# A NEW AND SUPERIOR ADRENAL SCANNING AGENT, NP-59

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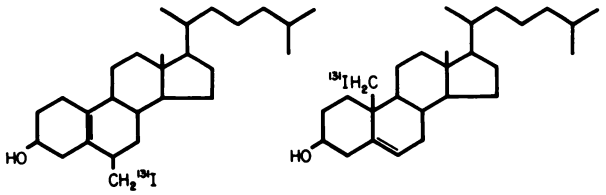
***The first synthesis of $^{131}$I-19-iodocholesterol had a 10–25% radiochemical impurity that was not iodide ion. This impurity has been identified as 6β-$^{131}$I-iodomethyl-19-nor cholest-5(10)-en-3β-ol (NP-59) and has been synthesized. Tissue distribution studies with $^{131}$I-NP-59 in rats and dogs revealed a higher adrenal uptake and adrenal-to-tissue ratios compared to $^{131}$I-19-iodocholesterol, probably less in vivo deiodination, and superior adrenal images. A high uptake was seen in the adrenal medulla in addition to that in the cortex. Iodine-131-NP-59 is being evaluated for the early detection of adrenal–cortical disorders and as a potential scanning agent for detecting structural abnormalities of the adrenal medulla.***

Radioiodinated 19-iodocholesterol was first synthesized by Counsell, et al (*1*). Following the subsequent report by Blair, et al (*2*) of adrenal imaging in dogs using $^{125}$I-19-iodocholesterol, the first visualization of human adrenals using this agent was reported by Beierwaltes and coworkers (*3*). Since then, several reports from this institution have demonstrated the value of $^{131}$I-19-iodocholesterol as an adrenal cortex scanning agent in the assessment of patients suspected of having Cushing's syndrome (*4–6*), aldosteronism (*7–9*), pheochromocytoma (*10*), and adrenal remnants following "total" adrenalectomy (*11*).

During developmental research on 19-iodocholesterol, while the radiopharmaceutical was being prepared for distribution, an "impurity" that was not iodide ion was noticed which accounted for 10–25% of 19-iodocholesterol. Basmadjian, et al (*12*) have identified this "impurity" as 6β-$^{131}$I-iodomethyl-19-nor cholest-5(10)-en-3β-ol (NP-59) and reported its synthesis. We now report that a tissue distribution study with $^{131}$I-NP-59 in rats and dogs shows a higher adrenal concentration and superior adrenal images compared to $^{131}$I-19-iodocholesterol.




$^{131}$I-NP-59 ($6\beta$-$^{131}$I-IODOMETHYL-19-NOR CHOLEST-5(10)-EN-3β-OL)

$^{131}$I-19-IODOCHOLESTEROL ($^{131}$I-19-IODOCHOLEST-5(6)-EN-3β-OL)

**FIG. 1.** Formulas of $^{131}$I-NP-59 and $^{131}$I-19-iodocholesterol.

## MATERIALS AND METHODS

**Radiopharmaceutical.** Figure 1 shows the chemical structures of $^{131}$I-NP-59 and $^{131}$I-19-iodocholesterol.

Using the method of Basmadjian, et al (*12*), NP-59 was synthesized from cholest-5-en-3β, 19-diol-19-toluene-p-sulphonate (*1*) by refluxing for 4 hr in absolute alcohol. After purification, $^{131}$I-NP-59 was obtained by isotope exchange with Na$^{131}$I in absolute alcohol to give a specific activity of 1.3 mCi/mg. It was formulated in 6.6% EtOH, 1.6% Tween 80, q.s. bacteriostatic saline.

Iodine-131-NP-59 in absolute alcohol was stable from −20°C to 4°C for more than a month. In formulation, NP-59 was stable at 4°C for 2 weeks while at room temperature (20°C) 20% deiodination occurred in 4 days.

