ESTIMATING THYROID IODINE BY ACTIVATION ANALYSIS IN VIVO

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The biological applications of activation analysis have been based almost entirely on the estimation of trace elements in tissue and other materials where the excellent sensitivity of the method can be fully exploited. A typical sample of biological material contains several elements which can be made radioactive by bombardment with neutrons or other particles. When the separate contributions to the induced radioactivity are of the same order of magnitude, gamma-ray spectrometry allows the determination of individual constituents quantitatively in a quick, simple and nondestructive way. More often, however, the induced radioactivity contributed by major elements such as sodium and chlorine is many times greater than that of the trace elements under investigation. In these cases chemical separation is necessary.

Activation analysis *in vivo* has intriguing possibilities. If the radiation dose delivered to the subject can be kept within satisfactory limits, the technique will be particularly valuable in offering a direct method of measuring the total quantity of an element in the body or in a particular organ.

Anderson *et al* (*1*) used activation analysis for the simultaneous estimation of chlorine, sodium and calcium *in vivo* in man after whole-body irradiation by 14-Mev neutrons which were partly moderated by being passed through 3 cm of polyethylene. The object of our experiment was to estimate the total quantity of iodine in the thyroid gland *in vivo* using thermal-neutron irradiation of the neck followed by gamma-ray spectrometry. The reaction used is ^{127}I $(n, \gamma)^{128}I(T_{1/2} = 25$ min). In principle, the quantitative determination is made by comparing the induced activities from ^{128}I in the thyroid with that of a standard irradiated under identical conditions. In practice, however, it is not possible to obtain this identity because of the heavy absorption of neutrons in the tissues.

Fairchild (*2*) found that a flux of thermal neutrons was reduced by a factor of 2.15 when it passed through 2 cm of tissue. In a comparable experiment, we used a beam of thermal neutrons 5 cm in diameter to irradiate a phantom similar in dimensions and elemental composition to a human neck. The beam was attenuated by a factor of 3.2 as it passed through 2 cm of tissue. It is clear that the flux of neutrons reaching the thyroid gland in different subjects (some normal and some suffering from thyroid disease) exposed to the same beam of thermal neutrons will vary considerably.

To avoid this difficulty we used $^{129}I(T_{1/2} = 1.6 \times 10^7$ yr) as an internal standard. After it is ingested, this isotope can be detected easily in the thyroid gland by its 28-kev x-ray emission. The actual amount of the isotope concentrated in the thyroid can be estimated indirectly by measuring urinary excretion after administration of a known dose. When it is exposed to a flux of thermal neutrons, ^{129}I is partly converted to $^{130}I(T_{1/2} = 12.5$ hr) which emits gamma rays with energies between 0.41 and 1.15 Mev. During *in vivo* activation analysis of the thyroid, the ^{129}I acts as a flux monitor, allowing an accurate determination of the ^{127}I content of the gland even though the neutron flux at each point in the thyroid is not accurately known.

Before using this method in humans, it is necessary to assess the validity of these observations by making investigations with animals. For this purpose we have used the sheep, an animal which is relatively easy to handle and which has a thyroid iodine content not much smaller than that found in man. In a
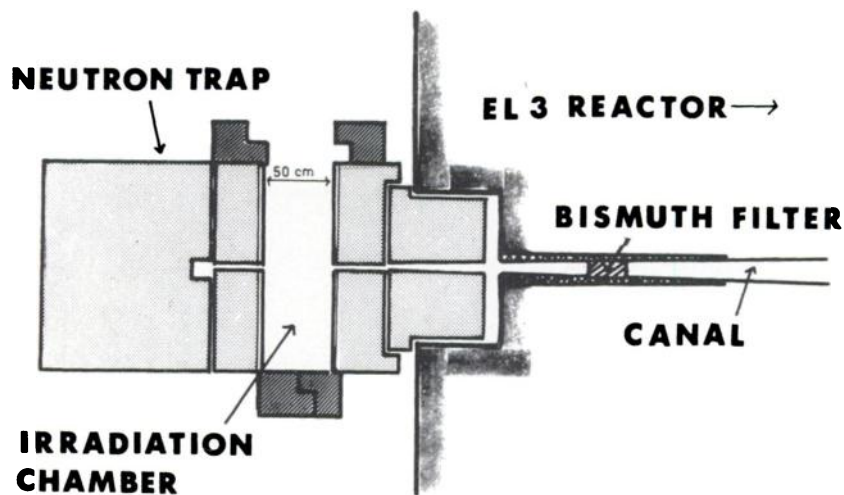
FIG. 1. Experimental arrangement used for irradiating sheep thyroid in vivo.

