AN APPROACH TO DEVELOPING ADRENAL-GLAND SCANNING

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Recent advances in radioisotope scanning have brought about diagnostic localization of organs which up to a few years ago could only be visualized by surgery. These include the pancreas, parathyroid glands, prostate (*1*) and thymus (*2*). The adrenal gland remains one of the few organs left to be scanned.

The difficulty of delineating the adrenal glands radiographically has stimulated considerable interest in radioisotope scanning for clinical diagnosis. This report describes our experience in animal distribution studies and clinical scanning with a new compound, ^{131}I-labeled stigmasterol, with a suitably high specific activity and sufficient adrenal-gland specificity to be useful for external scanning.

METHODS

^{3}H-labeled stigmasterol was prepared by catalytic reduction with ^{3}H gas, and ^{131}I-labeled stigmasterol and cholesterol were prepared by iodination with ^{131}I-sodium iodide and chloramine-T. The specific activity of the ^{131}I-stigmasterol was in the range of 95–140 μCi/mg of the compound. These compounds were dissolved in a surface-active agent and distilled water, and the final materials were then passed through a sterilizing membrane filter.

Whole-body autoradiography was carried out on ^{3}H- and ^{131}I-stigmasterol. Twenty microcuries of ^{131}I-stigmasterol or 600 μCi of ^{3}H-stigmasterol were injected intravenously into each mouse. Two mice were sacrificed at 3 hr and four mice at 24 hr after administration. They were immersed in acetone at solid-CO_2 temperature. Sagittal sections were freeze-dried, and autoradiographic exposure was made by contact with photographic films (x-ray film for ^{131}I and Fuji ET-7A nuclear plate for ^{3}H). Exposure time was 2 weeks for ^{131}I and 1 month for ^{3}H.

A group of Wistar rats was injected intraperitoneally with a dose of 10 μCi/100 gm body weight of ^{3}H-stigmasterol. The liver, adrenals, kidneys and ½-ml whole-blood samples were obtained from the rats. The radioactive material was extracted by homogenizing and repeated centrifuging with a mixture of acetone:alcohol:ether (4:4:1) solution. An aliquot of the supernatant extracts was evaporated in a warm-water bath under nitrogen gas, and the residue was redissolved in scintillator solution, toluene-POPOP-PPO. Radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer.

Another group of Wistar rats was injected with ^{131}I-stigmasterol intraperitoneally at a dose of 5–18 μCi/rat. Tissue samples were obtained, and radioactivity was measured in a well-type scintillation counter before and after the extraction with the acetone:alcohol:ether solution.

To determine the value of ACTH to stimulate the adrenal uptake of the compound, four rats received 0.5–1.0 unit of ACTH intramuscularly 30 min before injection of the labeled compound.

One millicurie of ^{131}I-stigmasterol was injected intravenously into a rabbit which was sacrificed 24 hr later. The lipids of the organs were extracted with chloroform:methanol (2:1) solution, separated by chromatography on florisil (*3*) and then assayed for radioactivity.

To evaluate the usefulness of ^{131}I-stigmasterol for scanning, three rabbits were injected with 50 μCi of the compound and scanned in the prone position at varying times after intravenous injection. Scans of the extirpated organs were also performed, followed by measurements with a Packard Armac counter.

Serial scans of six patients with nonadrenal disease (uterine cancer) and three patients with suspected

Received May 31, 1967; revision accepted Oct. 11, 1967.

