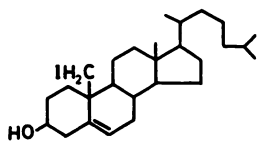
# NEW ADRENAL-SCANNING AGENT

Masaharu Kojima, Minoru Maeda, Hiroshi Ogawa, Kazuo Nitta, and Takayuki Ito

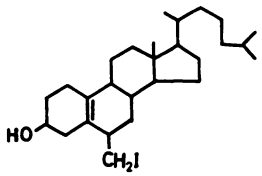
*Kyushu University, Fukuoka, and Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan*

***A new adrenal-specific compound, 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I), which is derived from 19-iodocholesterol (CL-19-I), has been found. Tissue distribution studies have revealed that the rat adrenal gland accumulates ten times more NCL-6-$^{131}$I than CL-19-$^{131}$I. The advantage of NCL-6-$^{131}$I as a possible adrenal-scanning agent is discussed.***

The radioiodinated analog of 19-iodocholest-5-en-3β-ol (CL-19-I) has been widely recognized as a clinically useful diagnostic agent for scintiscanning of the adrenal gland and associated tumors (*1–6*). Radioiodinated CL-19-I was first synthesized by Counsell, et al (*7*) by isotope exchange of CL-19-I with radioactive sodium iodide, Na$^{125}$I or Na$^{131}$I in refluxing acetone for 4 hr under nitrogen and purified by chromatography on deactivated alumina eluting with petroleum ether:ether (1:1). As a part of our research on a possible agent for adrenal scintigraphy, we attempted to confirm the synthesis and selective localization in the adrenal gland of CL-19-$^{131}$I. Unexpectedly, we have found a new adrenal-specific compound which is a radioiodinated steroid analog derived from CL-19-$^{131}$I.




CL-19-I




NCL-6-I

## EXPERIMENTAL

**Detection and identification.** After isolating CL-19-$^{131}$I according to Counsell's procedure (*7*), its purity was checked by thin-layer chromatography using a radiochromatogram scanner (TLC-aluminum sheet-silica gel F254, Merck Co., Ltd.). A single radioactive spot, with an $R_f$ value of 0.70 [Counsell (*7*), 0.66], was found using a chloroform-ethanol (1:1) solvent system. However, when chloroform was used as solvent, a radioactive spot from an unknown compound with an $R_f$ value slightly higher ($R_f$ 0.27) than that of CL-19-$^{131}$I ($R_f$ 0.20) was detected. The ratio of the new compound to CL-19-$^{131}$I determined by radioactivity counting was 0.3. Such a phenomenon was also observed on a cold run and preparative thin-layer chromatography (PLC-plates silica gel 60 F254, Merck Co., Ltd.) eluted with chloroform gave a pure new compound [glass, $[\alpha]_D^{23}$ +39° (cyclohexane)] together with pure CL-19-I. The structure of the new compound was identified as 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I) by spectroscopic methods [ultraviolet 228 (ε 7,290) and shoulder 259 nm (ε 3,020); NMR ($CDCl_3$, 100 MHz) 0.68 (3H, singlet, 18-Me), 2.07 (OH, $D_2O$ exchangeable), 3.97 (1H, m, 3-H), 3.08 (1H, t, J 10 Hz, 6-$CH_2$I), and 3.50 ppm (1H, dd, J 10 Hz, 2 Hz, 6-$CH_2$I)] (*8*).

Furthermore, heating of CL-19-I in boiling isopropanol for 7 hr gave NCL-6-I in 65% yield. Thus, the formation of NCL-6-I is readily interpreted by the rearrangement reaction of CL-19-I through a homoallylic cation intermediate that is known in the steroid series (*9,10*). It is evident that the preparation of CL-19-$^{131}$I according to Counsell's procedure always contains a considerable amount of NCL-6-$^{131}$I as a byproduct. This new compound was found to be more stable than CL-19-$^{131}$I. The radiochemical purity of NCL-6-$^{131}$I after 30 days at 5°C or after 10 days at 20°C was 94% while the radiochemical purity of CL-19-$^{131}$I was 60% after 30 days at 5°C or after 4 days at 20°C.

