The Combined Use of Iodine-125 and Iodine-131 in Autoradiographic Studies of Nodular Goiter

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The study of the thyroid scintigram as influenced by suppression of and stimulation by TSH has advanced our knowledge of the pathophysiology of the thyroid (1,2,3). Especially in enlarged glands, autoradiography is required for critical interpretation of the activity of various groups of follicles or nodules. The use of a single isotope of iodine limits the postoperative autoradiograph identifying activity of various areas of the thyroid as they functioned at the time of its administration. By using two isotopes, each administered during a different physiologic state of the thyroid, more information can be obtained from a specimen obtained at surgery.

Iodine-131 has been used for thyroid autoradiography for approximately 25 years. Iodine-125 with low-energy conversion and Auger electrons (4) has been noted to be superior to 131I in the resolution attainable with such preparations (5, 6). We have also observed its appreciably greater efficiency in blackening of both Kodak Ltd. AR 10 stripping film and Kodak Radiatized dental film. The former has a 5-micron thick emulsion and the latter a 9.5 micron thick emulsion on both sides of a 165-micron thick plastic base. The energy transfer of the 22.5 and 31 keV extranuclear electrons of 125I is greater in these thin films than that of the β particles of 131I with maximum energy of 608 keV and an average energy of 188 keV. The 125I electrons do not traverse the base of the dental film, but the 131I β particles do so and appreciably blacken the second emulsion. The electromagnetic radiation of 125I also passes through and affects this emulsion. Attempts to quantitate the increased efficiency of 125I using fixed, undeveloped photographic film as a carrier for both isotopes were satisfactory for the dental film but not for the stripping film.

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The experiment was carried out in the following manner. Undeveloped, fixed dental film was cemented to a glass slide and then dipped in a solution of the desired isotope. After drying, it was pressed firmly against the dental film, or against stripping film which had been wetted, expanded, and dried emulsion side up on a glass slide. The dental film preparations reflected the degree of increased efficiency of $^{125}$I that had been noted in routine studies; the stripping film preparations did not. To explain this discrepancy, serial 7-micron-thick thyroid sections containing $^{125}$I were used to expose stripping film in two different ways. The first was the routine procedure in which the wet, expanded film was floated emulsion side down onto the tissue section and dried. The second was that described above in which the tissue section was pressed against the dried emulsion on another slide. The blackening of the emulsion by the first procedure was more than twice that by the second. The 22.5 keV Auger electrons and the 31 keV conversion electrons of $^{125}$I have a range in water of 8 and 15 microns respectively. Therefore, with 7-micron thick tissue sections and a 5-micron thick emulsion, the spatial tolerances are small. The close apposition of the tissue and emulsion in the routine procedure was not duplicated by the experimental routine.

Failing in the search for a sliceable dispersion medium for the isotopes, sections of normal extranodular thyroid tissue were used for comparison of the film blackening efficiency of the two isotopes. The radioactivity in microcuries retained in a gram of wet tissue was first determined. Then visually equal film densities were evaluated on the basis of microcuries decayed per gram of tissue. An example of such comparisons is presented in Figure 1. In several experiments $^{125}$I was approximately three to four times as efficient as $^{131}$I in the blackening of dental film and at least ten times as efficient with AR 10 stripping film.

The sevenfold difference in the half lives of these two isotopes makes it possible to process autoradiographs after a short early exposure interval or after a long late exposure interval, with partial but distinct separation of the areas of blackening from each radioisotopic. Thus, an autoradiograph resulting from exposure during the first 72 hours would have four times the blackening from the

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Fig. 1. Autoradiographs of normal extranodular tissue. A. 0.18 $\mu$C decay of $^{125}$I per gram of wet tissue. (Dental film). B. 0.36 $\mu$C decay of $^{131}$I per gram of wet tissue. (Dental film)
Fig. 1c. 0.49 μC decay of 125I per gram of wet tissue. (Stripping film)

Fig. 1d. 4.3 μC decay of 131I per gram of wet tissue. (Stripping film)

131I as would one exposed for 21 days beginning 32 days from time 0 (*vide infra*). The blackening from 125I, on the other hand, would be four times as great in the second autoradiograph as in the first. A successful preparation depends on proper selection of initial tissue concentration of both isotopes. Table I is a simple expression of the foregoing. In this example the concentration of 131I in microcuries is four times that of iodine-125. The amount of blackening from each isotope at each time interval is expressed numerically as related to 100, an arbitrary figure for the blackening produced by 131I in three days from time zero using dental film.