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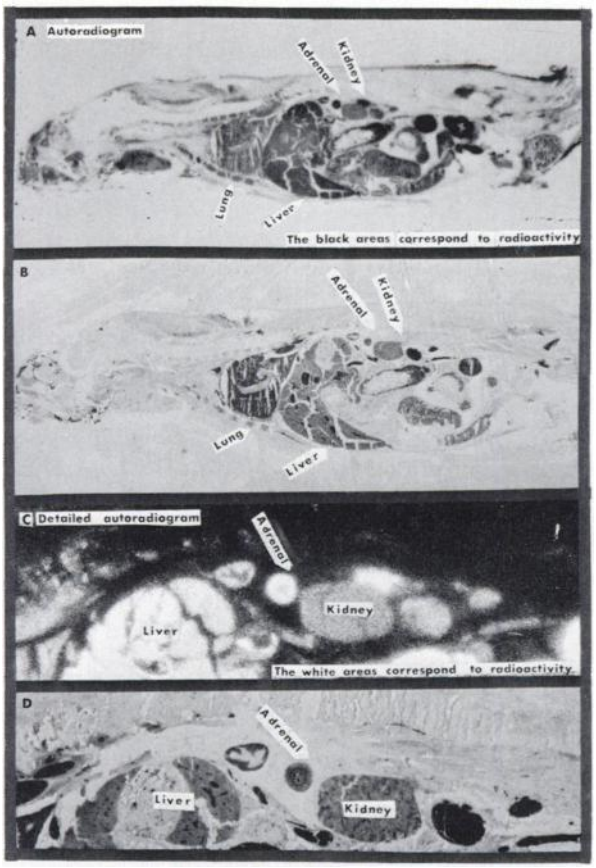

FIG. 1. Whole body autoradiogram showing distribution of ^{131}I-labeled stigmasterol in mouse 24 hr after intravenous injection.

adrenal tumor (one patient with primary aldosteronism and two patients with Cushing's syndrome) whose adrenal tumors were not apparent radiographically were performed to demonstrate the value of this procedure in clinical scanning. The patients were scanned 3 or 5, 24 and 48 hr after intravenous injection of 400–800 μCi ^{131}I-stigmasterol.

Scanning was carried out in the prone position using a Picker Magnascanner-5 with a 3-in. fine-focus, 163-hole collimator and a color attachment. Generally the thyroid gland was not blocked with sodium iodide solution to prevent the accumulation of free ^{131}I in the stomach. Renal scans using ^{203}Hg-chlormerodrin were performed before adrenal scanning to overcome positional difficulties.

In three patients with adrenal disease the scans were followed by surgery.

RESULTS

As demonstrated by whole-body autoradiography (Fig. 1), all mice had a high concentration in the liver and a very pronounced accumulation in the adrenal cortex.

Table 1 and Fig. 2 summarize the data on the relative radioactivity per unit weight of the organs (expressed as the concentration ratio). The adrenal-to-liver concentration ratio rose significantly in 24 hr with a further slight increase. The adrenal-to-kidney ratio increased up to 24 hr but registered a slight reduction at 48 hr. The adrenal-to-blood ratio rose markedly. Much higher concentration ratios were obtainable with animals given ^{3}H-stigmasterol.

These data, which show that the concentration of ^{131}I in the adrenal glands was as great as six times the concentration in the liver and 30–50 times the concentration in the blood and kidneys, indicate that this labeled compound is suitable for scanning purposes.

Figure 3 shows the mean uptake of the whole organs in percentages of administered dose 24 hr after intraperitoneal injection. The adrenal glands contained about 0.3% of the dose.

TABLE 1. MEAN CONCENTRATION RATIO

	No.	Time (hr)	Concentration ratio		
			Adrenal/liver	Adrenal/kidney	Adrenal/blood
^{131}I-stigmasterol	3	1	1.52±0.03	2.48± 0.67	1.15±0.14
	3	3	1.67±0.12	6.72± 1.33	1.36±0.20
	3	5	1.69±0.14	9.70± 0.41	2.03±0.07
	10	24	5.69±0.80	49.48±10.09	35.10±7.14
	6	48	7.47±1.78	44.00±11.22	61.54±9.00
^{131}I-cholesterol	4	24	2.70±0.40	15.70± 4.58	
^{3}H-stigmasterol	3	1	2.52±0.33	15.38± 7.07	
	3	3	2.61±0.05	19.50± 3.19	
	3	5	4.40±0.42	27.52±14.17	
	12	24	20.45±5.29	73.44±17.46	30.90±6.92*
	6	48	22.07±5.29	64.50±13.63	37.68±6.63

* 8 rats.