preliminary experiment we obtained from a slaughter-house the thyroid glands of ten male and female sheep 12 to 24 months old and measured the iodine content of each by *in vitro* activation analysis and gamma-ray spectrometry. The iodine contents ranged between 1.0 and 3.8 mg with a mean value of 2.5 mg. The iodine content of a human thyroid, according to Heedman and Jacobson (*3*), is between 4.5 and 31.5 mg with a mean value of 12.9 mg.

MATERIALS AND METHODS

Preparing the animals. Two rams, approximately 1 year old and 50 kg in mass, each received an intramuscular injection of 2 mg of ^{129}I in the form of sodium iodide 1 week before irradiation. About 30 min before irradiation, each animal was given a tranquillizer (Droperidol, 25 mg intravenously and 25 mg intramuscularly). Each animal was placed in a prone position and tied to a stretcher with its head extended and fixed in a position which allowed only the region of the neck around the thyroid gland to be irradiated. The position of the gland was determined previously by externally counting the ^{129}I with a sodium iodide crystal 30 mm in diameter and 5 mm thick. A piece of gold foil (100 mg/cm^2) was placed on the skin over the thyroid to act as a flux monitor to verify the immobility of the animal during irradiation.

TABLE 1. GAMMA DOSE RATES IN SHEEP DURING THERMAL-NEUTRON IRRADIATION

Energy (kev)	Exposure dose rate (R/min)
30 to 400	2.6
400 to 1,300	6.2
Skin-equivalent chambers	2

Irradiation. The irradiation was made in one of the horizontal beam ports of the EL3 reactor at Saclay. This port goes through the biological shield and the graphite reflector, terminating at the heavy-water tank. The intense gamma radiation from the core of the reactor was attenuated considerably by placing a bismuth block 40 cm thick in the canal at the level of the concrete shield. A rotating collimator made of concrete 1 meter thick allowed the port to be opened or closed. The neutron beam emerged into a shielded enclosure in which the animals were placed for irradiation (Fig. 1). The diameter of the neutron beam was 3 cm at the collimator and 5 cm at the neck of the sheep about 20 cm beyond the collimator. Measurements of the activities induced in plain gold foils and in gold foils wrapped in cadmium indicated a thermal neutron flux of 3×10^8 n/cm^2/sec and a flux of 3×10^5 n/cm^2/sec at an energy above 0.5 ev.

Gamma-ray ionization chambers were placed at several points within the enclosure. Chambers outside the neutron beam recorded no measurable amount of gamma radiation; the gamma-ray dose rates within the neutron beam are shown in Table 1.

The stretcher carrying the sheep was placed so that the axis of the collimator was perpendicular to the neck of the animal. The neutron beam fell first on the left lobe of the thyroid; the right lobe, on the other side of the trachea, received a much smaller dose of neutrons. The irradiation time was 10 min; each animal was irradiated on two occasions, separated by a 24-hr interval.