**Rats.** Fifteen mature female Sprague-Dawley rats, weighing 210–260 gm and fed a regular diet, were each given 25 μCi (19.0 μg in a volume of about 0.2 ml) of $^{131}$I-NP-59 through a femoral vein after intraperitoneal sodium pentobarbital anesthesia (0.05 mg/gm). For comparison, six additional female rats were similarly injected with $^{131}$I-19-iodocholesterol (specific activity, 1.3 mCi/mg). None of the animals received Lugol's iodine or potassium perchlorate.

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**TABLE 1. RAT TISSUE DISTRIBUTION OF $^{131}$I FROM 6β-$^{131}$I-IODOMETHYL-19-NOR CHOLEST-5(10)-EN-3β-OL (NP-59) AND $^{131}$I-19-IODOCHOLESTEROL (% KG DOSE/GM ±1 s.e.m.)***

| | $^{131}$I-NP-59 | | | | | Iodocholesterol | |
|---|---|---|---|---|---|---|---|
| | Time after dose | | | | | | |
| Tissues | 2 hr | 24 hr | 5 days | 10 days | 15 days | 24 hr | 5 days |
| Adrenals | 0.4615 ±0.0535 | 10.1289 ±0.8670 | 8.5809 ±1.6850 | 8.4221 ±0.5959 | 7.1680 ±0.5650 | 2.3470 ±0.1306 | 2.5081 ±0.1871 |
| Thyroid | 0.4323 ±0.0159 | 22.7413 ±4.7587 | 7.4256 ±1.4251 | 8.2817 ±0.8137 | 5.6238 ±0.3750 | 43.6035 ±4.1738 | 17.9832 ±2.0032 |
| Liver | 0.1180 ±0.0037 | 0.1936 ±0.0224 | 0.0255 ±0.0034 | 0.0129 ±0.0012 | 0.0093 ±0.0015 | 0.1197 ±0.0280 | 0.1227 ±0.0007 |
| Spleen | 0.1572 ±0.0312 | 0.2973 ±0.0448 | 0.0435 ±0.0093 | 0.0214 ±0.0012 | 0.0147 ±0.0027 | 0.1413 ±0.0268 | 0.0107 ±0.0008 |
| Kidneys | 0.0153 ±0.0006 | 0.0939 ±0.0116 | 0.0300 ±0.0077 | 0.0185 ±0.0005 | 0.0124 ±0.0013 | 0.0496 ±0.0112 | 0.0079 ±0.0005 |
| Ovaries | 0.0863 ±0.0088 | 3.3243 ±0.1847 | 1.7798 ±0.2967 | 1.2910 ±0.0842 | 1.2608 ±0.1361 | 1.0239 ±0.0734 | 0.6422 ±0.0584 |
| Stomach | 0.0119 ±0.0007 | 0.0808 ±0.0060 | 0.0296 ±0.0056 | 0.0259 ±0.0073 | 0.0168 ±0.0019 | 0.0354 ±0.0110 | 0.0124 ±0.0048 |
| Blood | 0.1854 ±0.0524 | 0.1127 ±0.0115 | 0.0112 ±0.0015 | 0.0061 ±0.0006 | 0.0030 ±0.0004 | 0.0803 ±0.0239 | 0.0034 ±0.0017 |

* Intravenous injection.

**Dogs.** Eighteen mature female mongrel dogs, weighing 6–12 kg and fed a regular commercial dog food, were each given 60 μCi (46 μg in an average volume of 0.5 ml) per kilogram body weight of $^{131}$I-NP-59 through a cephalic vein.

**Adrenal scans.** An effort was made to perform adrenal scintiscans on the dogs at various time intervals after dosing, just prior to sacrifice. The scans were posterior views obtained with the dogs in the prone position, under intravenous sodium pentobarbital anesthesia, using a Picker rectilinear scanner equipped with a 5-in. crystal and a medium-energy 3-in. fine-focus collimator.