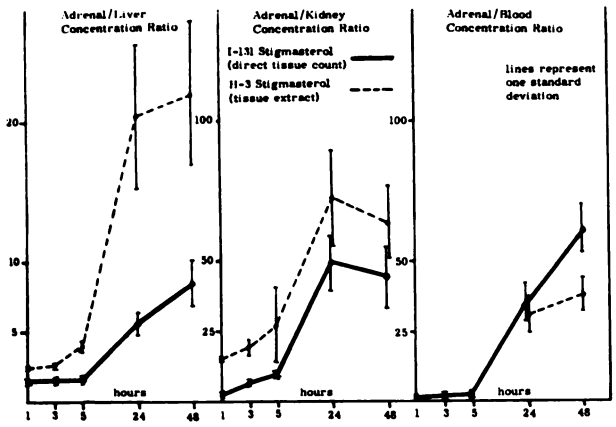

FIG. 2. Mean concentration ratio.

As Table 2 shows, 70–90% of the total tissue radioactivity in these organs was in lipid fractions.

Table 3 demonstrates that an attempt to enhance the relative concentration ratios with ACTH was valueless in rats.

Table 4 is a presentation of the tissue counting data of rabbits using an animal counter. The adrenal-to-kidney concentration ratios in rabbits were less than the ratios in rats, but a favorable concentration was found in the adrenal glands.

TABLE 2. LIPID-BOUND ^{131}I

No.	Time (hr)	Lipid-bound ^{131}I in % total activity			
		Adrenal	Liver	Kidney	Blood
3	1	83	86	84	90
3	3	85	90	87	89
3	5	90	89	86	87
10	24	83	89	85	80
6	48	68	80	74	82

TABLE 3. CONCENTRATION RATIO IN ACTH-TREATED ANIMALS

	No.	Time (hr)	Adrenal/liver concentration ratio
Without ACTH	10	24	5.69±0.80
With ACTH	4	24	5.34±0.68

TABLE 4. CONCENTRATION RATIO IN RABBITS

No.	Time (hr)	Concentration ratio	
		Adrenal/liver	Adrenal/kidney
1	3	2.73	1.97
1	24	9.12	6.16
1	32	5.66 (R:5.88 L:5.43)	5.90 (R:3.96 L:5.84)

Figure 4 shows radioactivity found in a stigmasterol ester, a triglyceride, free stigmasterol, a diglyceride, and monoglyceride separated from lipids of the rabbit's adrenal glands. The percentages of recovered ^{131}I radioactivity in the lipid fractions are presented in Table 5. The maximum radioactivity was usually found in the diglyceride or monoglyceride fractions, and a considerable amount of radioactivity was present in the stigmasterol ester extracted from the adrenal glands.

The scans in normal rabbits were poor and inadequate for interpretation. This was mainly because of the uncertainty of the location of the organs. Scans of the extirpated organs, however, showed that the relative concentration in the adrenal glands was sufficient for visualization by external scanning as Fig. 5 shows.

All scans in patients with normal adrenal glands failed to indicate the adrenal glands. This means that histologically normal human-adrenal tissue could not be seen by this procedure (Fig. 6).

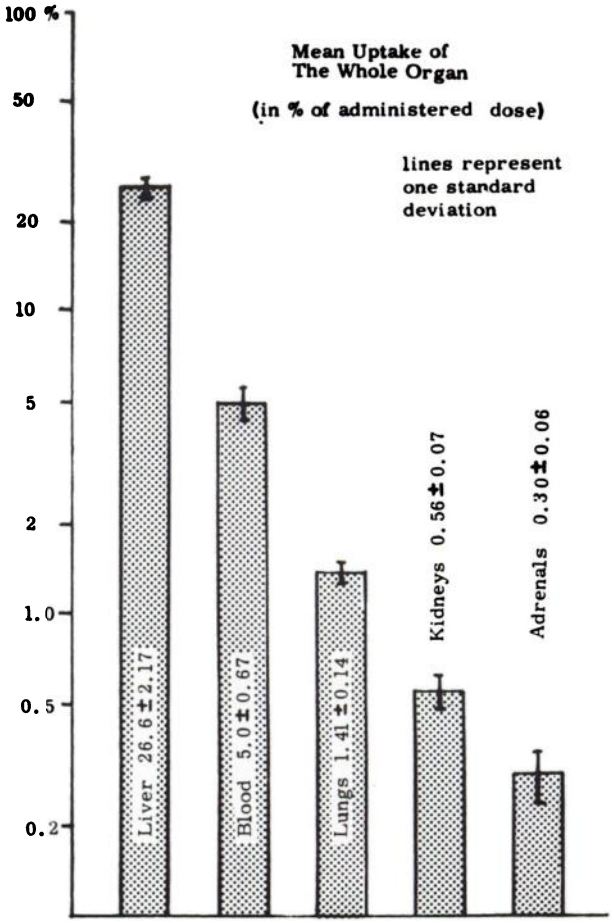

FIG. 3. Mean uptake of whole organ. Blood uptake was based on estimated blood volume of 8% of body weight.