Measurement of induced radioactivity. After irradiation, the animals were taken to a counting room. The detecting system included a sodium iodide crystal 20 cm in diameter and 10 cm thick with a collimator made of lead, 4 cm thick, in the shape of a cone tapering to an aperture of 6 cm in diam-
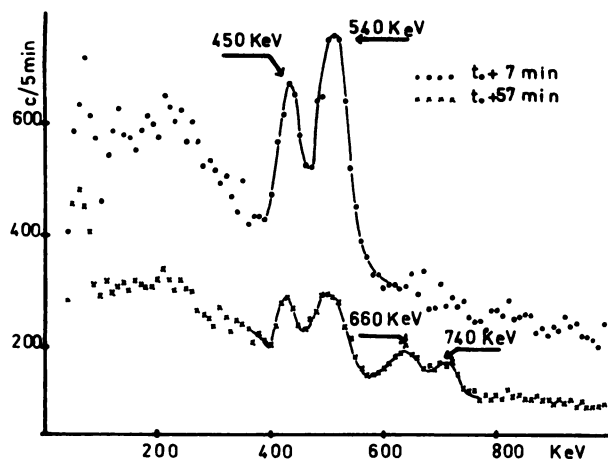
FIG. 2. Gamma-ray spectra obtained from 20 × 10-cm collimated crystal after neutron irradiation in vivo of sheep thyroid gland, 7 and 57 min after end of irradiation.
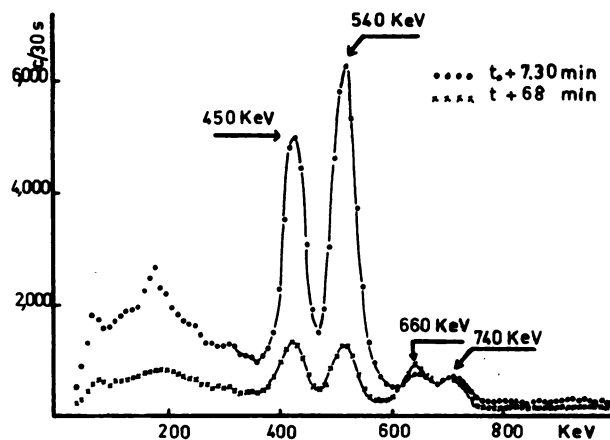
FIG. 3. Gamma-ray spectra obtained after neutron irradiation in vitro of thyroid gland in Fig. 2. Two spectra were recorded 7.5 and 68 min after end of irradiation.

eter. A 400-channel pulse-height analyzer was used for gamma-ray spectrometry. When the animal was in position for radioactive assay, the thyroid gland was about 10 cm from the sodium iodide crystal. The first measurements were made approximately 5 min after irradiation; further counts for 2–5 min were made during the succeeding hour to follow the decay of the various photoelectric peaks identified in the gamma-ray spectra in the 0–1-Mev energy range.

Finally, the animals were sacrificed and the thyroids removed. The amounts of [127]I and [129]I were measured by activation analysis using a 10-sec irradiation in a thermal neutron flux of 4×10^{12} n/cm²/ sec.

Interpretation of gamma-ray spectra. Figure 2 shows gamma-ray spectra obtained by *in vivo* meas-

FIG. 4. Decay curves of 540-kev photopeak in Fig. 2. Curve I: after subtraction of Compton background; Curve II: after subtraction of activity attributable to [130]I; Curve III: after correction for annihilation radiation associated with gamma emitters of energy above 1 Mev; Curve IV: growth of activity of [130]I under peaks at 660 and 740 kev.

urements 7 and 57 min after the end of the irradiation of one of the sheep. The upper curve shows the peak at 450 kev corresponding to [128]I as well as a peak at 540 kev. The lower curve shows additional peaks at 660 and 740 kev corresponding to [130]I.

Figure 3 shows the gamma-ray spectra obtained from the thyroid gland of the same animal irradiated *in vitro*. The photopeak at 540 kev, decaying with a half-life of about 10 min, is attributed to an isomeric state of [130]I (*4,5*) which decays to [130]I. It is therefore possible to estimate the amount of [129]I in the thyroid gland by measuring the induced activity from [130]I or from [130m]I. In principle, the assay of [130m]I will be more sensitive because this isotope will be formed in greater quantity than [130]I after an irradiation of 10 min. In practice, however, the resolution of the detecting system is not sufficient to distinguish between the 540-kev peak of [130m]I and the 510-kev peaks associated with annihilation radiation from [24]Na, [38]Cl, [42]K and [49]Ca. These four activities are induced not only in the thyroid itself but also in the extracellular fluids in the neighboring muscle and in the thyroid cartilage.

