

Iodine Analysis of Biological Samples by Neutron Activation of ^{127}I , with Scintillation Counting of Cerenkov Radiation¹

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To measure the small amounts of iodine in tissues, organelles, and subfractions it is necessary to use very sensitive and specific analytical methods. Neutron activation analysis meets these criteria; the measurement of ^{129}I after irradiation of the naturally occurring isotope, ^{127}I , has a sensitivity of about 0.08 nanogram (1,2). However, neutron activation analysis has the disadvantage of requiring instrumentation not generally available in biological laboratories; given the availability of a nuclear reactor, β -counters or γ -spectrometers have generally been necessary to measure radioactivity and ensure specificity.

We have measured nanogram quantities of iodine in small biological samples using a combination of postirradiation extractions to separate Cl and Br (1,2) with timed counting of the resultant ^{129}I in aqueous solution, utilizing Cerenkov radiation.

MATERIALS AND METHODS

Standards

Standards for iodine analysis were prepared in double-distilled water from analytical-grade dry ammonium iodide (Matheson, Coleman & Bell) or potassium iodide (G. T. Baker Chemical Co.). Although NH_4I is relatively unstable, similar results were obtained from both standards. All reagents used in preparing mitochondria and in the postirradiation treatment of samples were of analytical or reagent grade.

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Sample Preparation

Male rats weighing 150–200 gm (Charles River Breeding Laboratories, Boston, Mass.) were fasted overnight before use. Mitochondria were prepared from their livers in 0.25 *M* sucrose. The final suspension was made in sucrose, to give a final concentration of 15 mg protein/ml. Protein was determined by a biuret method (3). An aliquot of 0.7 ml mitochondrial suspension was heat-sealed in a 2/5 dram polyethylene flip-top vial. Two such sample vials were placed in a 2 dram polyethylene vial, which was in turn heat-sealed, giving a leakproof container for irradiation.

Irradiation

Two samples were irradiated for 15 min in pneumatic tube PL3 of the Phoenix Memorial Laboratory nuclear reactor; the thermal neutron flux was $\sim 2 \times 10^{12}$ *n*/cm²/sec. The beginning of irradiation was timed, and correlated with subsequent radio-assay data.

Postirradiation Chemistry

The procedure of Duce and Winchester (2) was used to separate I from Cl and Br and, for convenience, is given in detail here: Immediately after irradiation, 0.5 ml of each mitochondrial suspension was removed from the sealed vial with a 1 ml syringe and hypodermic needle, and added to a 125 ml Erlenmeyer flask containing 25 mg KI in 10 ml water. To promote radiochemical exchange, 1 drop phenolphthalein, 8 drops 12 *M* NaOH, and 10 drops 5% NaClO (laundry bleach) were added, and swirled 60 sec to ensure complete oxidation of iodine. Then 8 drops 16 *M* HNO₃ were added to acidify, and 10–15 drops 0.5 *M* Na₂S₂O₅ (until the solution changed to a pale yellow); the solution was swirled 30 sec to ensure reduction to I⁻. NaOH was added dropwise until the solution became basic to phenolphthalein; then 2.5 ml glacial acetic acid (to prevent possible oxidation of Br⁻ by HNO₂) and the contents of the flask were poured into the first of a series of 125 ml separatory funnels (A) set up in advance, as follows:

- (A) 20 ml CCl₄ + 5 drops 1 *M* NaNO₂
- (B) 50 ml H₂O + 2 ml 1% (NH₃OH)₂SO₄
- (C) 20 ml CCl₄
- (D) 50 ml H₂O + 2 ml 1% (NH₃OH)₂SO₄
- (E) 10 ml H₂O

Funnel (A) was shaken for 60 sec (I⁻ is oxidized to I₂ and extracted into the CCl₄ layer) and the CCl₄ phase was added to funnel (B) and the aqueous layer to funnel (C). Funnel (C) was shaken for 30 sec to give the aqueous layer a second CCl₄ wash and remove any remaining

I_2 , and the CCl_4 layer was returned to funnel (B). Funnel (B) was shaken for 10 sec (to remove any Br in CCl_4) and the CCl_4 layer run into funnel (D); funnel (D) was shaken for 10 sec and CCl_4 was run into funnel (E). It was important not to shake (B) or (D) too long because hydroxylamine sulfate reduces I_2 slowly. Then 10 to 15 drops 0.5 M $Na_2S_2O_5$ was added to (E) until the CCl_4 was colorless (indicating all the I_2 was reduced). The CCl_4 layer was discarded and the aqueous layer was put into a glass counting vial for Cerenkov counting.