TABLE 5. RECOVERY OF ^{131}I IN TISSUE LIPID FRACTIONS SEPARATED BY CHROMATOGRAPHY ON FLORISIL

	Percentages of recovered ^{131}I in:					
Lipid fraction	Adrenal	Liver	Lung	Pancreas	Kidney	Blood
Stigmasterol ester	15.2	9.4	7.3	8.1	12.5	12.9
Triglyceride	10.7	13.7	9.4	10.0	16.4	12.8
Stigmasterol	13.8	15.9	25.3	11.0	22.9	20.1
Diglyceride	30.6	24.8	22.9	33.1	16.6	25.1
Monoglyceride	23.7	26.5	25.4	30.9	21.1	21.4

The scans of three patients with adrenal disease could be interpreted. Tumors found at subsequent operation were identified histologically as cortical adenomas, and the removed tissues were not available for radioactivity measurement.

There were no clinical side effects.

Case 1. In a 54-year-old female with Cushing's syndrome, scans performed 5 and 24 hr after injection showed an area of concentration which corresponded to a tumor in the left adrenal gland as Fig. 7 indicates. On the basis of the findings on the scans, the diagnosis of the location of the tumor was recorded in the chart preoperatively. Figure 8 shows the cortical adenoma removed at surgery.

Case 2. In a 32-year-old female with Cushing's syndrome, the scans were poor, but an adrenal tumor corresponded to an area of relatively increased radioactivity on the scans.

Case 3. In a 41-year-old female with primary aldosteronism, scans were inadequate for interpretation.

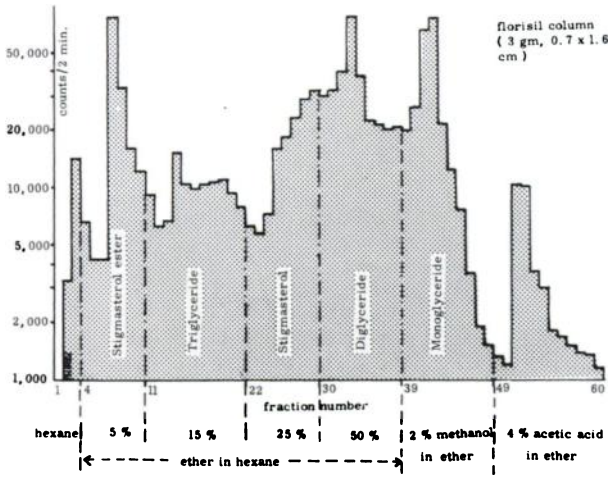

FIG. 4. Separation of lipids from adrenal glands by extraction with chloroform:methanol 2:1.

DISCUSSION

During the course of an investigation studying the incorporation of ^{3}H- and ^{14}C-cholesterol into the aorta of rabbits with experimental hypertension (*4*), we noticed that the radioactivity was deposited in the adrenal glands and that the concentration in the adrenal glands was several times that of other organs.

On the basis of this finding, we had devised a technique of visualizing the adrenal glands using ^{131}I-cholesterol. The adrenal-to-liver concentration ratio of ^{131}I-cholesterol, however, was only 2.70.