Estimates of the induced radioactivity corresponding to the three isotopes [130]I, [130m]I and [128]I were made in the following way. The areas under the photopeaks which are characteristic of the three isotopes of iodine were measured after background subtraction, calculated by extrapolating the Compton spectrum between 360 and 800 kev. Area measurements on the peaks at 450 kev and 540 kev were corrected for the contributions from [130]I and from annihilation radiation at 510 kev. The contribution of [130]I to these two peaks was estimated from a study of the spectra recorded approximately 1 hr after thyroid irradiation; these spectra had conspicuous peaks
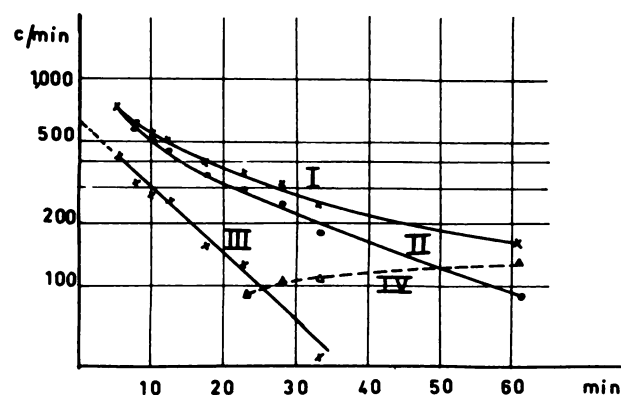
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at 660 kev and 740 kev. Knowing the gamma-ray spectrum given by an isolated sample of [130]I and the rate of formation of this isotope by [130m]I decay, it was possible to calculate for each spectrum measured *in vivo* the correction to be applied to the peaks at 450 kev and 540 kev for activity contributed by [130]I. This correction proved to be negligible for the 450-kev peak but was significant for the 540-kev peak.

In correcting for annihilation radiation, we assumed that the 540-kev peak shown on the spectra recorded 1 hr after the end of the irradiation is attributed to [130]I and annihilation radiation exclusively; the activity due to [130m]I with its 9.2-min half-life has almost entirely disappeared by this time. The area under the 540-kev peak, reduced by an amount corresponding to the activity of [130]I (estimated and explained in the previous paragraph), represents the contribution from annihilation radiation. We have assumed that the decay of this annihilation radiation peak is matched by the decay of the Compton continuum radiation in the 800–900-kev range; this radiation comes almost entirely from gamma emitters with energies above 1 Mev.

Figure 4 illustrates the corrections made to the 540-kev peak. Curve I shows the decay of this peak after the Compton background is subtracted. Curve II was obtained by subtracting the contribution from [130]I, and Curve III was made by eliminating the contribution from annihilation radiation. The decay of this final peak has a half-life of 9 min, corresponding well to that of [130m]I. Curve IV shows the growth of the peaks at 660 kev and 740 kev corresponding to [130]I.

Calculation of results. A sample containing 2 mg of [127]I and 500 μg of [129]I in a 3-ml volume was irradiated for the same time as the animals were and assayed with the same detecting equipment. Measurement of the activity contributed by the different isotopes of iodine in the sample and in the experimental animals provides the information required to estimate the amount of iodine in the thyroids of the living sheep.

Let a_s and a_t be the specific activities of [129]I in the standard and the thyroid *in vivo*, expressed as counts per minute of [130]I or [130m]I per microgram of [129]I. Let b_s be the specific activity induced in the standard, expressed as counts per minute of [128]I per microgram of [127]I. Let C be the counting rate due to [128]I in the thyroid of the animal irradiated *in vivo*. The quantity of [127]I in the thyroid, measured in micrograms, is equal to $C(a_s/b_s a_t)$.

Table 2 shows the results obtained by calculating the iodine content of the thyroids of the two sheep. In each case we have calculated the [127]I content of the left lobe of the thyroid since this part of the

TABLE 2. ESTIMATES OF THYROID IODINE CONTENT OF TWO SHEEP MADE *IN VIVO* AND *IN VITRO*[*]			
	[127]I (μg) in left lobe		
Internal Standard	In vivo		In vitro
	[130]I	[130m]I	
Baptiste (1)	1,300	1,560	1,520
(2)	1,380	1,580	
Auguste	500	470	520

[*] For further explanations, refer to text.

organ was irradiated preferentially. During subsequent *in vitro* estimations on the same thyroids, we were able to verify that the specific activity of iodine ([129]I/[127]I) was the same in both lobes of the gland. The results obtained for the left lobe can therefore be extrapolated to give the total iodine content of the gland.