Scintillation Counting

The aqueous solution containing the $^{128}I^-$ and the carrier $^{127}I^-$ was put in a glass counting vial and the Cerenkov radiation was measured using a liquid scintillation counter (Beckman Instrument Co., type CPM 100). About 20 sequential 1 min counts were measured alternately on two irradiated samples and the time for each was noted. The total

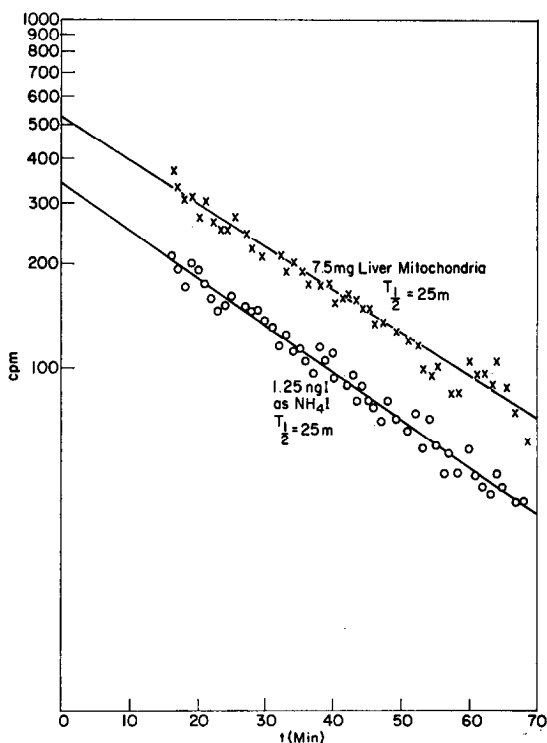


Fig. 1. Liquid scintillation counting (Cerenkov radiation) of neutron activated samples of rat liver mitochondrial iodine and NH_4I , as function of time after irradiation.

counting time was 30–45 min. Background counts (about 30 cpm) were subtracted. The specific radioactivity at $t = 0$ (end of irradiation) was calculated by plotting the decay curve (\log [radioactivity] vs. time) and extrapolating back to zero. Figure 1 shows decay curves for an NH_4I sample and a mitochondrial sample. Linearity was closely approximated, and the apparent half-life for each sample was close to the 25 min for ^{128}I .

Correction for Recovery

After radioassay, the iodine in each sample was precipitated and weighed, after (2). To the sample was added, in sequence, 1 drop 6 N HNO_3 , 5 ml 0.1 M AgNO_3 , and 5 ml 16 M HNO_3 (to destroy any $\text{Ag}_2\text{S}_2\text{O}_5$ or Ag_2SO_3 that may have formed). The silver iodide was retained on a glass-fiber filter paper which had been pretreated with H_2O , ethanol, and ethyl ether and preweighed. The precipitate was washed with H_2O , ethanol, and ethyl ether, dried, and weighed. In general, from 65 to 75% of the iodine was recovered. The extrapolated zero-time counting rates were corrected in each sample by the measured recovery factor.

RESULTS

The radioactivity, measured as cpm above background, and corrected for decay and chemical recovery, of 30 consecutive samples of KI or NH_4I standard solutions is plotted against amount of iodine, in nanograms, in Fig. 2. Linearity is approximated over the range of 0 to 30

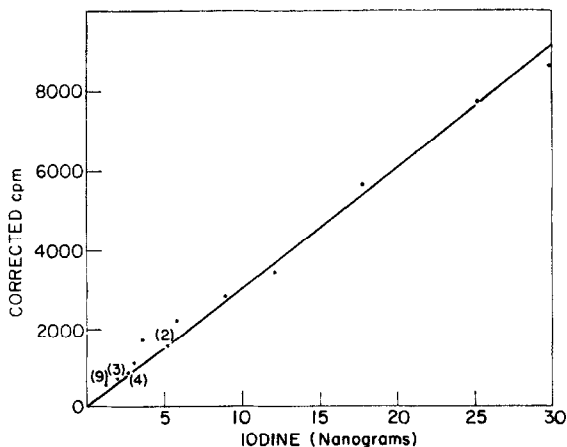


FIG. 2. Radioactivity as function of iodine content in standard solutions of KI and NH_4I . Radioactivity is corrected for decay and chemical recovery. Parentheses indicate number of samples; means are shown. The line is calculated by a method of least mean squares.

nanograms; the solid line is calculated by a method of least mean squares, and has a slope of 292 cpm/ng, a zero intercept of 126 cpm, and a correlation coefficient equal to 0.995. With greater amounts of iodine standards than those shown in Fig. 2, the linearity is not constant; for 59.6 ng, the slope is 284 cpm/ng and, for 258.0 ng, 227 cpm/ng.

