A NEW METHOD OF ANALYSIS OF PROTEIN CONTENT IN GRAIN BY PROTON ACTIVATION[1]

D. A. DOHAN and K. G. STANDING, Department of Physics, and W. BUSHUK, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

ABSTRACT   Cereal Chemistry 53(1): 91-100

This new technique measures the total nitrogen in a sample like the classical Kjeldahl method, but uses a nuclear reaction instead of a chemical reaction. A beam of 16 MeV protons strikes a sample of grain and produces radioactive ^{14}O nuclei through the reaction $^{14}N+p \rightarrow {}^{14}O+n$. The ^{14}O decay (half-life 71 sec) is detected off-line by its characteristic 2.31 MeV γ-ray. The ratio of the number of γ-rays counted to the total number of protons striking the sample determines the abundance of nitrogen. The γ-rays provide a unique identification of this nuclear reaction and thus an unambiguous measure of the nitrogen content of the sample. The method is applicable to either whole or ground grain. Weighing the sample is unnecessary since its effective thickness is determined by the proton range (≈ 0.23 g/cm^2). Samples of barley, common wheat, durum wheat, oats, rapeseed, rye, triticale, and high-protein flour have been tested. Accuracy is comparable to the accuracy of the Kjeldahl method. When developed, the technique should be capable of analyzing ~ 10 samples per min, with resulting advantages in cost.

For many years, the standard method of measuring the protein content of cereals has been the Kjeldahl technique, a measurement of total nitrogen, which is related to protein through an accepted conversion factor. The method is time-consuming and becomes very cumbersome when applied to a large number of samples. This difficulty has stimulated developments in several directions. First, the Kjeldahl method itself has been automated (1,2), so that a single unit can process up to about 20 samples per hr. Similar improvements have been made in the traditional Dumas method (3). At the same time, new techniques have been

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introduced, *e.g.*, infrared reflectance spectroscopy, which is capable of analyzing about 400 samples per hr (4).

Nuclear techniques have also been applied. Nitrogen content may be measured by the intensity of particles or γ-rays emitted directly in nuclear reactions on nitrogen-14: γ-rays from $^{14}N(n, \gamma)^{15}N$ (5), deuterons from $^{14}N(p, d)^{13}N$ (6), protons from $^{14}N(d, p)^{15}N$ (7), or α-particles from $^{14}N(d, \alpha)^{12}C$ (7). More commonly, the disintegration of the radioactive end product of a nuclear reaction is observed. Most work has been done with nitrogen-13, produced in the reactions $^{14}N(n, 2n)^{13}N$ (8,9,10) or $^{14}N(\gamma, n)^{13}N$ (11,12). The nitrogen-13 decays with a half-life ($\tau_{1/2}$) of 10 min by emission of a positron, which in turn annihilates, producing two γ-rays each of energy 0.51 MeV. It is this "annihilation radiation" which is detected. Carbon-11 from the $^{14}N(p, \alpha)^{11}C$ reaction has similar properties (13); it decays by positron emission also, with a half-life of 20 min. Unfortunately, positrons are emitted by the radioactive products of many other nuclear reactions in the target material. Background of annihilation radiation from these activities can be distinguished from the radiation due to the desired activity only by differing lifetimes. Thus, a waiting period $\sim$ 10 min between irradiation and counting has been helpful, but the background is still troublesome.

We have investigated the use of a different nuclear reaction (14,15) for protein analysis, a reaction which we believe has significant advantages[2]. In our measurements, nitrogen content is measured by observing the radioactivity of oxygen-14, produced in the reaction $^{14}N+p \rightarrow ^{14}O+n$. The radioactive ^{14}O decays by emission of a 2.31 MeV nuclear γ-ray as well as a positron. Its half-life is $\approx$ 71 sec. The 2.31 MeV γ-ray identifies ^{14}O uniquely. *No* other reaction leading to ^{14}O is energetically possible at our proton energy, so the intensity of 2.31 MeV γ-rays also provides an unambiguous measure of the amount of nitrogen in the sample. The short half-life enables rapid processing of samples. This report describes an investigation of the use of the $^{14}N(p, n)^{14}O$ reaction for analysis of protein content in grain.

