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Preliminary Note

^{99m}Tc Labeled Serum Albumin for Scintillation Scanning of the Placenta[1]

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A technique for accurate placental localization is important to differentiate placenta praevia from other causes of third trimester bleeding in pregnancy. Soft tissue radiographical placentography provides only indirect evidence of placenta praevia when the placenta cannot be identified in the uterine fundus and is totally inaccurate in posterior implantations of the placenta. Radioisotopic localization has been achieved by "multiple point counting" over nine to fifteen arbitrarily selected sites of the abdomen by manual positioning of a collimated scintillation detector. In the beginning, ^{24}Na (3) was used but soon was discarded because of its rapid diffusion. Now, only agents which persist intravascularly are employed, such as ^{131}I albumin (9), ^{132}I albumin (10), ^{51}Cr labelled red blood cells (15), and ^{51}Cr albumin (12). Theoretically, ^{131}I cholografin (14) could also be used.

Harper *et al* (7) first called attention to the nuclide ^{99m}Tc as an ideal agent for human diagnostic studies where reduction of radiation dosage is of prime importance. Because of its short physical half-life of six hours, essentially monochromatic gamma radiation of 140 kev, lack of beta emission, relatively high radiochemical purity (16), and ready availability from a ^{99}Mo generator (19), it has been administered with safety in 10 mc doses as pertechnetate for brain scanning (7, 13). Harper and other workers (1, 5, 11) demonstrated also that technetium in a reduced state could label a variety of organic compounds.

In this paper, a reliable method of preparation of ^{99m}Tc labelled human serum albumin with high yield and stability is described which permits the direct visualization of the placenta. This material may be used also for scanning the mediastinum or other vascular structures.

METHOD OF PREPARATION OF ^{99m}TC ALBUMIN

Details of this procedure will be provided in a subsequent paper. The material may be prepared immediately prior to use, or on the preceeding day if allowance is made for four half-lives of physical decay.

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One mg $FeCl_3 \cdot 6\ H_2O$ and one mg ascorbic acid are added to the desired activity of carrier-free ^{99m}Tc pertechnetate eluted in 0.1 N HCl from a ^{99}Mo generator.[1] By pH meter, the pH is adjusted to 4.5 to 5.0 with 1 N NaOH. While stirring, this tagging solution is added, dropwise, to the protein solution containing 2 cc 25 per cent human serum albumin, 3 cc 10 per cent dextrose, 5 cc 0.1 N acetate buffer at pH 5.6, and one drop of Dow-Corning antifoam AF agent. The pH of this reaction mixture is then decreased to 2.5 with 1 N HCl and allowed to incubate for two minutes. The nonprotein bound TcO_4 is removed from the product by passage through a 15 cm column of Amberlite IRA-400 (Cl^-) anion exchange resin overlaid with 4 cm Dowex 1 x 2 (Cl^-) resin, 50–100 mesh, thoroughly washed with distilled water. The column is eluted with distilled water at a rate of 0.5 to 0.8 cc per minute; the first 5 cc of eluate are discarded and the next 20 cc collected. After passage through the resin, the pH of the eluate will be approximately 5.5.

For human use, only pyrogen-free distilled water and other reagents may be used. The apparatus is sterilized by autoclaving at 260° F, 121 psi for 15 minutes: the final solution is sterilized by transfer from one sterile disposable polypropylene syringe to another through a Millipore filter.[2] Radioassay is performed quickly in a large well ionization chamber[3] by comparison with a 10 mc ^{57}Co standard. The total time required to complete this preparation is 90 minutes.

Analysis of protein binding experiments by descending paper chromatography in 85 per cent methanol (Table I) has indicated the following:

1. Both ascorbic acid and Fe^{+++} ions are essential for this particular method of tagging in approximately equal amounts.
2. The adjustment of the tagging solution pH to 4.5–5.0 is important to increase the per cent binding of the technetium.
3. The subsequent reduction of the pH to 2.5 increases the yield of the protein-bound label and prevents the formation of unknown oxidation states or possible complex formations with ferric ion.
4. The tagging reaction is virtually instantaneous.