**MATERIALS AND METHODS**

From the experience gained in the autoradiographic study of over 100 thyroids using either 125I, 131I, or both, the following protocol was evolved for
The combined use of the two isotopes. The normal, suppression, and TSH uptakes and scintigrams were usually obtained prior to administration of the doses of the isotopes for autoradiography. An oral dose of $^{125}$I was given first, either after seven days of 75 mcg of liothyronine, or 24 hours after ten units of TSH. Iodine-131 was given 24 hours before surgery. If TSH was given first, suppression was started 24 hours after the administration of the $^{125}$I and continued for seven days. If suppression was the initial procedure, after administration of $^{125}$I at least 48 hours elapsed before TSH was given. As the rapid decay of the $^{131}$I was compensated partially by its relatively poor film blackening efficiency, a dose of $^{131}$I, that would deliver to the tissue three to four times the number of microcuries anticipated for $^{125}$I, was selected. The actual numerical value depended on the uptake and anticipated volume of distribution of each isotope. In a thyroid gland of normal size with a 25% 24-hour $^{131}$I uptake it was found that 200 µC of $^{125}$I gave satisfactory autoradiographs on either film, when exposure was started 32 days after administration and continued 14-21 days. All dosage calculations were made from this baseline. The autoradiograph dose was calculated as of the time of initial film exposure, i.e. one or two days following surgery. This has been and will be subsequently referred to as time zero. No exposure was continued for over 30 days because of the latent image fading of the stripping film.

**Table 1**

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Day 0 to 3</th>
<th>Day 32 to 53</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>14</td>
<td>62</td>
</tr>
<tr>
<td>Stripping Film</td>
<td>$^{125}$I</td>
<td>20</td>
</tr>
<tr>
<td></td>
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<td>14</td>
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**Results**

The application of the above procedure to an individual case is best illustrated by one in which a functioning area, independent of TSH, is clearly separated from areas of tissue in which function is suppressed. The following case of a 50-year-old woman is in this category. A diffuse multinodular goiter was present that was estimated to weigh 80 grams. The liothyronine suppression and the TSH stimulation scans comprise Figure 2. The 24-hour radiiodine uptake after suppression was 25%, and after stimulation it was 80 per cent. The volume of distribution of the $^{125}$I in the tissue stimulated by TSH was estimated to be six times that of the $^{131}$I in the tissue not suppressed after liothyronine administration. Twenty-four hours after ten units of TSH were given intramuscularly, 500 µC of $^{125}$I were administered orally. One day later 100 mg of liothyronine were given orally and continued as a daily dose for seven days. One millicurie of $^{131}$I was then administered orally 24 hours prior to surgery. Seventy-five grams of tissue comprising 90% of the thyroid were removed and 11 areas were sampled and sectioned in
Fig. 2 A. Scintigram after 100 μg of liothyronine daily for 7 days. Uptake of $^{131}$I at 24 hours was 25%. Neither the scintigram pattern nor the uptake were appreciably different from those done before suppression.

Fig. 2 B. Scintigram after 10 units of TSH. Uptake of $^{131}$I at 24 hours was 80%.
the usual manner. A series of autoradiographs was prepared using both dental and stripping film. The autoradiographs made with dental film were used as a guide for the stripping-film exposures, and they will be discussed here because of the simplicity of illustration.

In Figure 3A and B, the autoradiographs of the nodule and the extra-nodular tissue are compared at 46 hours from time zero. This represents 15% of the decay of the $^{131}$I and two per cent of the $^{125}$I present. In Figure 3 C and D, a 72-hour exposure of the same tissue was begun 31 days later. This resulted in about the same decay of $^{125}$I but only one per cent of the original iodine-131. From these the relative proportion of the effect of the two isotopes in the original autoradiographs can be estimated.

The amount of $^{125}$I present in this tissue was sufficient to allow a longer time interval between preparations and a second autoradiograph almost devoid of $^{131}$I effect could be made. Figures 4A and B are photographs of dental-film autoradiographs made from the same tissue sections as in Figure 3. Exposure was for four days beginning 31 days from time zero. The nodule is slightly more active than the extranodular tissue. When exposure was started 86 days from time zero and continued 12 days, the relative activity is reversed as seen in Figures 4C and D. In these two autoradiographs $^{131}$I has produced less than one per cent of the blackening that it caused in Figure 3A and B, while the blackening from $^{125}$I is 250 per cent.