**Tissue samples.** Three rats were sacrificed at 2 hr, 24 hr, 5 days, 10 days, and 15 days after the dose. Each animal was deeply anesthetized with ether and sacrificed by cutting out the heart. Seventeen tissues were obtained, including the adrenals, thyroid, liver, spleen, kidneys, ovaries, stomach, blood, lungs, heart, small intestines, large intestines, pancreas, fat, muscle, brain, and parotids. Urine samples were obtained at 2 hr. The samples were cleaned of adipose and connective tissue, weighed, and placed in tubes to which 2.5 ml of distilled water was added. Samples of tissue were similarly obtained on Days 1 and 5 from the six rats given $^{131}$I-19-iodocholesterol.

Two dogs were sacrificed at 2 hr after dosing and at Days 1, 2, 3, 5, 7, 10, 15, and 20 by intravenous injection of a lethal quantity of sodium pentobarbital. Duplicate samples were obtained from the 17 tissues; samples of urine, bile, and gallbladder were also obtained. The adrenals were placed on dry ice immediately after removal, and the cortex and medulla were separated by sectioning the adrenal sagittally and scooping out the medulla. All the dog tissue samples were processed as described above for rats.

**Measurement of radioactivity and expression of concentrations.** Tissue samples were counted in an automatic gamma well counter for 10 min and corrections made for radioisotope decay and counter efficiency. The concentration in each tissue was expressed as percent kilogram dose per gram (% kg dose/gm), which was calculated as follows:

$$\frac{\mu\text{Ci in organ/gm} \times \text{kg body wt} \times 100}{\mu\text{Ci administered dose}} = \%\ \text{kg dose/gm}$$

This unit normalizes mass variation and provides an adequate means of extrapolating tissue distribution data between species (*13*). The percent dose per total organ can be obtained from it as follows:

$$\%\ \text{dose/total organ} = \frac{\%\ \text{kg dose/gm} \times \text{wt of organ (gm)}}{\text{kg body wt}}$$

**Extraction and separation of radioactive products from adrenals.** Total lipid extraction was carried out on several adrenal samples from dogs using Folch's procedure (*14*). Samples of adrenal cortex and medulla were separated as described earlier, weighed, and homogenized for 3 min in 7.0 ml absolute methanol per gram of tissue. Fourteen milliliters of chloroform per gram of original tissue was added to the homogenates, and the sample was allowed to extract for 1 hr under constant agitation. The lipid and

TABLE 2. DOG TISSUE DISTRIBUTION OF $^{131}I$ FROM 6$\beta$-$^{131}I$-IODOMETHYL-19-NOR CHOLEST-5(10)-EN-3$\beta$-OL (% KG DOSE/GM ±1 s.e.m.)*