METHOD OF SCANNING

200 mg of potassium perchlorate dissolved in water are given orally one to two hours before injection, and on the evening of the procedure to block the maternal and fetal thyroid. One mc of the sterile ^{99m}Tc albumin is administered intravenously. Immediately thereafter, the patient is positioned prone on the scanning table, with an eight-inch diameter NaI (Tl) crystal detector mounted beneath. Large cushions of foam rubber five inches thick support the patient's weight above and below the abdomen, so that the uterus is not subjected to external pressure and the patient remains comfortable. Rectilinear scanning is performed over a 12 x 12 inch area extending upwards from the symphysis pubis.

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[1]Brookhaven National Laboratory, Upton, N. Y.

[2]Swinny hypodermic adapter or microsyringe filter holder with autoclaved HA type filter (mean pore size 0.45 μ), Millipore Filter Corp., Bedford, Mass.

[3]NE 014 beta-gamma ionization chamber and NE 503A electrometer D. C. amplifier. General Radiological, Ltd., Nuclear Engineering Div. Middlesex, England.

TABLE I

EFFECT OF VARYING CONDITIONS ON THE BINDING OF ^{99m}Tc TO HUMAN SERUM ALBUMIN

Reaction No.	Fe^{+++}*	Tagging Solution: Ascorbic Acid, mg	Tagging Solution: Initial pH	Tagging Solution: Adjusted pH	Albumin Solution + Tagging Solution: Initial pH	Albumin Solution + Tagging Solution: Adjusted pH	Percent ^{99m}Tc Protein Bound**
1	10	10	< 2.5	4.5–5.0	7.1	2.5	92***
2	0	10	< 2.5	4.5–5.0	7.0	2.5	1.2
3	1	0	< 2.5	4.5–5.0	7.1	2.5	3.0
4	1	10	< 2.5	4.5–5.0	7.0	2.5	73
5	10	10	< 2.5	6.8	7.2	2.5	2.8
6	10	10	< 2.5	3.5–4.0	6.8	2.5	2.5
7	10	10	< 2.5	4.5–5.0	6.7	5.5	55
8	1	1	< 2.5	4.5–5.0	7.1	7.1	40
9	1	1	< 2.5	4.5–5.0	7.1	4.7	54
10	1	1	< 2.5	4.5–5.0	7.1	2.5	93
11	2	2	< 2.5	2.5	6.1	6.1	1
12	2	2	< 2.5	2.5	6 0	2.5	2.9
13	2	2	< 2.5	4.5–5.0	7.0	7.0	28
14	2	2	< 2.5	4.5–5.0	7.1	2.5	89

*Expressed as mg $FeCl_3 \cdot 6H_2O$.
**Radioactivity at origin of chromatogram.
***Average of 10 preparations for clinical use.

With a linear scanning speed of 96 inches per minute and an index spacing of $\frac{1}{8}$ or $\frac{3}{16}$ inches, the total scanning time is 7.5 to 12 minutes.

A 199-hole focusing collimator designed according to the criteria of Beck (7) for optimal counting of ^{131}I with a radius of resolution of 0.5 inches was used with the eight-inch crystal. The sensitivity of detection was approximately six times that of a three-inch crystal scanner with a 19-hole collimator with the same radius of resolution. The counting rates over the placenta and precordium ranged from 30 to 50,000 cpm when the range of the pulse height analyzer extended from 120 to 160 kev.

RESULTS

In 18 patients, the position, size, and discoid configuration of the placenta were well visualized by scintillation scanning in all but one. The single failure was due to poor labelling of the serum albumin which caused high free pertechnetate levels in the bladder to obscure the pelvic area. The ovoid uterine wall could be identified surrounding the "avascular" fetus, together with increased areas of radioactivity in the liver and precordium. Faint concentrations were seen in the iliac vessels and perineum. The activity in the lateral portion of the lower

pelvis was frequently asymmetrical. This series of patients was too small for evaluation of diagnostic accuracy; nevertheless, no misdiagnoses of placenta praevia have yet occurred. Scans in Fig. 1 show (a) a placenta normally positioned in the left fundus, (b) complete placenta praevia verified at caesarean section, and (c) right anterior low-lying placenta which had no affect on the subsequently uneventful pelvic delivery.

