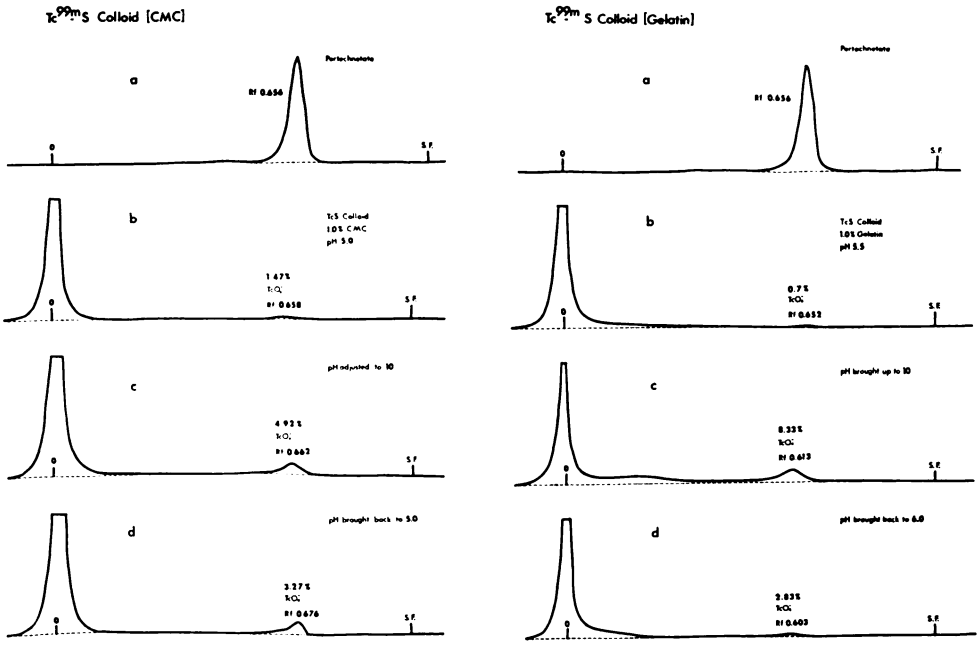

Fig. 1. Effect of pH on the chromatographic distribution of CMC stabilized ^{99m}Tc-S "colloid". Curve (a) shows $^{99m}TcO_4^-$ as eluted from a ^{99}Mo - ^{99m}Tc generator. Curves (b) through (d) show the effect of pH adjustment on the final product. ^{99m}Tc-S "colloid" remains at the origin. Chromatography was carried out in 85% methanol (descending).

Fig. 2. Effect of pH on the chromatographic distribution of gelatin stabilized ^{99m}Tc-S "colloid". Curve (a) shows free pertechnetate ion ($^{99m}TcO^-$) with an Rf of 0.65. Curves (b) through (d) show the effect of pH on the final product. The ^{99m}Tc-S "colloid" remains at the origin. Chromatography was carried out in 85% methanol (descending).

Method I. Carboxymethylcellulose stabilizer

CMC is slightly soluble in water at room temperature and forms a highly viscous solution. A two per cent CMC solution is many times more viscous than water and therefore impossible to pipet accurately. Since it is important for the final volume of the colloid to be kept at a minimum, it is advisable to add CMC as a solid material (the same will apply to gelatin in Method II).

(1) In a 20-30 cc^3 serum vial containing a teflon-coated bar magnet, partially dissolve 90 milligrams of CMC in 5 cc isotonic saline containing $Tc^{99m}O_4$ for 15 minutes at 15 psi.

(2) Remove the cap, place the vial in a water bath at 60 to 62° C, begin stirring, and add exactly 1 cc^3 of 3.0 N sulfuric acid.

(3) While stirring the acidified pertechnetate solution, add 0.5 cc^3 of 0.8 per cent sodium thiosulfate solution (4 milligrams) and continue to stir for 15-20 minutes.

(4) Cool the colloid and *carefully* adjust to pH 6.0 with 1.0 N sodium hydroxide.