| Tissue | Time after dose | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 hr | 24 hr | 2 days | 3 days | 5 days | 7 days | 10 days | 15 days | 20 days |
| Adrenal cortex | 0.7025 ±0.0461 | 3.1324 ±0.2209 | 5.1470 ±0.5342 | 4.9153 ±0.5823 | 4.8775 ±0.5036 | 6.1473 ±0.7710 | 8.3796 ±1.2719 | 4.9706 ±0.6858 | 6.5010 ±0.5840 |
| Adrenal medulla | 0.4662 ±0.0614 | 1.9357 ±0.2245 | 2.7090 ±0.4719 | 2.8742 ±0.8710 | 3.4690 ±0.0807 | 3.4942 ±1.0624 | 7.0728 ±2.6310 | 2.5692 ±0.7600 | 2.5227 ±0.5286 |
| Thyroid | 1.1165 ±0.0407 | 13.1145 ±1.8874 | 18.7181 ±3.5724 | 10.5649 ±3.9256 | 8.5395 ±4.4288 | 15.1040 ±1.4314 | 12.4048 ±0.7732 | 14.5409 ±1.6136 | 12.2015 ±3.5737 |
| Liver | 0.4041 ±0.0025 | 0.1984 ±0.0136 | 0.1378 ±0.0082 | 0.0559 ±0.0023 | 0.0480 ±0.0061 | 0.0431 ±0.0012 | 0.0244 ±0.0016 | 0.0191 ±0.0019 | 0.0159 ±0.0030 |
| Bile | 0.9763 ±0.0431 | 1.0945 ±0.4538 | 1.0102 ±0.3384 | 0.2155 ±0.0591 | 0.0799 ±0.0575 | 0.0640 ±0.0091 | 0.0778 ±0.0281 | 0.0436 ±0.0181 | 0.0131 ±0.0065 |
| Spleen | 0.3770 ±0.0046 | 0.3323 ±0.0094 | 0.2109 ±0.0179 | 0.0642 ±0.0195 | 0.0769 ±0.0199 | 0.0475 ±0.0079 | 0.0243 ±0.0051 | 0.0121 ±0.0028 | 0.0087 ±0.0016 |
| Ovary | 0.1210 ±0.0063 | 0.1889 ±0.0084 | 0.2043 ±0.0318 | 0.1148 ±0.0027 | 0.6227 ±0.2987 | 0.1565 ±0.0328 | 0.5678 ±0.2366 | 0.3281 ±0.1161 | 1.6136† ±0.8293 |
| Kidney | 0.0713 ±0.0038 | 0.1070 ±0.0049 | 0.1435 ±0.0042 | 0.0680 ±0.0072 | 0.0488 ±0.0140 | 0.0338 ±0.0052 | 0.0160 ±0.0043 | 0.0088 ±0.0011 | 0.0055 ±0.0017 |
| Stomach | 0.0331 ±0.0082 | 0.0682 ±0.0132 | 0.1206 ±0.0465 | 1.7197 ±1.6569 | 0.4755 ±0.3771 | 0.0795 ±0.0165 | 0.0444 ±0.0130 | 0.0376 ±0.0122 | 0.0268 ±0.0103 |
| Lung | 0.1780 ±0.0198 | 0.1733 ±0.0293 | 0.1432 ±0.0134 | 0.0916 ±0.0221 | 0.0637 ±0.0238 | 0.0452 ±0.0112 | 0.0190 ±0.0039 | 0.0215 ±0.0020 | 0.0053 ±0.0016 |
| Blood | 0.1981 ±0.0045 | 0.1204 ±0.0050 | 0.0873 ±0.0066 | 0.0301 ±0.0027 | 0.0153 ±0.0060 | 0.0150 ±0.0033 | 0.0136 ±0.0093 | 0.0071 ±0.0040 | 0.0019 ±0.0021 |

* Intravenous injection; female dogs.
† One of two dogs was pregnant.

nonlipid biphasic system was then obtained with the addition of 0.2 ml water/ml sample (assume 1 gm tissue = 1 ml) and centrifugation at 500 rpm for 10 min. Aliquots of the chloroform (lipids) and methanol–water (nonlipid) fractions were counted in an automatic gamma well counter. The percentage of radioactivity in the lipid fraction was calculated.

## RESULTS

Table 1 presents the relative distribution of $^{131}I$ from NP-59 and 19-iodocholesterol in rats. Table 2 shows the distribution of $^{131}I$ from NP-59 in dogs.

**Rats.** *Adrenal uptake.* The peak adrenal concentration of $^{131}I$ from NP-59 was 10% kg dose/gm (1.7% dose per total organ) and occurred at 1 day. The uptakes at 1 and 5 days were respectively fivefold and threefold greater than those from 19-iodocholesterol.

*Adrenal-to-liver ratio.* The highest adrenal-to-liver concentration ratio for NP-59 was 771 (at 15 days). At 1 day, this ratio was 52 as compared with 19 for 19-iodocholesterol. At 5 days, it was 337 and 20 for NP-59 and 19-iodocholesterol, respectively.

*Thyroid uptake.* The concentrations of $^{131}I$ from NP-59 in the thyroid at 1 and 5 days were half of those from 19-iodocholesterol.

*Uptake in other tissues.* The concentrations in the adrenals, thyroid, liver, spleen, kidneys, ovaries, stomach, and blood are given in Table 1. Peak uptakes (at 1 day) in the lungs and small intestines were 0.31 ± 0.05 (% kg dose/gm ± s.e.m.) and 0.16 ± 0.03, respectively. In the heart, pancreas, large intestines, parotids, fat, muscle, and brain, peak concentrations (also occurring at 1 day) were 0.08% kg dose/gm or less.