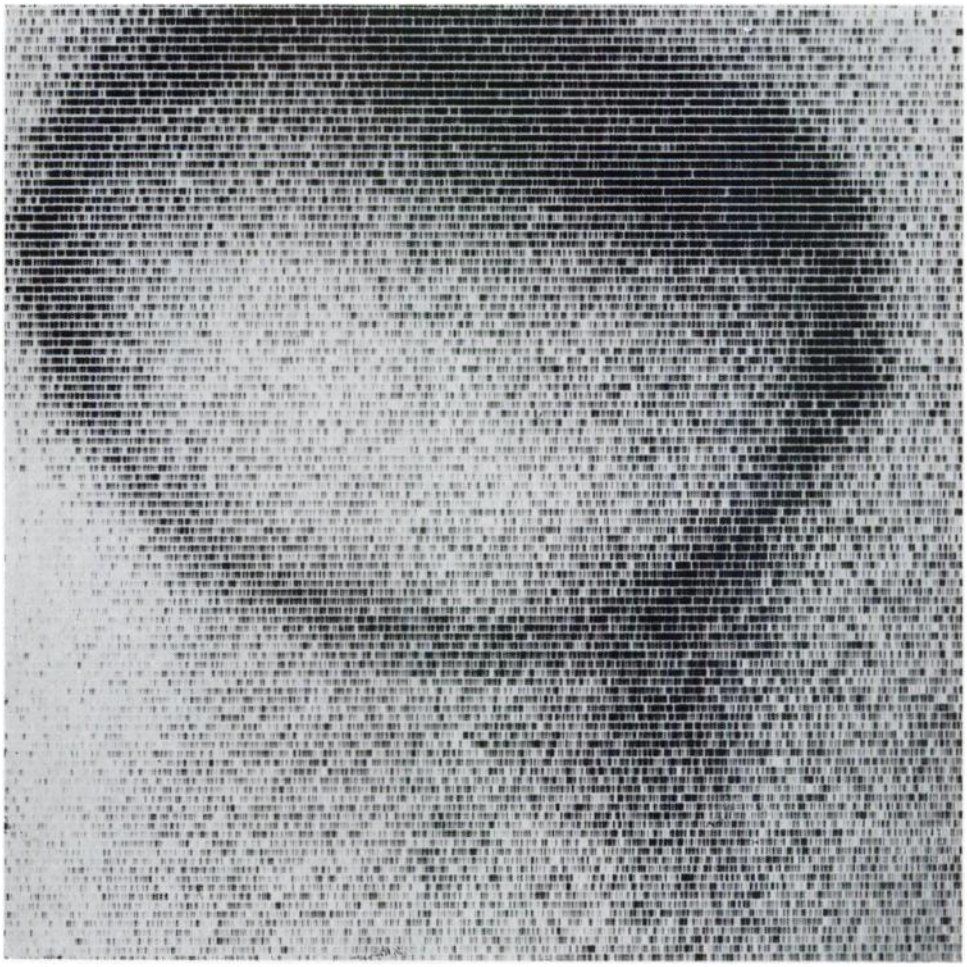

Fig. 1a. Placental seen on 8-inch diameter NaI (TI) crystal scanner, following 1 mc ^{99m}Tc albumin intravenously. Placenta in left fundus.