MATERIALS AND METHODS

The method is very simple in principle. A proton beam of constant energy is produced by a cyclotron and directed into a sample of grain. The number of reactions is measured by the number of 2.31 MeV γ-rays emitted after irradiation. The ratio of the number of γ-rays detected to the number of protons hitting the sample determines the nitrogen content. In the work reported here, the total number of protons hitting the sample was measured by the electric charge collected.

The Nuclear Reaction

The most probable nuclear reaction produced in nitrogen by protons of energy $\sim$ 16 MeV is the (p, n) reaction, $^{14}N+p \rightarrow ^{14}O+n$. The radioactive ^{14}O decays back to ^{14}N by emission of a positron (β^+) and a nuclear γ-ray of energy 2.31 MeV. Only 0.6% of the decays go directly to the ^{14}N ground state without γ-ray emission. No other reaction on any target nucleus can yield ^{14}O until the proton energy exceeds

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[2]This reaction has received some attention for the detection of trace amounts of nitrogen (16,17); see also Mazitov *et al.* (18).

$\sim$ 20 MeV; therefore, the amount of ^{14}O produced unambiguously determines the abundance of ^{14}N.

Figure 1 gives the yield of the ^{14}N(p, n)^{14}O reaction as a function of energy for a target thick enough to stop the protons ($\gtrsim$ 0.28 mg/cm^2 at 16 MeV). The increase in yield with proton energy is an argument for the use of higher energy protons. However, an upper limit on the proton energy is set by the threshold of the ^{16}O(p, t)^{14}O reaction at 21.7 MeV, the first possible reaction to yield ^{14}O background. Furthermore, the only noticeable background activity in the vicinity of 2.31 MeV γ-ray energy comes from potassium-38, whose yield relative to oxygen-14 increases with energy. A satisfactory compromise between these requirements was achieved at 16 MeV proton bombarding energy.

Materials

Samples of barley, common wheat, durum wheat, oats, rapeseed, rye, triticale, and high-protein flour were obtained, some from the Canadian Grain Commission Grain Research Laboratory, and some from the stocks of the Plant Science Department. The samples were selected to cover a fairly large range of protein content. Part of each sample was set aside for analysis by the new method; the remainder was analyzed for moisture and protein content in the Kjeldahl laboratory of the Plant Science Department.

Some of the samples for nuclear analysis were ground; others were left as whole grain. They were then placed in cylindrical containers 3.8 cm diam $\times$ 3.0 cm long. One end of the cylinder was covered with thin aluminum foil (0.7 mg/cm^2). Protons entered the sample through this foil parallel to the axis of the cylinder. Since the range of 16 MeV protons in a grain kernel is $\lesssim$ 2.5 mm, the sample was amply thick enough to stop all protons.

It is not necessary to weigh the sample to determine the percentage of nitrogen. Each proton has the same range in a given material ($\approx$ 0.28 g/cm^2), so the material irradiated is equivalent to a solid disc of diameter equal to the diameter of the proton beam and thickness equal to the range. [More accurately, the effective thickness is equal to the difference in range between protons of energy 16 MeV and protons at the reaction threshold ($\approx$ 6.4 MeV—see Fig. 1). This amounts to $\approx$ 0.23 g/cm^2.] The irradiated mass is, therefore, determined by the proton energy.
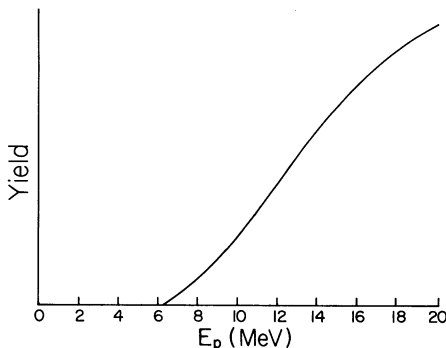

Fig. 1. Yield of the reaction ^{14}N(p, n)^{14}O as a function of proton energy for a thick target. The yield up to 12 MeV was obtained by integrating the cross-sections of Kuan and Risser (19). The yield beyond 12 MeV was obtained from our own measurements.