Figures 3 and 4 present double-isotope autoradiographs that can be interpreted. The nodule is the major area of uptake when TSH is suppressed, but visual subtraction of 3D from 3B indicates some degree of autonomus function in the extra-nodular tissue as well. The total uptake of the section of nodule in the

Fig. 3 A. Hot nodule. Exposed 46 hours from time zero. B. Extranodular tissue. Same exposure as “A”. C. Hot nodule. Exposed 72 hours beginning 31 days from time zero. D. Extranodular tissue. Same exposure as “C”.

Fig. 4 A. Hot nodule. Exposed 86 days from time zero. B. Extranodular tissue. Same exposure as “A”. C. Hot nodule. Exposed 98 days beginning 31 days from time zero. D. Extranodular tissue. Same exposure as “C”.
presence of exogenous TSH is appreciably less than that of the extra-nodular tissue Fig. 4C, D. The above information can be correctly inferred from the scintigrams because the different types of pathology are gross and obvious. It is necessary to prove the experimental method using this type of case, however, before applying it to microscopic lesions. Even in this case, study of stripping-film autoradiographs of the extra-nodular tissue gave interesting information on the character of this tissue, functionally dependent on TSH, but anatomically independent of it. (The large mass of tissue had remained in spite of prolonged thyroxine administration.) This is to be reported.

The greater sensitivity of the stripping film to $^{125}$I enhances its value for demonstration of pure $^{125}$I distribution in autoradiographs exposed beginning three to five weeks from time zero. Figure 5 pictures autoradiographs from one of 12 blocks of a thyroid gland from a patient with toxic autonomous goiter. The dental film in Figure 5A was exposed from time zero to demonstrate the uptake of $^{131}$I by the autonomous functioning tissue. Figure 5B was exposed beginning 22 days from time zero to demonstrate relative activity of the follicles to exogenous TSH, identified by the distribution of iodine-125. Attention is called to the 2 mm hot spot which is relatively and actually darker on Figure 5A. In Figure 5C and D, the same area is compared on stripping film. The contrast between the hot area and the adjacent tissue in Figure 5C is similar to Figure 5A. In Figure 5D the contrast is considerably less than on the dental film in Figure 5B. The $^{131}$I effect, therefore, was still too great at 22 days in Figure 5B for good identification of the $^{125}$I distribution on the dental film. In Figure 5D the greater sensitivity of this film to $^{125}$I minimizes the persistent $^{131}$I effect and made this an acceptable preparation when Figure 5B was not.

Fig. 4 A. Hot nodule. Exposed 4 days beginning 31 days from time O. B. Extranodular tissue. Same exposure as “A”. C. Hot nodule. Exposed 12 days beginning 86 days from time O. D. Extranodular tissue. Same exposure as “C”.

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Fig. 5 A. Dental film exposed 4 days from time O. B. Dental film exposed 10 days beginning 22 days from time O.

Fig. 5 C. Stripping film exposed 14 days from time O. (x 55)

Fig. 5 D. Stripping film exposed 30 days beginning 22 days from time O.
The use of two films with different sensitivities to the two isotopes allows compensation for error in estimating the dose or the exposure intervals.

In the study of the autonomous thyroid, with proper experimental design, autoradiographs made with both $^{125}\text{I}$ and $^{131}\text{I}$ present initially, can provide the following information with only one operative procedure: 1) the identity of that tissue which is not functional with or without circulating TSH. 2) the identity of tissue normally responsive to TSH and suppressed in its absence. 3) the identity of autonomously functioning tissue and its comparative response to TSH. If one isotope is administered before any medication, and the second after TSH, then the relative function of two or more types of thyroid tissue in response to two different degrees of TSH stimulation can be demonstrated, i.e. normal vs exogenous excess TSH.

SUMMARY

The sevenfold difference in the half lives of $^{125}\text{I}$ and $^{131}\text{I}$ permits the interpretation of autoradiographs prepared from tissue in which both are present. With proper selection of dosage, autoradiographs exposed from time zero can be made to reflect predominantly the distribution of $^{131}\text{I}$; those from time 32 days or longer, mainly the distribution of iodine-125. This technique has proved practical for the study of nodular goiters containing autonomous functioning tissue when one isotope is administered during suppression by endogenous or exogenous thyroxine, and the other during stimulation by exogenous TSH.

The decay of one microcurie of $^{125}\text{I}$ is equivalent to 3-4 $\mu$C of $^{131}\text{I}$ in blackening dental film, and is equivalent to at least ten $\mu$C in blackening AR 10 stripping film. With a given tissue mixture of these isotopes, the relative effect of $^{131}\text{I}$ will be greater when using dental film, and that of $^{125}\text{I}$ greater when using stripping film.

REFERENCES