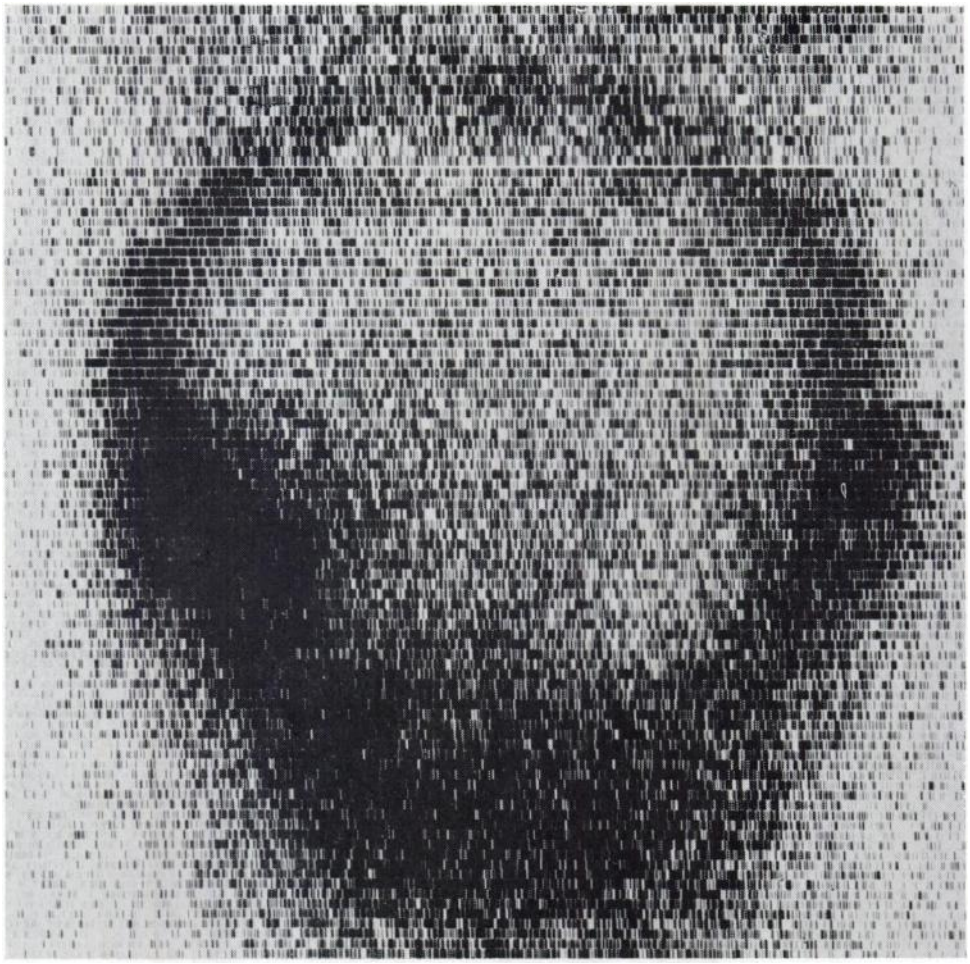

Fig. 1b. Central placenta praevia.