Received Oct. 1, 1974; revision accepted Jan. 23, 1975.

For reprints contact: Masaharu Kojima, Faculty of Pharmaceutical Sciences, Kyushu University, Maedashi 3-chome 1-1, Higashi-ku, Fukuoka 812, Japan.

**TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN MALE RATS AT VARIOUS TIMES FOLLOWING INJECTION OF CL-19-$^{131}$I***

| | Day 1 | Day 3 | Day 7 | |
|---|---|---|---|---|
| | Rat 1 | Rat 1 | Rat 1 | Rat 2 |
| Adrenal | 16,830 | 6,554 | 5,098 | 5,034 |
| Liver | 511 | 60 | 22 | 23 |
| Kidney | 297 | 62 | 17 | 14 |
| Lung | 824 | 92 | 18 | 22 |
| Spleen | 937 | 76 | 21 | 15 |
| Testicle | 113 | 24 | 6 | 3 |
| Blood | 305 | 33 | 10 | 10 |
| Thyroid† | 448,154 | 346,600 | 157,018 | 280,779 |

* Radioactivity in disintegrations per minute per milligram of tissue.
† Thyroid weight was estimated as 30 mg.

**TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN MALE RATS AT VARIOUS TIMES FOLLOWING INJECTION OF NCL-6-$^{131}$I***

| | Day 1 | Day 3 | Day 7 | |
|---|---|---|---|---|
| | Rat 1 | Rat 1 | Rat 1 | Rat 2 |
| Adrenal | 141,305 | 76,984 | 56,676 | 61,616 |
| Liver | 1,249 | 233 | 82 | 56 |
| Kidney | 830 | 377 | 169 | 151 |
| Lung | 2,313 | 704 | 156 | 168 |
| Spleen | 2,070 | 421 | 97 | 89 |
| Testicle | 310 | 121 | 67 | 69 |
| Blood | 706 | 109 | 30 | 28 |
| Thyroid† | 89,427 | 115,860 | 47,813 | 13,916 |

* Radioactivity in disintegrations per minute per milligram of tissue.
† Thyroid weight was estimated as 30 mg.

**Tissue distribution studies of CL-19-$^{131}$I and NCL-6-$^{131}$I.** The above facts, therefore, induced us to make a comparison of the accumulation of very pure CL-19-$^{131}$I and NCL-6-$^{131}$I in the rat adrenal gland. Radioiodination of NCL-6-I was readily achieved by isotope exchange with sodium iodide-$^{131}$I in refluxing acetone. Four Wistar male rats weighing 140–150 gm were injected intravenously with approximately 50 $\mu$Ci of CL-19-$^{131}$I and the second group with approximately 50 $\mu$Ci of NCL-6-$^{131}$I (the specific activity of both compounds was 1 mCi/mg). Both compounds were in saline solution (250 $\mu$Ci/ml). Chemical and radiochemical purity of both compounds in saline solution were established by thin-layer chromatography. Animals from both groups were sacrificed at 1, 3, and 7 days, respectively, after administration. The liver, kidney, adrenal, thyroid, and other organs were excised, weighed, and placed in small counting vials. Counting was done in a well counter maintaining the same geometric efficiency. The radioactivity is expressed in disintegrations per minute per milligram of tissue or calculated to percent administered dose per gram of tissue.

## RESULTS AND DISCUSSION

Tables 1 and 2 compare the concentration of radioactivity (dpm/mg) in the individual organs following administration of CL-19-$^{131}$I and NCL-6-$^{131}$I, respectively. Figures 1 and 2 show graphically the average accumulation in each organ connected for radioactive decay (% dose/gm).

The rat adrenal accumulates ten times more NCL-6-$^{131}$I than CL-19-$^{131}$I and retains a higher concentration. The average concentration, therefore, in the adrenal gland of CL-19-$^{131}$I-dosed rats was 9.7%/gm at 7 days while the NCL-6-$^{131}$I-dosed rats averaged 136%/gm.