The reproducibility of the method can be inferred from results obtained with the sheep, Baptiste, which was irradiated on two occasions separated by 24 hr. The sheep, Auguste, was also irradiated twice, but only one estimation of thyroid iodine content was possible. During one of the irradiations the animal moved, and the thyroid gland received only a small dose of neutrons. The three columns of Table 2 indicate the values found *in vivo* using [130]I and [130m]I as internal standards as well as the estimates made by subsequent activation analysis on the excised thyroids.

DISCUSSION

These results indicate that:

1. The method for estimating thyroid [127]I requires a knowledge of the amount of [129]I in the gland. For convenience in handling the animals this quantity was determined in thyroid glands removed after sacrificing the sheep. In any future application of the technique to humans, the amount of [129]I held in the thyroid will be estimated as the difference between the quantity of [129]I ingested and the quantity eliminated in the urine during the first three days after ingestion.

2. As we learned from tests with phantom necks, an internal standard is essential to obtain an accurate estimate of the [127]I content of the thyroid *in vivo* after activation by thermal neutrons. In the phantom tests, the volume of the phantom and the geometrical relationship of the simulated thyroid gland to the incident neutron beam varied within the limits to be expected for a typical group of normal and abnormal human subjects. Measurement of
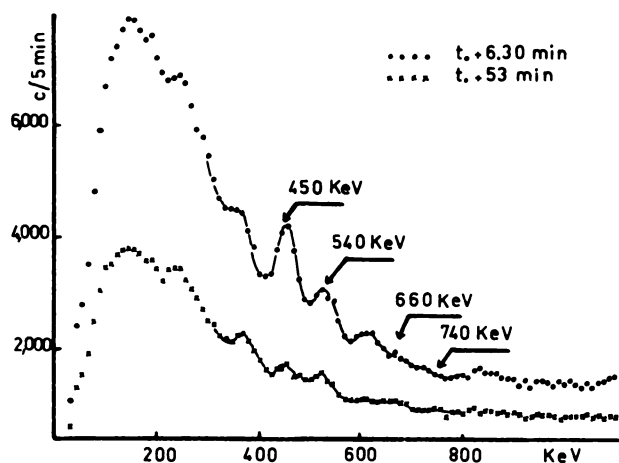
FIG. 5. Gamma-ray spectra obtained from 20 X 10-cm uncollimated crystal after neutron irradiation *in vivo* of sheep thyroid gland.

the induced radioactivity in [128]I did not give accurate estimates of the amount of iodine irradiated.

The use of [129]I as an internal standard is valid only if the amount of this isotope held in the thyroid does not appreciably alter the iodine content of the gland. The amount of [129]I retained in each lobe of the thyroid in the two sheep used in this experiment was 265 μg (Baptiste) and 117 μg (Auguste). These quantities were detected easily by activation analysis *in vivo*. In a normal human thyroid, increase of the iodine content by an additional mass of about 200 μg would have a negligible effect. The distribution of [129]I in the gland must also be identical to that of [127]I. This condition was satisfied, at least on a macroscopic scale, in the animals used in our experiment.

It is generally accepted that the human thyroid is not a homogeneous tissue. Structural heterogeneity on a microscopic scale does not invalidate the technique of the internal standard. However, the method can be subject to error when it is applied to large thyroid glands where well-defined regions which differ in iodine uptake are revealed by scintillation scanning. In such cases it cannot be presumed that the concentration ratio [129]I/[127]I is the same throughout the thyroid gland.

3. The specification of equipment for radioactive assay and in particular the choice of a large sodium iodide crystal is influenced by a knowledge of the radioactive elements formed by neutron activation of the neck *in vivo*. [24]Na, [38]Cl, [42]K and [49]Ca, which are abundant in the tissue after irradiation, emit high-energy gamma rays which contribute to the production of a substantial Compton background in the energy range that includes the photoelectric peaks of [128]I, [130m]I and [130]I. This effect is diminished by the use of a large crystal. In some preliminary ex-

periments we used a detector fitted with a 5 X 4-cm sodium iodide crystal and a cylindrical collimator with a 3-cm wall thickness. The Compton background between 400 kev and 800 kev was so great that the iodine peaks could not be detected.