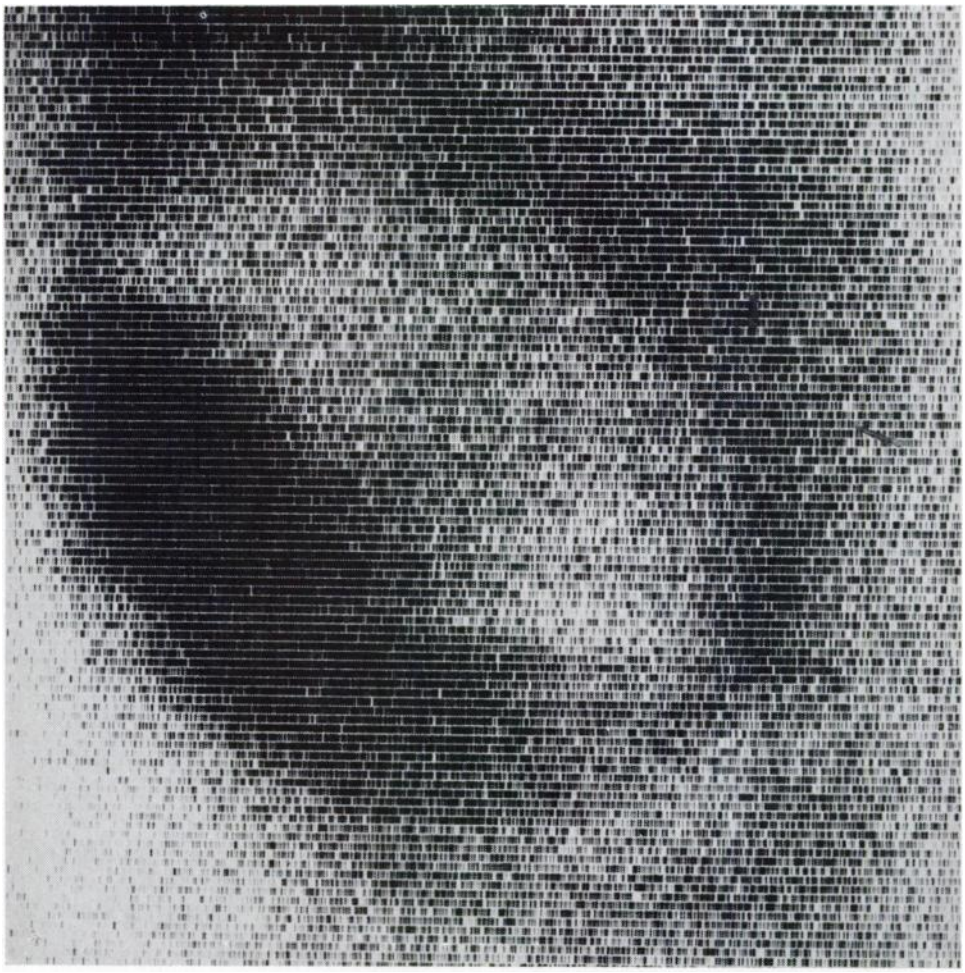

Fig. 1c. Right anterior low-lying placenta.

TISSUE DISTRIBUTION OF ^{99m}TC ALBUMIN

In both animals and man, ^{99m}Tc albumin persists in the blood stream better than ^{51}Cr albumin, but not as well as ^{131}I albumin. This biological behavior may be evaluated in two hours by obtaining serial blood samples from the orbit of white mice following intravenous injection (Fig. 2a). The addition of six per cent dextran to the preparations improves the retention of ^{131}I albumin in the blood, presumably by increasing the electro-negativity, but has no affect on ^{99m}Tc

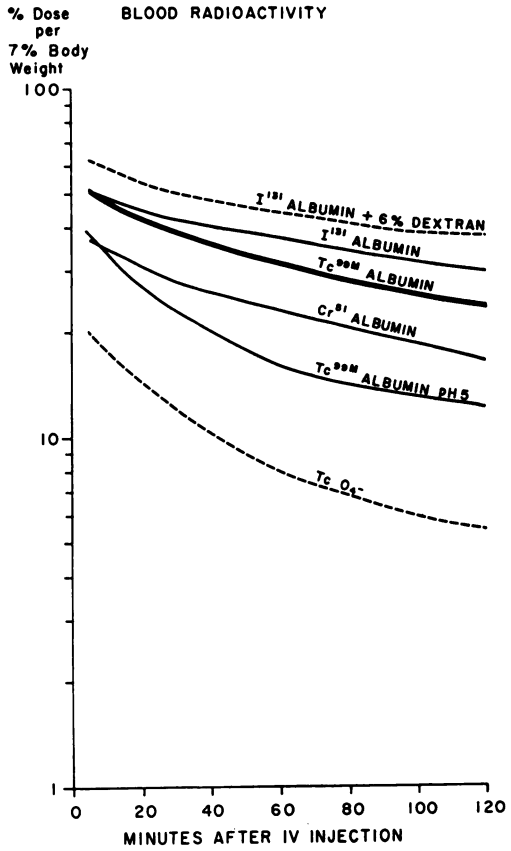

Fig. 2a. Comparison of rate of loss of blood radioactivity for labeled proteins and free pertechnetate in white mice (corrected for radioactive decay).

or ^{51}Cr labelled albumin. In pregnant women, 30 minutes after injection at the completion of a placental scan, from 50 to 75 per cent of the radioactivity is still present in the blood volume. After five minutes following injection, the first biological half-time in the bloodstream is about six hours, with subsequent half-times of three days.

The concentration of ^{99m}Tc in the thyroid, salivary and gastric glands and excretion in both urine and feces observed with pertechnetate (13) does not occur with ^{99m}Tc albumin. In three normal volunteers, less than 0.5 per cent of

the injected radioactivity was recovered in either the urine or feces within the first 24 hours after injection.