PRECAUTION. If the colloid is overneutralized beyond pH 6.5 dissolution will take place (Fig. 1C) and one cannot readjust to a lower pH and expect similar biological distribution.

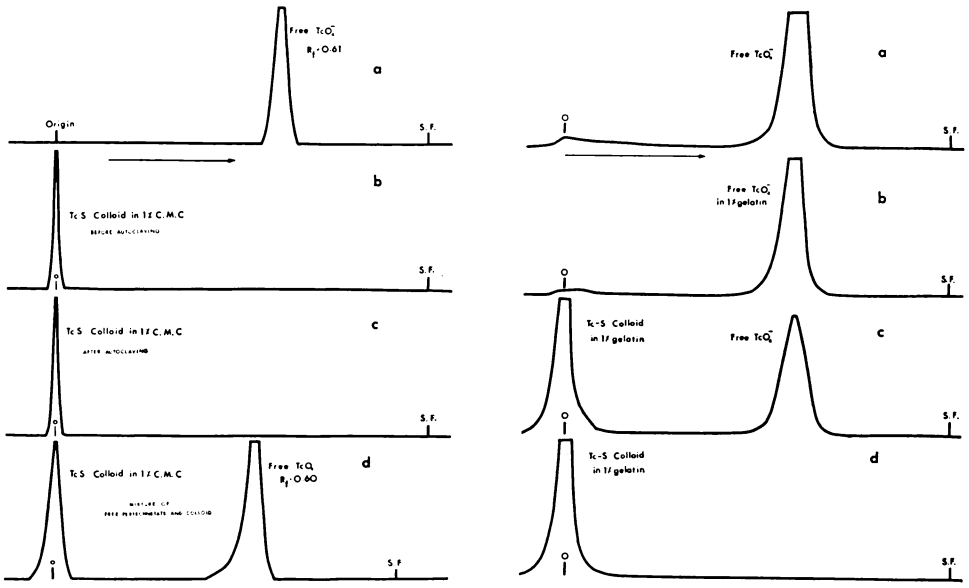

Fig. 3. Effect of autoclaving on the chromatographic distribution of CMC stabilized ^{99m}Tc-S "colloid". Curve (a) free $^{99m}TcO_4^-$ with an Rf of 0.61 Curves (b) and (d) show the colloid before and after autoclaving, respectively. Curve (d) shows the separation achieved when admixtures of "colloid" and pertechnetate ion are chromatographed together.

Fig. 4. ^{99m}Tc-S "colloid" with gelatin stabilizer. Curve (a) shows free pertechnetate ion ($^{99m}TcO_4^-$). Curve (b) depicts all reaction components before thiosulfate addition. Curve (c) shows separation of admixture of ^{99m}Tc-S "colloid" and free $^{99m}TcO_4^-$. Curve (d) is final product neutralized to approximately pH 6.0.

(5) Autoclave for 20 minutes at 15 psi, cool and assay in a suitable instrument against a standard ^{57}Co source (3).

(6) Chromatograph in 85% methanol as described previously for ^{99m}Tc serum albumin (2).

Method II. Gelatin Stabilizer

(1) The desired quantity of $^{99m}TcO_4^-$ (in 5 cc^3 saline) is added to a serum vial containing a teflon-coated bar magnet and 100 mg gelatin, U.S.P., granular form, and is sterilized by autoclaving.

(2) Add 1 cc^3 of sterile 3.0 N sulfuric acid and place in a water bath at 60-62° C for five minutes.

(3) While stirring the contents of the vial, add 0.5 cc^3 of sterile 0.8 per cent sodium thiosulfate and continue to stir for 15-20 minutes.

(4) Cool and adjust to pH 6.0 with sterile 1.0 N sodium hydroxide. Use the same precaution for pH adjustment as described in Method I above (Fig. 2C).

(5) Assay against a ^{57}Co standard and chromatograph in 85% methanol as described in text.