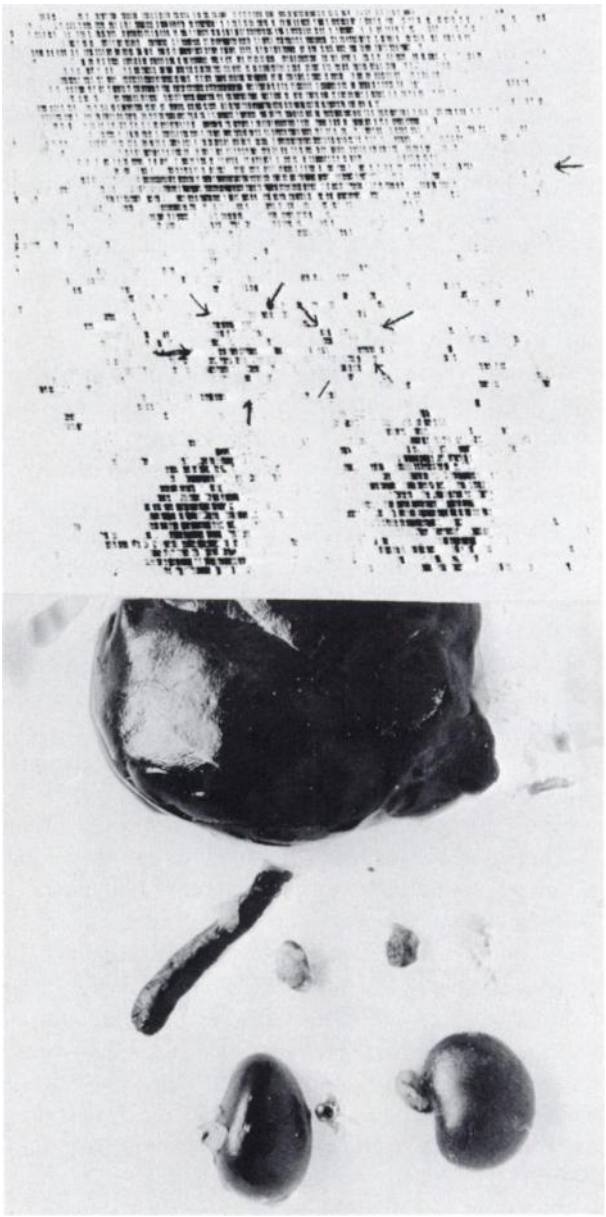

FIG. 5. In vitro scan and photograph of extirpated rabbit organs showing accumulation of ^{131}I-stigmasterol in the adrenal glands. Scan was reproduced from color scan taken 24 hr after injection.

TABLE 6. CLINICAL DATA OF PATIENTS WITH ADRENAL DISEASE

Case	Age	Sex	Disease	Scan interpretation	Weight of gland (gm)	Surgical finding
Case 1	54	Female	Cushing's syndrome	Fair	11	Cortical adenoma of left adrenal gland (2.2 × 2.2 × 2.2 cm)
Case 2	32	Female	Cushing's syndrome	Poor	17.5	Cortical adenoma of left adrenal gland (3.4 × 3.2 × 2.6 cm)
Case 3	41	Female	Primary aldosteronism	Inadequate	6.2	Cortical adenoma of left adrenal gland (1.7 × 1.3 × 1.0 cm)
Cases 4–9		Female	Cancer of cervix	Negative		

We had doubts about the usefulness of this compound for scanning purposes because the target-to-nontarget ratio seemed to be insufficient to distinguish the adrenal glands from adjacent organs.

As the next step, we considered stigmasterol as an agent because it is easily available and suitable for labeling with ^{131}I. Although the mechanism by which ^{131}I is incorporated into the adrenal glands is not clear, one might hypothesize that the adrenal gland actively uses stigmasterol in its metabolism.

Despite the encouraging results obtained in animal experiments, the limitation of this procedure was considerable in clinical scanning mainly because of the confusing patterns of radioactivity in the liver and stomach. Radioactivity in the liver creates a problem in delineating the right adrenal gland, and radioactivity accumulated in the stomach may superimpose over the left adrenal gland.

More precise visualization of the adrenal glands than could be provided by this procedure must await the development of other labeled compounds with more selective adrenal specificity. Iodinated compounds are known not to maintain the original biochemical properties, and the ^{131}I label can be removed before its concentration in the target organ. We are now concerned with substituting ^{18}F for one of the hydrogen atoms in the structure of stigmasterol and cholesterol. Recently we have succeeded in synthesizing ^{18}F-cholesterol with suitably high specific activity (*5*) but we have not yet used it for scanning because of the low yields of the compound.