Localization studies were performed in the rabbit, because the placenta is hemochorial as in the human, and transfers comparable quantities of sodium and other ions. In pregnant rabbits near term, the tissue distribution of ^{99m}Tc albumin (Fig. 2b) administered without thyroid blocking agents was similar to ^{131}I albumin (Fig. 2c) except that the blood and placental levels were slightly lower,

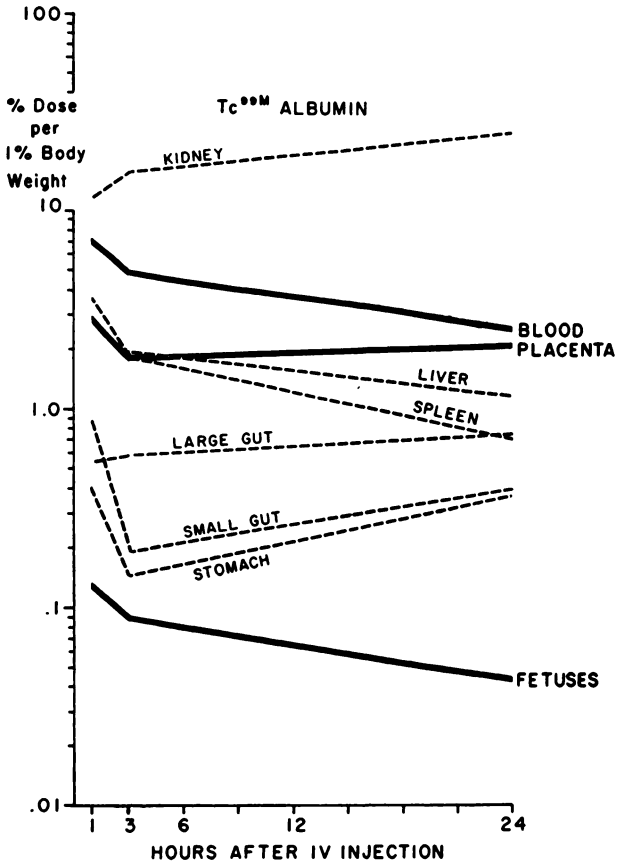

Fig. 2b. Organ distribution of ^{99m}Tc albumin in 9 pregnant rabbits near term (corrected for radioactive decay).

and the concentrations within the fetuses much lower. The highest tissue concentration of ^{99m}Tc albumin was observed in the kidney.

The body of two infants delivered approximately one and four hours following the intravenous administration of 1 mc ^{99m}Tc albumin to the mother contained 0.4 per cent of the maternal radioactivity as determined by external counting and comparison with a phantom. The technetium concentration of cord blood was two per cent of the maternal blood, similar to the average figure of 1.74 per cent of maternal plasma activity previously reported for ^{131}I albumin (20). The

amniotic fluid contained 0.37 per cent of the maternal blood concentration. The placenta contained about one per cent of the administered dose per one per cent of the maternal body weight.

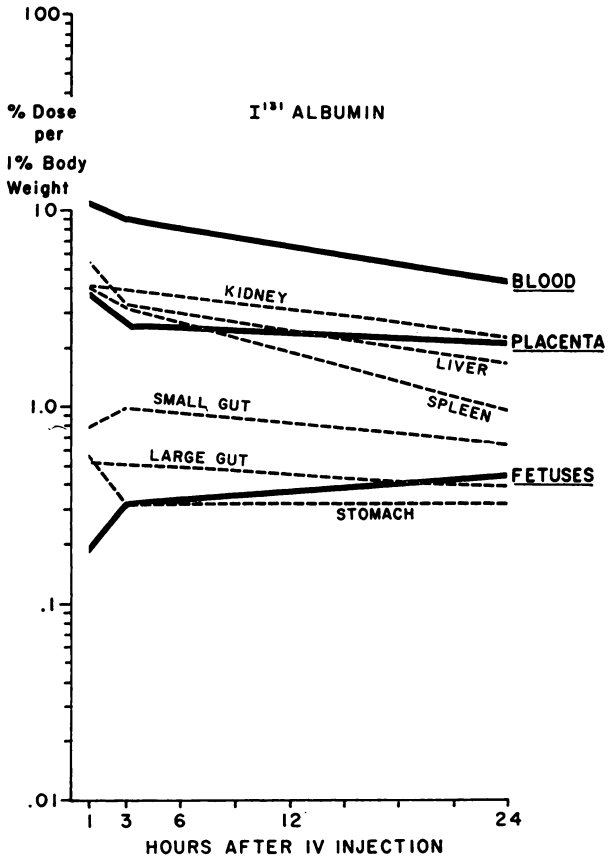

Fig. 2c. Organ distribution of ^{131}I albumin in 9 pregnant rabbits near term (corrected for radioactive decay).