The efficiency of the scintillation counting is shown in Fig. 3 as (cpm/ng)/(dpm/ng). Efficiency decreases from about 50% in the range 1 to 3 ng to about 40% in the range 5 to 25 ng. An attempt was made to increase the efficiency of the radioassay of the Cerenkov radiation by adding to the radioactive solution a wavelength shifter, 7-amino-1,3-naphthalenedisulfonic acid (4). Although this compound increased the counting rate about 20%, it interfered with the precipitation of AgI after counting and so made it impossible to estimate recovery. Other wavelength shifters were not investigated, but might be of use in extending this method to the measurement of samples containing less than 1.0 to 0.1 ng of iodine.

Recovery of added iodine was tested. A mitochondrial sample containing 0.392 ng/mg protein gave an extrapolated value of 1055 cpm above background at zero time. A sample of NH_4I , containing 1.88 ng of I, had 675 cpm above background at zero time. A combined sample gave 1560 cpm, representing a recovery of 90%.

The precision of the method, when measuring samples of standards (KI and NH_4I), in the range 1.3 to 30 nanograms, is expressed by the correlation coefficient of 0.995 obtained from the data in Fig. 2. In another assay of precision, 26 consecutive samples of 1.25 ng of NH_4I gave a mean of 368 cpm above background, ± 9.5 cpm standard error;

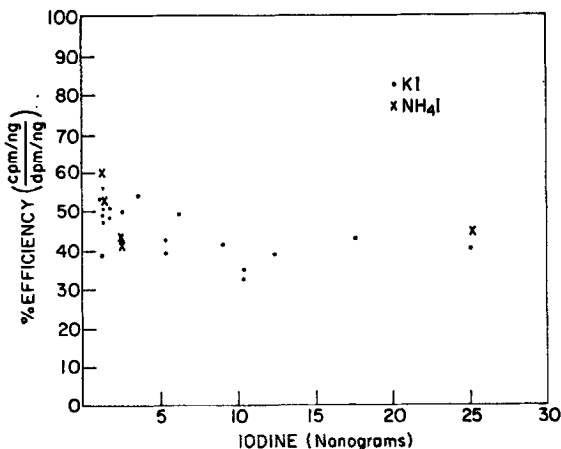


FIG. 3. Efficiency of scintillation counting of Cerenkov radiation.

TABLE 1
Duplicate Analyses of Iodine Content per Milligram Protein of Different Batches of Rat Liver Mitochondria

Mitochondria source	I, ng/mg protein	\pm % deviation from the mean
Normal rat		
1	0.354, 0.357	0.56
2	0.358, 0.388	4.02
3	0.210, 0.214	0.94
Hypothyroid rat		
1	0.211, 0.197	6.87
2	0.106, 0.163	20.1
3	0.170, 0.156	8.59

the coefficient of variation ($100 \times \text{S.E.}/m$) was 2.6%. The precision of duplicate analyses of six different batches of liver mitochondria is shown in Table 1. The average deviation from the mean for the three samples obtained from normal rats was $\pm 1.84\%$, and for the three samples obtained from hypothyroid rats, which have lower iodine contents, $\pm 10.8\%$; these values indicate the precision of sampling the mitochondrial suspensions as well as that of the method of analysis.

A larger series of single analyses of the iodine content of mitochondria obtained from the livers of rats is shown in Table 2, and compared with other published values. The current values are higher for mitochondria from hypothyroid rats and lower for those from normal rats. Biological variations might account for these differences, but these data also seem to throw doubt on the accuracy of either the present or the chemical method.