RESULTS AND DISCUSSION

Paper Chromatography of ^{99m}Tc-S "Colloid"

Descending chromatography in 85% methanol gives identical results regardless of the stabilizer used, viz. CMC or gelatin (Figs. 3, 4). The ^{99m}Tc-S "colloid"

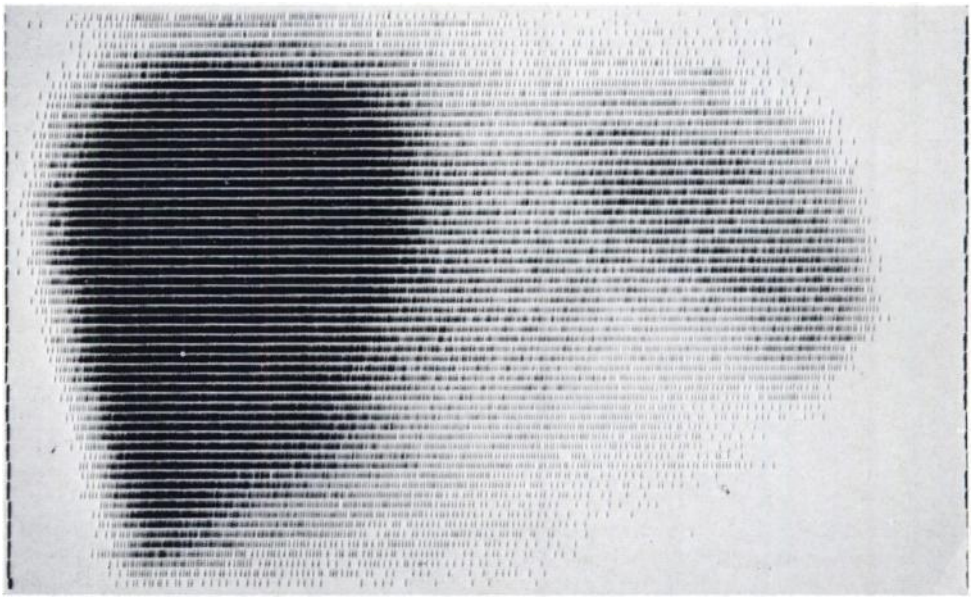

Fig. 5. Normal liver scan using approximately 2 mC of ^{99m}Tc-S "colloid" stabilized with CMC. Posterior supine view showing normal liver (left) and spleen (right).

remains at the origin of the chromatogram while free pertechnetate ion ($^{99m}TcO_4^-$) migrates with an Rf of approximately 0.6 to 0.65.

Pertechnetate ion is readily separated and distinguished from its reduction product which combines with sulfur as shown by the chromatography of admixtures of "colloid" and $^{99m}TcO_4^-$ (Figs. 3D, 4C). When the CMC stabilized "colloid" is autoclaved, (Fig. 3C), there is little, if any, liberation of pertechnetate ion as shown by the absence of radioactivity at Rf 0.6.

Effect of sodium thiosulfate concentration

Although the original parameters of the reaction were investigated using gelatin as the stabilizer, reinvestigation with CMC stabilizer gave similar results with respect to concentrations of reactants, temperature and time.

The incorporation of ^{99m}Tc into the sulfur "colloid" shows little variation in radioactive yield when from 3 to 12 mg of thiosulfate were used (Table I). The liberation of free sulfur is 20% by weight (0.2 mg S for each 1.0 mg thiosulfate) as shown by the reaction,

$$Na_2S_2O_3 + H_2SO_4 \longrightarrow \underset{\downarrow}{H_2S_2O_3} + Na_2SO_4$$
$$S\downarrow \quad + \underset{\downarrow}{H_2SO_3}$$
$$H_2O + SO_2\uparrow$$

In all subsequent studies to determine the optimum conditions for the reaction with respect to acid concentration, temperature and time, 5.0 mg of sodium thiosulfate were used.

TABLE I

TECHNETIUM-SULFUR 'COLLOID' FORMATION AS A FUNCTION OF THIOSULFATE CONCENTRATION

Reaction No.[1]	*mg Thiosulfate*	*Yield*[2]
A	1.0	86.7%
B	2.0	88.3
C	3.0	94.8
D	4.0	95.3
E	5.0	94.6
F	6.0	94.4
G	12.0	96.9

[1]All reactions were carried out in 0.5N sulfuric acid and contained 100 mg gelatin. The reaction time was 30 minutes at 60-62° C. Final volume 10 cc[3] neutralized to pH 6.0.