It is known that ^{14}C-serotonin and 5-hydroxytryptophane can be incorporated into the adrenal glands of experimental animals (*6*), but we have no experience with these agents labeled with a gamma emitter.

Although our procedure with ^{131}I-stigmasterol is not yet entirely successful in clinical scanning, we have summarized our efforts to visualize the adrenal glands by scanning and we hope that this attempt may stimulate renewed interest in the technique.

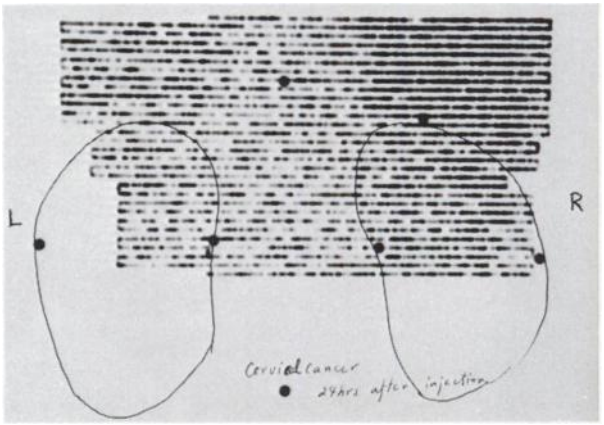

FIG. 6. Photoscan of abdomen taken 24 hr after intravenous injection of ^{131}I-stigmasterol in patient with normal adrenals shows no concentration in areas of adrenal glands. Scan was performed in prone position. Marks identify outline of kidneys which was obtained from renal scan with ^{203}Hg-chlormerodrin.

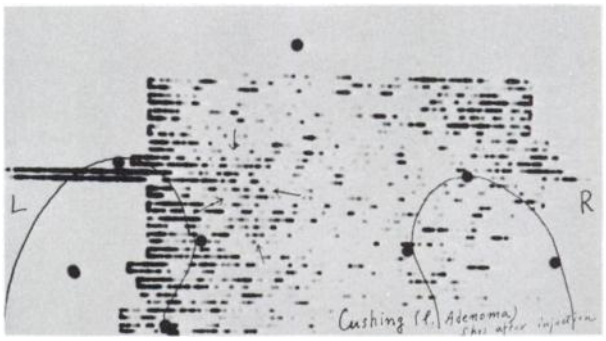

FIG. 7. Photoscan taken 5 hr after intravenous injection of ^{131}I-stigmasterol in Case 1 shows radioisotope concentration in the area of left adrenal gland. Diagnosis of location was recorded on chart preoperatively.

SUMMARY

^{131}I-labeled stigmasterol administered to animals selectively localizes in the adrenal glands. Although the degree of localization is insufficient for actual clinical scanning, in two out of three patients with adrenal tumor there was good correlation between the preoperative scans and subsequent surgical findings. The search for a practical technique to scan the adrenal glands is being continued.

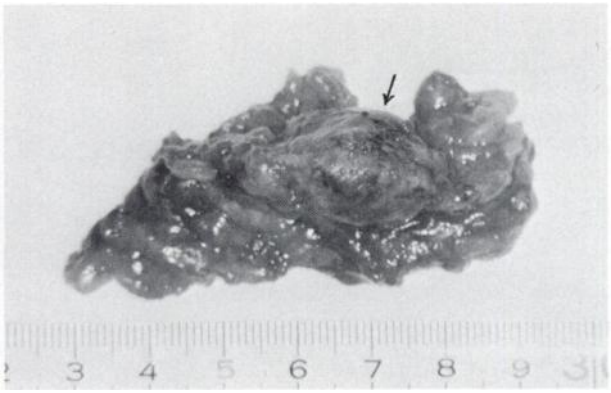

FIG. 8. Photograph of surgically removed cortical adenoma of left adrenal gland in Case 1.

ACKNOWLEDGMENT

We express our gratitude to M. Hasegawa and R. Goto of the Daiichi Kagaku Co., Tokyo, for preparing the labeled compounds and to S. Ichii, W. Matsuoka and M. Kashima for technical advice. We are also indebted to H. Takayasu and his staff at the Dept. of Urology, University of Tokyo, for their cooperation.

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