Accordingly, aliquots of the same mitochondrial suspension were analyzed by neutron activation and by a chemical method (5) (Table

TABLE 2
Iodine Content of Mitochondria Obtained from Livers of Normal and Hypothyroid Rats, as Measured by Neutron Activation Analysis, Compared with Previous Chemical Analyses

Method	I, ng/mg protein, \pm S.E. (n)	
	Normal rats	Hypothyroid rats
Neutron activation	0.28 \pm 0.02 (17)	0.19 \pm 0.006 (11)
Chemical (ref. 10)	0.43 \pm 0.03 (13)	0.10 \pm 0.02 (9)
Chemical (ref. 11)	0.50 \pm 0.08 (9)	

3). Both methods give reasonably similar values for the iodine content of the various samples. It appears, then, that the disparities between the results of the current and the previous analyses shown in Table 2 arise from real differences in iodine content of the mitochondrial preparations, and that the accuracy of neither method need be doubted on the basis of these analyses.

Although the separation of the halogens according to Duce and Winchester (2) is satisfactory for their atmospheric and sea water samples, and appears to be equally satisfactory for our samples of liver mitochondria (as implied in Table 3), difficulties in estimating the iodine

TABLE 3
Iodine Content of Mitochondria Obtained from Livers of Normal Rats, and of L-Thyroxine, as Measured on Aliquots of Same Batch by Neutron Activation and by a Chemical Method (5)

Sample	I, ng/ml	
	by activation anal.	by chem. anal.
Liver mitochondria	3.2	4.0
L-Thyroxine		
prepn. 1	8.9	7.0
prepn. 2	9.6	10.0

content of rat skeletal muscle mitochondria by neutron activation seem to arise occasionally because of an interference by Cl. Muscle mitochondria are isolated in a medium that contains, among other ingredients, 100 mM KCl and 1 mM MgCl₂ (6). Adding 100 mM KCl to 3 ng I as KI raises the cpm from 110 to 6250, and changes the apparent half-life of the irradiated product from 25.4 to 34.0 min.

DISCUSSION

The sensitivity and specificity of neutron activation analysis have been applied in a number of methods for measuring iodine in biological samples. Some of these methods have employed the reaction $^{129}\text{I} (n, \gamma) ^{130}\text{I}$, ^{130m}I , in which the irradiation products have half-lives of 12.6 hr and 9.2 min, respectively. In blood samples, organic moieties containing I have been measured with a sensitivity of 0.01 ng of iodine (7). Total I in the thyroid glands of live rams has been estimated by irradiation of the animals (8). These methods involve the feeding of ^{129}I to animals for a period long enough to equilibrate the isotope with the natural ^{127}I . Although they are very sensitive, they may suffer from the disadvantage that equilibration is not attained, and therefore less than the total I

content is measured. Such lack of equilibration is evident when animals are fed ^{125}I as a step in a procedure to measure the subcellular distribution of thyroid hormones (9). By this method, only one-third or less the amount of iodine is found in rat liver mitochondria as by neutron activation in the present report, or by previous chemical analyses (10-12).

The reaction used here, $^{127}\text{I} (n,\gamma) ^{128}\text{I}$, has a capture cross-section of 6.4 barns and a product with a half-life of 25.0 min. The relatively short half-life of ^{128}I is inconvenient, making postirradiation fractionation and resolution of components difficult, or requiring timed counting by a high-resolution γ -spectrometer. Part of these disadvantages is circumvented, however, by using the relatively high β -emission of ^{128}I (1.67 and 2.12 MeV) for scintillation counting of the Cerenkov radiation produced in aqueous solutions. Such counting offers the advantages of high sensitivity (especially for hard β -emitters like ^{128}I), minimal self-absorption, and simplicity of sample preparation. Since Cerenkov radiation is not produced in water by β -emissions of less than about 0.26 MeV energy, the procedure used here in effect eliminates some interfering elements.

SUMMARY

The naturally occurring isotope, ^{127}I , has been measured by neutron activation, postirradiation extraction of ^{128}I plus ^{127}I (added carrier), and timed scintillation counting of aqueous solution utilizing Cerenkov radiation. With extrapolation of the counts to zero time (end of irradiation) and measurement of chemical recovery by weighing of precipitated AgI , there are about 300 cpm above background per nanogram of I, in the range of 0-30 ng. The precision for samples of NH_4I is $\pm 2.6\%$ coefficient of variation. The accuracy is good when standards, rat liver mitochondria, or thyroxine solutions are measured both by activation analysis and by a chemical method. The use of Cerenkov radiation simplifies sample preparation, eliminates the need for a γ - or β -spectrometer, and acts as an energy band-pass.

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