[2]Taken as the per cent radioactivity remaining at the origin when chromatographed in 85% methanol (descending).

TABLE II

^{99m}Tc-S 'COLLOID' FORMATION AS A FUNCTION OF SULFURIC ACID CONCENTRATION

Reaction No.	*Initial H_2SO_4 Concn. Normality*	*Yield*[1]
A	0.1	83.0
B	0.3	88.7
C	0.5	98.6
D	1.0	93.8
E[2]	3.0	62.1

[1]Per cent radioactivity remaining at the origin of the chromatogram.
[2]At 50° C.

Effect of sulfuric acid concentration

Using 5 mg of sodium thiosulfate, 100 mg of gelatin and heating at 60° C, the sulfuric acid concentration was varied as shown in Table II.

The maximum incorporation of radioactivity into "colloid" occurred at an initial sulfuric acid concentration of 0.5 N as shown by reaction C, Table II. At a concentration of 3.0 N, either the gelatin was hydrolyzed or the CMC was broken down, even at reduced temperatures, yielding approximately 60% bound ^{99m}Tc.

TABLE III

EFFECT OF TEMPERATURE AND TIME ON THE FORMATION OF ^{99m}Tc-S COLLOID

Reaction No.[1]	*Temperature ° C*	*Time in Minutes*	*Yield Per Cent*[2]
A	50	30	88.1
B	60	30	96.3
C	80	30	88.7
D	60	0.5	10.6
		3.0	95.4
		5.0	94.5
		10.0	94.9
		15.0	95.3
		20.0	94.7
		30.0	96.2

[1]All reactions contained 100 mg gelatin, 5.0 mg sodium thiosulfate and an initial sulfuric acid concentration of 0.5 N. Final volume, 10 cc[3].
[2]Per cent radioactivity remaining at the origin of the chromatogram.

TABLE IV

PARTICLE SIZE DISTRIBUTION OF ^{99m}Tc-S 'COLLOID'

Size of millipore filter millimicrons	*Per cent radioactivity passing through filter*
10	9.9
10-50	5.4
50-450	7.6
450-1200	13.6
1200 and above	63.5

The reaction was carried out with 5 mg sodium thiosulfate, 100 mg gelatin, 0.5 N sulfuric acid (initial concentration), at 60° C while stirring for 30 minutes.

Effect of temperature and time on ^{99m}Tc-S colloid formation

Although the previous data indicate standardized temperature and time parameters when the reactant concentrations were under study, it must be evident these two conditions were studied separately.

TABLE V

^{99m}Tc-S 'COLLOID' DISTRIBUTION IN WHITE MICE AFTER INTRAVENOUS ADMINISTRATION

Stabilizer	*Time of Sacrifice*	*Per cent of injected dose in organ*					
		Blood	*Liver*	*Spleen*	*Kidney*	*Lung*	*Stomach*
Gelatin	15 min	4.3	78.7	1.0	0.8	1.2	0.06
	15 min	4.5	79.8	1.0	0.8	0.9	0.03
	1 hour	3.3	77.4	1.2	1.3	0.7	0.04
	1 hour	3.2	75.9	0.9	1.3	0.8	0.05
CMC (not	15 min	4.6	76.6	2.1	0.4	2.6	0.06
autoclaved)	15 min	5.7	74.3	1.6	0.7	2.1	0.05
	1 hour	2.1	76.3	2.5	0.3	1.6	0.07
	1 hour	1.4	83.7	3.0	0.4	1.4	0.09
CMC	15 min	5.7	74.4	0.8	1.2	2.8	0.11
(autoclaved)	15 min	4.1	75.1	0.6	1.2	1.8	0.05
	1 hour	3.1	69.2	0.7	0.8	1.8	0.08
	1 hour	2.1	66.0	0.7	1.2	1.7	0.06