Figures 1 and 2 demonstrate that the excretion of CL-19-$^{131}$I from organs other than the adrenal and thyroid glands seems to be faster than the excretion of NCL-6-$^{131}$I. The ratio of adrenal gland-to-liver concentrations at 7 days was 851 for NCL-6-$^{131}$I while that for CL-19-$^{131}$I was 225. The latter is similar to the value of 157 (in rats) reported by Counsell, et al (*11*).

The original idea for CL-19-$^{131}$I as an adrenal-specific agent was developed from the observation that cholesterol has a tendency to accumulate in the

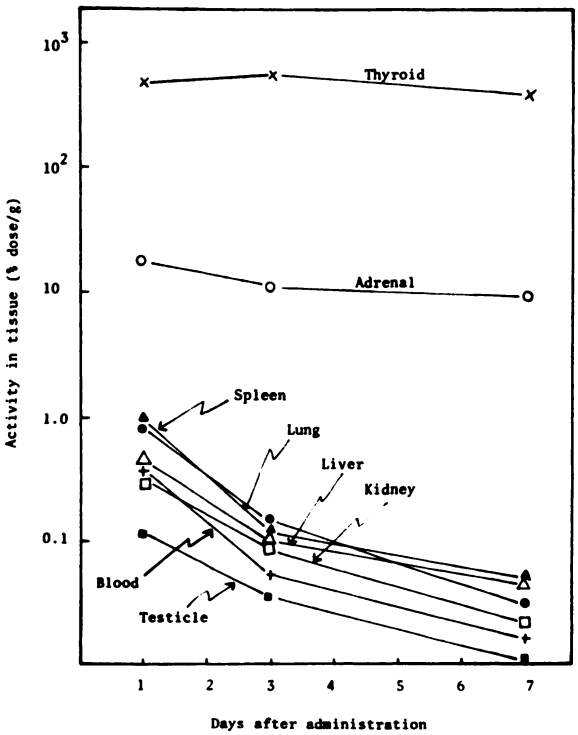



**FIG. 1.** Tissue accumulation of 19-iodocholest-5-en-3$\beta$-ol-$^{131}$I.

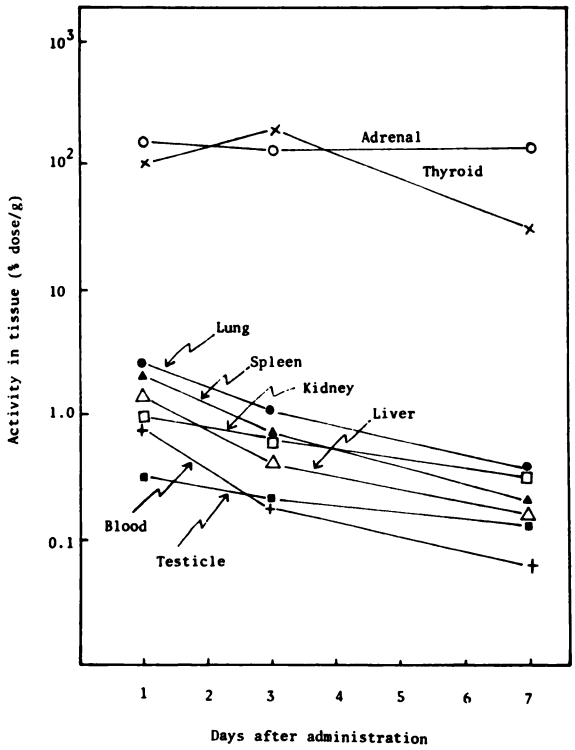



**FIG. 2.** Tissue accumulation of 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol-$^{131}$I.

adrenal cortex (*12*). Autoradiography and tissue radioassays established the "fact" (*13*) that $^{14}$C-labeled cholesterol concentration in the adrenal cortex exceeded that of any other tissues and that cholesterol concentrated to a greater extent than the other steroid analogs. The accumulation of NCL-6-$^{131}$I in the (rat) adrenal gland is much greater than CL-19-$^{131}$I and, therefore, is more suitable as an adrenal-scanning agent. The high selective concentration of CL-19-$^{131}$I in the adrenal cortex previously reported is now questionable since it was not known at that time that CL-19-$^{131}$I usually includes NCL-6-$^{131}$I.