The radioactive nuclides formed during activation do not all remain at the place of irradiation. Extracellular elements such as sodium and chlorine are taken up in the circulating blood and removed from the range of detection of an adequately collimated counter. This desirable removal of interfering activity is shown by the spectra in Fig. 5, obtained after activation *in vivo* of a sheep thyroid and radioactive assay using a large uncollimated crystal. These spectra should be compared with those in Fig. 2 which were obtained under the same conditions but with a collimated crystal. One can see that the Compton background is significantly reduced by shielding the crystal against radiation emitted from the rest of the body in which a significant proportion of the induced radioactivity is circulating. On the other hand, the organic iodine in the thyroid is not subject to removal in this way, despite the possibility of detachment in the Szilard-Chalmers process.

4. Analysis of gamma-ray spectra to estimate the activity corresponding to the different isotopes of iodine is complicated by the variety of activities formed during irradiation. In particular the estimation of induced activity corresponding to [130m]I under the 540-kev photoelectric peak involves several successive corrections. As already indicated, one of these involves subtracting the radioactivity corresponding to 12.5-hr [130]I. This correction can only be made when the [130]I peaks at 660 kev and 740 kev are clearly visible on the final spectra. If the amount of [129]I retained in the thyroid is too small to produce accurately measurable peaks at 660 kev and 740 kev, it is not possible to calculate directly the contribution of [130]I activity to the 540-kev peak. In these circumstances reference should be made to the gamma-ray spectra in the region above 1 Mev; by studying the photoelectric peaks associated with [24]Na, [38]Cl, [42]K and [49]Ca, it is possible to estimate the contribution to the 540-kev peak of [130m]I made by annihilation radiation attributable to these elements. In any substantial program involving the method we described, the use of computers for resolving gamma-ray spectra would clearly be attractive.

5. The use of these techniques in humans will be reasonable only if the radiation dose both to the thyroid and to the neighboring tissues is of the same order of magnitude as that incurred during a tracer test of thyroid function with [131]I.

Because our main concern in this investigation was to establish the validity of the technique in general

terms, the radiation doses given to the sheep were not measured very precisely. More detailed investigations on this point will be necessary before the method is applied to humans. However, it is possible to give an approximate indication of the dose received by the animals, based on measurements of the fast-neutron, thermal-neutron and gamma-ray fluxes. For an irradiation of 10 min, the mean dose to the thyroid from fast and thermal neutrons was approximately 45 rads. In this calculation we assumed that the thyroid gland lies at a depth of 1 cm below the skin surface and is 1 cm thick. For thermal neutrons, unit flux corresponds to an absorbed dose of 6×10^{-10} rad (6).

On the skin surface the absorbed dose was 110 rads, and a dose of the same order was delivered at the level of the thyroid cartilage; with a well defined radiation field, the area of thyroid cartilage irradiated could be significantly reduced.

In the estimates of gamma-ray dose given in Table 1 no account has been taken of radiation doses attributable to the decay of the radioactive elements formed during the neutron irradiation; the dose contributed by [129]I in the thyroid gland is also negligible in relation to that associated with the irradiation flux.

The radiation dose delivered to a human could be reduced by using a shorter irradiation time and by placing additional bismuth shielding in the beam. These measures would result in appreciable reduction in the sensitivity of detection of iodine, but the technique would still be effective in a normal human subject with a thyroid iodine content of the order of 10 mg.

CONCLUSION

The technique which has been described gives satisfactory results when used for estimating the thyroid iodine content of the animals *in vivo*, and it is reasonable to envisage its extension to human subjects. By shortening the irradiation time and by adding additional shielding to reduce the gamma radiation from the reactor core which contaminates the neutron beam, the irradiation dose can be reduced to a level comparable with that accepted in a routine tracer test with [131]I. In the arrangement which we have described, the flux of epithermal and fast neutrons accompanying the thermal-neutron beam gives a negligible radiation dose.

Methods used previously for estimating the thyroid iodine content have depended on isotopic dilution during kinetic studies with the help of radioactive iodine (7). These techniques take into account only exchangeable iodine and do not always give accurate results, particularly in subjects suffering from disorders of thyroid function. Except for a method based on detecting the characteristic radiation emitted by iodine under x-ray bombardment (3), there has until now been no method for directly estimating thyroid iodine *in vivo*. On the basis of the experiments described here, we believe that the neutrons produced by the EL3 reactor at Saclay will allow an accurate estimate of thyroid iodine in human subjects using activation analysis *in vivo*.

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