It was found early in this investigation that heat was necessary to obtain satisfactory ^{99m}Tc incorporation into the sulfur "colloid." From Table III reaction B, it is observed that a temperature of between 60 and 65° C gave maximum radioactive yields. The time-course of the reaction was studied (reaction D) by removing samples for chromatographic analysis at the times indicated. The reduction of pertechnetate and liberation of free sulfur occurs in approximately two to three minutes. Although no increase in radioactive yield occurs by incubating greater than three minutes, the "colloid" is routinely heated and stirred for an additional 20 minutes after the initial opalescence. The physical properties of many colloids, specifically particle size, are effected by reaction conditions such as pH, reactant concentrations, temperature, time, stirring, and so forth. Consequently, uptake in various organs may vary from lot to lot if the conditions are not uniform. The following particle size distribution, Table IV, was observed when the Tc-S "colloid" was made under standardized conditions described in the text.

Distribution of ^{99m}Tc-S "Colloid" in White Mice

The data in Table V show the distribution of the "colloid" in white mice sacrificed 15 minutes and one hour after injection. The results are representative of many batches which were made according to the CMC method.

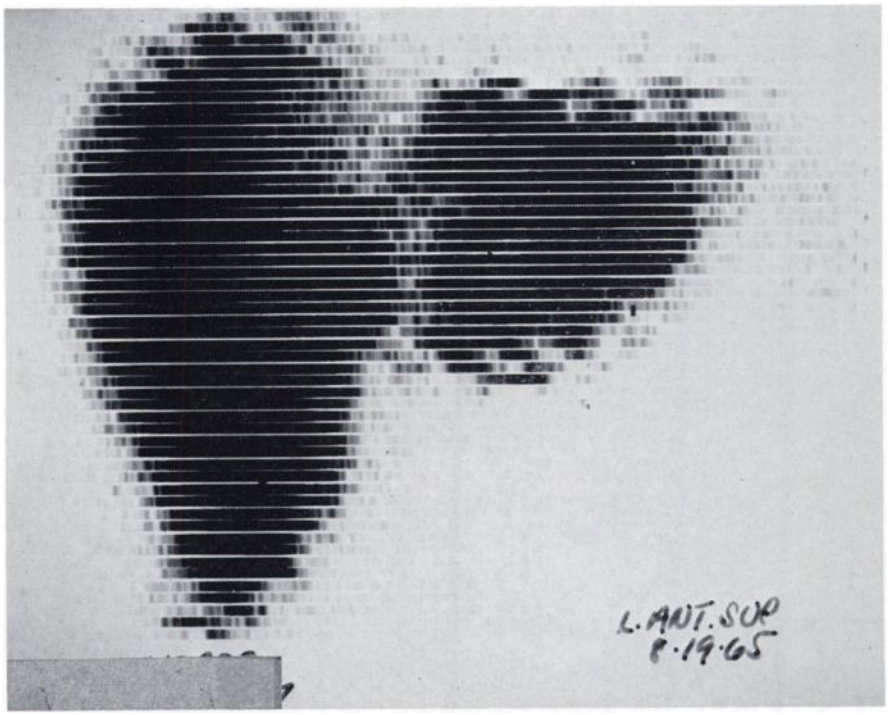

Fig. 6. Abnormal liver scan using approximately 2 mC ^{99m}Tc-S "colloid" stabilized with CMC. Anterior supine view showing abnormality or "hole" in liver at area of decreased uptake.