Our findings should also be of interest in connection with the role of cholesterol as the principal precursor of adrenocortical steroids (*14,15*). Due to the relation between the higher accumulation of NCL-6-$^{131}$I in rat adrenal gland and its chemical structure, the present cholesterol precursor theory seems to encounter some difficulties. Our finding is just one interesting fact about NCL-6-$^{131}$I, however, and it should be studied more deeply.

We can say that NCL-6-$^{131}$I is by far a more effective adrenal-concentrating agent and should be a better scanning agent than CL-19-$^{131}$I. We are presently investigating the possible diagnostic usefulness of NCL-6-$^{131}$I in man.

## REFERENCES

*1.* BLAIR RJ, BEIERWALTES WH, LIEBERMAN LM, et al: Radiolabeled cholesterol as an adrenal-scanning agent. *J Nucl Med* 12: 176–182, 1971

*2.* BEIERWALTES WH, LIEBERMAN LM, ANSARI AN, et al: Visualization of human adrenal glands in vivo by scintillation scanning. *JAMA* 216: 275–277, 1971

*3.* LIEBERMAN LM, BEIERWALTES WH, CONN JW, et al: Diagnosis of adrenal disease by visualization of human adrenal glands with $^{131}$I-19-iodocholesterol. *N Engl J Med* 285: 1387–1393, 1971

*4.* CONN JW, MORITA R, COHEN EL, et al: Primary aldosteronism: Photoscanning of tumors after administration of $^{131}$I-19-iodocholesterol. *Arch Intern Med* 129: 417–425, 1972

*5.* MORITA R, LIEBERMAN LM, BEIERWALTES WH, et al: Percent uptake of $^{131}$I radioactivity in the adrenal from radioiodinated cholesterol. *J Clin Endocrinol Metab* 34: 36–43, 1972

*6.* SCHTEINGART DE, CONN JW, LIEBERMAN LM, et al: Persistent or recurrent Cushing's syndrome after "total" adrenalectomy: Adrenal photoscanning for residual tissue. *Arch Intern Med* 130: 384–387, 1972

*7.* COUNSELL RE, RANADE VV, BLAIR RJ, et al: Tumor localizing agents IX. Radioiodinated cholesterol. *Steroids* 16: 317–328, 1970

*8.* KOJIMA M, MAEDA M, OGAWA H, et al: Homoallylic rearrangement of 19-iodocholesterol. *JCS Chem Comm*: in press

*9.* TADANIER J: Homoallylic rearrangements of 19-substituted steroids. *J Org Chem* 31: 2124–2135, 1966

*10.* MOUSSERON-CANET M, LANET JC: Stéroïdes fluorés. Transposition homoallylique du fluoro-3β,hydroxy-19 cholestène-5. *Bull Soc Chim Fr* 1745–1757, 1969

*11.* COUNSELL RE, KULKARNI PG, AFIATPOUR P, et al: Potential organ or tumor-imaging agents. 13. 19-radioiodinated sterols. *J Med Chem* 16: 945–947, 1973

*12.* COUNSELL RE, RANADE VV, KULKARNI PG, et al: Potential organ or tumor-imaging agents. 12. Esters of 19-radioiodinated cholesterol. *J Nucl Med* 14: 777–780, 1973

*13.* APPELGREN LE: Sites of steroid hormone formation. Autoradiographic studies using labeled precursors. *Acta Physiol Scand* 71: Suppl 301, 1–108, 1967

*14.* GOODMAN DS: Cholesterol ester metabolism. *Physiol Rev* 45: 747–839, 1965

*15.* SAMUELS LT, UCHIKAWA T: Biosynthesis of adrenal steroids. In *The Adrenal Cortex*, Einstein AB, ed, Boston, Little, Brown, 1967, p 61