Two male rats were also studied and showed a testicular concentration of 0.008 ± 0.002 (% kg dose/gm ± s.e.m.) at 5 days.

**Dogs.** *Adrenal uptake.* There was a rapid increase in the adrenal cortical concentration (Table 2) to 5% kg dose/gm as early as 2 days after the dose, reaching the highest value of 8% kg dose/gm at 10 days. The adrenal medulla also showed high concentrations, with a peak uptake of 7% kg dose/gm at 10 days. From these values, the calculated percent administered dose to an entire adrenal would be 0.8 for a dog weighing 10 kg.

*Uptake in other tissues.* The concentrations in adrenal cortex, adrenal medulla, thyroid, liver, bile, spleen, ovary, kidney, stomach, lung, and blood are given in Table 2.

It is worthwhile noting that one of two dogs studied at 20 days was pregnant and showed a high ovarian uptake (3% kg dose/gm) as compared to the ovarian concentration in the nonpregnant dog (0.2% kg dose/gm). The concentration in a fetus (about 4 weeks' gestation) was 0.003% kg dose/gm, and that in the placenta, 0.006% kg dose/gm.

TABLE 3. ADRENAL CORTEX-TO-TISSUE CONCENTRATION (% KG DOSE/GM) RATIOS OF $^{131}$I FROM $^{131}$I-NP-59 IN FEMALE DOGS

| | Days after dose | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 1 | 2 | 3 | 5 | 7 | 10 | 15 | 20 |
| Liver | 16 | 37 | 88 | 102 | 143 | 343 | 260 | 409 |
| Kidney | 29 | 36 | 72 | 100 | 182 | 525 | 563 | 1177 |
| Blood | 26 | 59 | 163 | 318 | 410 | 616 | 705 | 336 |
| Bile | 3 | 5 | 23 | 61 | 96 | 108 | 114 | 497 |
| Small intestine | 27 | 41 | 105 | 86 | 142 | 296 | 302 | 761 |
| Large intestine | 41 | 43 | 222 | 119 | 195 | 480 | 407 | 908 |

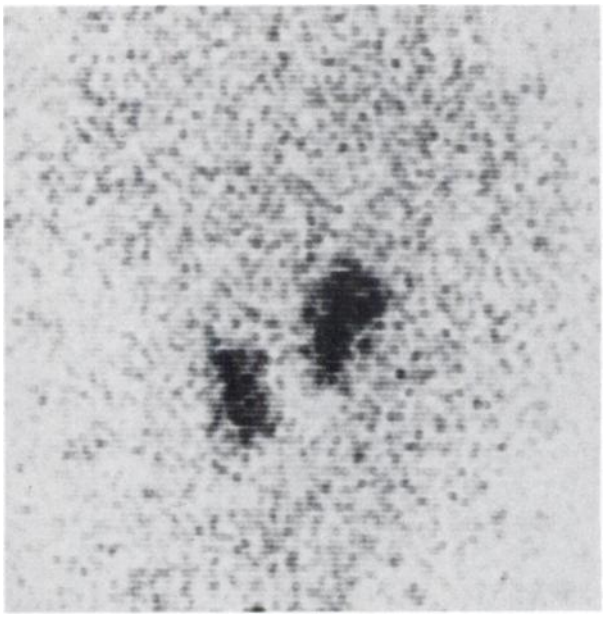

**FIG. 2.** Posterior adrenal image in dog 4 days after administration of $^{131}$I-NP-59.

Concentrations in the heart, small intestines, large intestines, gallbladder, pancreas, muscle, adipose tissue, brain, and urine were 0.1% kg dose/gm or less.

The testicular concentration in two male dogs was 0.066 ± 0.003 (% kg dose/gm ± s.e.m.) at 2 days.