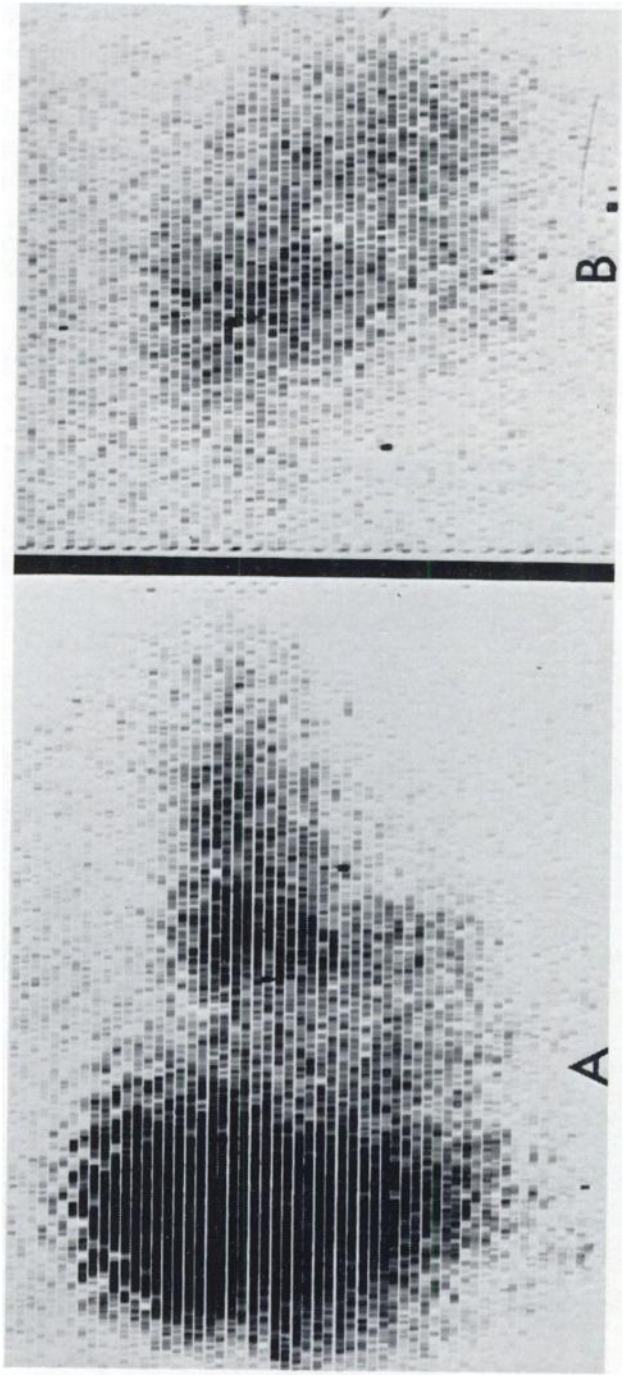

Fig. 7. Abnormal liver scans with 2 mC ^{99m}Tc-S "colloid" stabilized with CMC. View (A) is anterior supine showing prominent defect (area of decreased uptake) through entire vertical mid-section. View (B) is a right lateral scan verifying this defect.

The very low uptake of activity by the stomach, in all cases, correlates well with the absence of "free" pertechnetate ion on chromatographic analysis, and earlier reported concentration of TcO_4^- in the stomach, salivary glands, and thyroid glands (4). The liver uptake is not as dramatic as with colloidal gold and the kidney and lung show more infiltration than with gold colloid. The overall distribution of the ^{99m}Tc-S "colloid" parallels the distribution of chromic phosphate colloid, Type G reported by Lahr, *et al* (5). Although the average liver uptake appears to be decreased slightly when CMC-stabilized-Tc-S "colloid" is autoclaved, "colloid" made in this manner is perfectly satisfactory for liver, spleen, and/or bone marrow scans which are shown below, Figures 5-8.

DISCUSSION

A method for the preparation of a ^{99m}Tc-S "colloid" has been reported. Colloidal sulfur preparations have been used in medicine since 1921 (6), and some