RADIATION DOSIMETRY

The radiation dosimetry of ^{99m}Tc albumin and other substances will be considered in detail in a subsequent publication by Smith (17). Using conventional methods of calculation[1], an intravenous administration of 1 mc ^{99m}Tc albumin

[1]Assumptions in dosimetry calculations: ^{99m}Tc I_{gamma} 0.56 r/mc-hr at 1 cm, $\bar{E}_B$ 14 kev, effective half-time of ^{99m}Tc albumin = physical half-life = 6 hrs. Effective half-time ^{99m}Tc pertechnetate = 3 hrs. Retention of 50% ^{99m}Tc activity in the maternal bloodstream; maternal weight 65 kg, blood volume 60 ml/kg $\bar{g}$ 126, $\bar{g}_p$ maternal trunk 178. Fetal body, 3540 gm; $\bar{g}$ 64, radioactivity 0.5% of the maternal activity; fetal thyroid 2 gm., $\bar{g}$ 7, radioactivity 2% of total free pertechnetate.

without thyroid-blocking agents results in a maternal total body dose of approximately 13 mrads, a maternal blood dose of 43 mrads and a fetal body dose of 14 mrads. About 94 per cent of the total fetal dose is due to the gamma radiation from the maternal trunk, rather than from the small amounts of free pertechnetate which directly enters the fetal body through the placenta. In comparison, calculations of the radiation doses for 5 μc doses of ^{131}I albumin have been reported as follows—maternal total body dose—.015 rads (6), less than .017 rads (8), .044 rads (20); maternal blood dose—.073 rads (12), .087 rads (20); fetal body dose—.005 rads (10, 20), .0065 rads (6, 8). For 20 μc of ^{51}Cr labelled red blood cells, the maternal body dose has been estimated at less than .012 rads, and the fetal body dose .008 rads maximum (15). In these dosage assessments, the contributions of the radioactivity in the maternal trunk and in the placenta itself have often been omitted. All of these levels are much less than the fetal doses resultant from a single AP radiograph of the maternal abdomen (.2 to .3 r) (2, 4, 18).

In the event that thyroid-blocking agents are omitted, the radiation dosage to the maternal and fetal thyroid is probably much lower for ^{99m}Tc albumin than ^{131}I albumin. Assuming one per cent free ^{131}I iodide in the albumin preparation, the maternal thyroid dose would be approximately 0.13 rads per 5 μc. Following administration of ^{131}I albumin without Lugol's solution, two per cent of the administered radioactivity becomes concentrated in the fetal thyroid, resulting in a tissue dose of 5 rads per 5 μc. (10). Harper (7) has calculated the thyroid dose in adults for 1 mc ^{99m}Tc pertechnetate to be 0.1 rads. Assuming as much as five per cent free ^{99m}Tc pertechnetate in the albumin preparation, the maternal thyroid dose will be only .005 rads. Although the fetal thyroid uptake of ^{99m}Tc has not been measured as yet, it has been calculated that the maximum thyroid dose would be .07 rads and a more probable estimate, .02 rads.

CONCLUSIONS

Radioisotopic scanning promises to be the most accurate method for the diagnosis of placenta praevia, since the placenta is directly visualized. The results are easier to interpret and more objective than multiple count rate values obtained by manual manipulation of an external scintillation detector. The radiation dosage levels from 1 mc ^{99m}Tc albumin approximate those of other radioisotopic methods used previously. Automation of the radiochemical labelling procedure may permit studies on an emergency basis in the future. Scanning in both anterior and posterior projections, or in the lateral projection, should further improve the localization. With refinements of technique, it may be possible to detect placental abnormalities other than misplacements.

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