*Adrenal cortex-to-liver ratios.* The radioactivity in the liver showed a gradual decrease with time, while that in the adrenal cortex increased. The adrenal cortex-to-liver ratio (Table 3) thus increased rapidly from 16 at 1 day to 409 at 20 days. High ratios were obtained even at 3 and 5 days (88 and 102, respectively).

*Other adrenal-to-tissue ratios.* Table 3 presents the ratios of concentrations in the adrenal cortex to those of other organs. The adrenal cortex-to-bile ratio was 3 at 1 day and rose progressively to 497 at 20 days. The adrenal cortex-to-kidney ratio also showed a progressive increase from 29 at 1 day to 1,177 at 20 days.

*Radioactive products in adrenal cortex.* Adrenal cortical radioactivity was completely recovered in the chloroform phase (lipid fraction) and none appeared in the methanol phase. Thus, no free $^{131}$I-iodine was found in adrenal tissues after the administration of $^{131}$I-NP-59.

*Scintillation scans.* Adrenal images of excellent quality (Fig. 2) were obtained on all the dogs that were scanned. In our experience these images, obtained with half the usual administered radioactivity to dogs, were definitely superior to those obtained with $^{131}$I-19-iodocholesterol. Moreover, there was earlier visualization of the adrenals, a good image being obtained even at 24 hr after the dose.

**Toxicity.** Toxicity studies were not done as they would require large quantities of the cold compound which was unavailable. However, we found no apparent toxic effect in the animals following administration of $^{131}$I-NP-59, and no gross abnormality in any organ after their sacrifice.

## DISCUSSION

Our studies show a higher adrenal uptake, higher adrenal-to-tissue ratios, superior images, and probably less in vivo deiodination with $^{131}$I-NP-59 as compared with $^{131}$I-19-iodocholesterol.

There is a growing need for better diagnostic tests in adrenal disease. In Cushing's syndrome standard biochemical tests on random blood samples may not be diagnostic in the early stages. This might be related to the episodic nature of cortisol production (*15,16*). Using $^{131}$I-19-iodocholesterol scans, we have shown some evidence for the presence of functional adrenal nodules in patients suspected of having Cushing's syndrome but in whom no clear-cut biochemical abnormalities could be demonstrated (*6*). Recently Raux, et al (*17*) have described undetectable blood ACTH levels in patients with cortisol excess due to adrenocortical nodular hyperplasia. Iodine-131-NP-59, with its superior biologic localization, might enable a better delineation of adrenal abnormalities in these patients.

With $^{131}$I-19-iodocholesterol, we have discretely imaged aldosteronomas 2.2 cm in diameter, with a characteristic image not found in any other condition (*18*). It is logical that an iodocholesterol adrenal scanning agent with a higher percent uptake and higher adrenal-to-tissue ratios would allow us to detect smaller aldosteronomas and macronodules in patients with aldosteronism.

In this context, it is worthwhile noting that Gunnells, et al (*19*) and Grim, et al (*20*) have described the same continuum of micro- and macronodular hyperplasia and adenomas in patients with low renin hypertension as has been found in aldosteronism (*9*). There is evidence that low renin hypertension is associated with mineralocorticoid excess (*21,22*). We are now exploring the possibility that adrenal scanning with $^{131}$I-NP-59 could be helpful in detecting structural abnormalities of the adrenals in patients with low renin hypertension.

Although we have not performed autoradiography to determine the distribution of $^{131}$I from NP-59 in the adrenals, our finding of a high uptake in the adrenal medulla is not entirely unexpected since chromaffin granules in bovine adrenal medulla have been shown to be relatively rich in lipids (especially cholesterol), which represent 22% of their dry weight (*23,24*). Moreover, lysolecithin, one of the main components of the phospholipids of chromaffin granules, is thought to be involved in the release of catecholamines. Thus, we are also evaluating the possible use of $^{131}$I-NP-59 as a scanning agent for structural abnormalities of the adrenal medulla, especially in the detection of pheochromocytomas less than 3 cm in diameter (*10*).

## ACKNOWLEDGMENTS

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