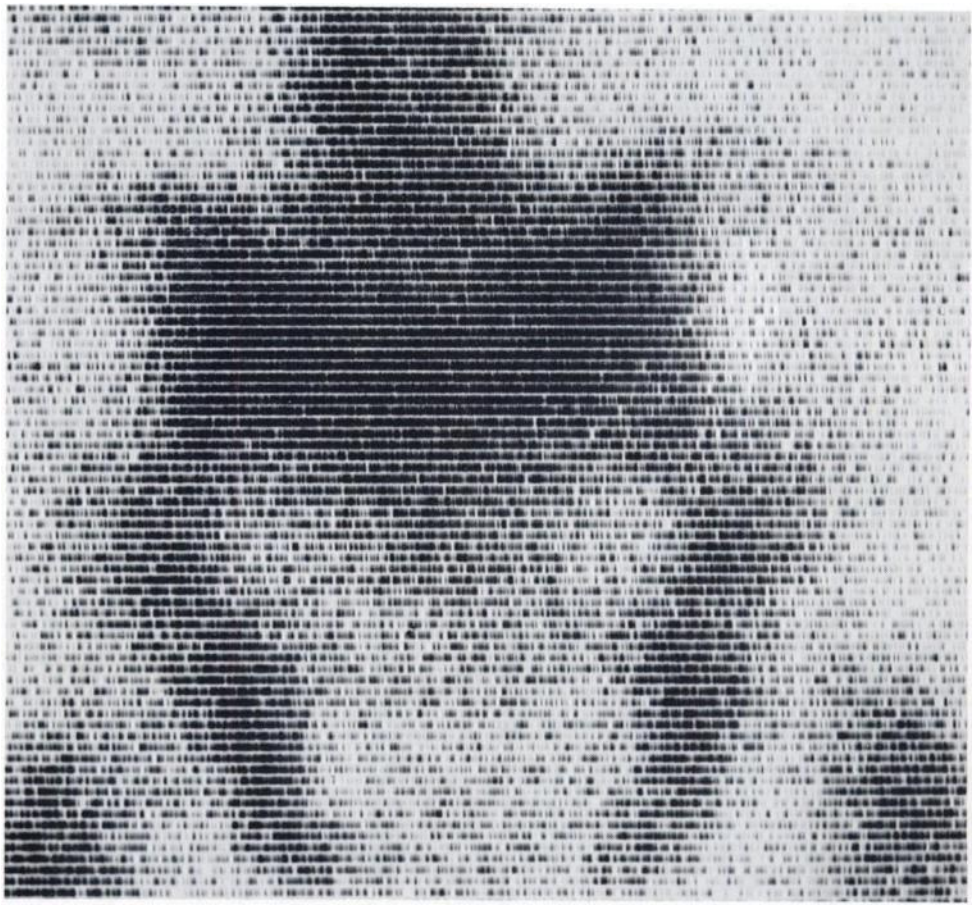

Fig. 8. Normal pelvic marrow scan in patient with systemic mastocytosis. Posterior supine view using approximately 10 mC ^{99m}Tc-S "colloid" stabilized with gelatin.

investigators have administered as much as 30 mg per day intravenously in cases of arthritis (7). Of the two methods described in the text, the authors prefer the "colloid" stabilized with CMC because of the advantage of terminal sterilization by autoclaving. However, if speed is important, sterile technique with gelatin as the stabilizer is the choice method. Although the liver uptake of ^{99m}Tc-S is not as great as with colloidal gold, it does resemble colloidal chromic phosphate, type G, with respect to organ distribution (5).

The important conditions for the preparation of a uniformly reproducible colloid appear to be the initial acid concentration, temperature at which the reaction is carried out, and the time the "colloid" is stirred after initiating the reaction.

The ^{99m}Tc-S "colloid" prepared in the manner described shows very little "free" pertechnetate ion as evidenced by negligible gastric accumulation and lack of a significant readioactive spot at Rf 0.6 when the "colloid" is chromatographed in 85% methanol. There is slight instability of the "colloid" above pH 6.5. Although this preparation of Technetium-99m and sulfur has been termed a "colloid" and mimics many known radiocolloids with respect to biological distribution, the true nature of the reaction and the compound formed remains elusive.

ADDENDUM

By increasing the temperature to 100° C and changing to a dextran stabilizer, ^{99m}Tc-Sulfur Colloid can be prepared from thiosulfate in four minutes (private communication, Dr. Wil B. Nelp, Seattle, Washington). The 'colloid' prepared in this manner reduces the lung uptake by a factor of two or more.

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