Sample Irradiation

Figure 2 shows the irradiation geometry. Protons were extracted from the University of Manitoba cyclotron at minimum energy ($\sim$ 22 MeV). They passed down an evacuated beam pipe and were bent through an angle of 45°. The proton energy was defined by slits before and after the bending magnet. After the bend, the beam pipe passed through an aperture in a concrete shielding wall. Magnetic quadrupole lenses focused the protons onto the carbon collimators whose apertures (3.2 cm in diam) determined the beam size. An aluminum absorber between the collimators reduced the proton energy almost to 16 MeV. The protons then passed into air through a thin window at the entrance to a metal container ("Faraday cup") which held the sample.

The Faraday cup was insulated from the beam line and connected to ground through a current integrator, which measured the total charge carried into the Faraday cup by the protons entering it. This measures the total number of protons striking the sample, since each proton carries a single electronic charge e $\approx 1.6 \times 10^{-19}$C. A suppressor ring at -1500 V was placed in front of the Faraday cup to bend secondary electrons back into the cup.

The beam transport quadrupoles were adjusted to give a fairly uniform beam over the collimator aperture. The effective mass of grain irradiated was then approximately π 1.6^2 cm^2 $\times 0.23$ g/cm^2 ≈ 1.8 g. It is estimated that between 100 and 200 individual kernels were sampled by the protons in the whole-grain wheat.

Experimental Procedure

A typical run began with the cyclotron beam stopped within the shielding wall around the cyclotron. After insertion of the sample, the beam was allowed to strike the remotely controlled shutter shown in Fig. 2. Raising the shutter started the irradiation, which lasted for 1 min at a proton beam current of $\sim$ 50nA. Dropping the shutter terminated the irradiation. With the beam again stopped
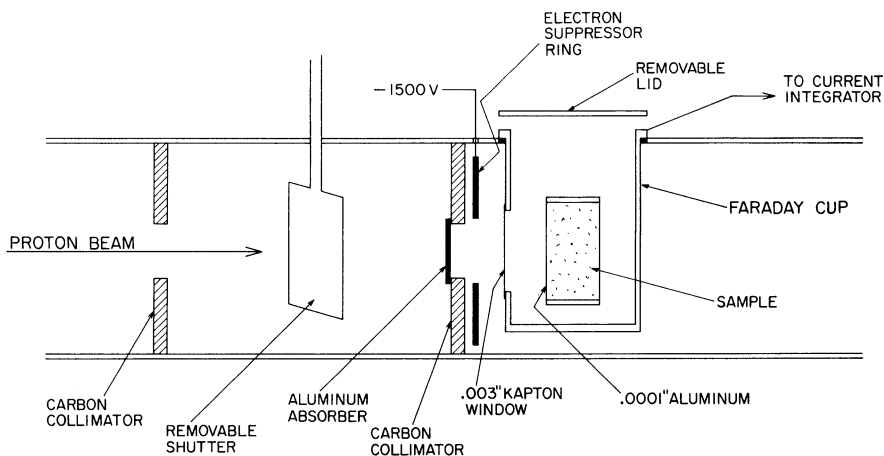

Fig. 2. Cross-section (side view) of the irradiation geometry.

within the shielding wall, the sample was transferred manually to a counting station behind another shielding wall. This took 1 min. The γ-rays were counted for 3 min, the results recorded, and the whole process repeated for the next sample. This rather primitive procedure thus required a total of about 6 min per sample.

γ-Ray Detection

The γ-ray counters were two NaI(T1) scintillation counters with crystals 5 cm diam × 5 cm long. Each counter was covered with 2.5 cm lead shielding to reduce the intensity of the annihilation radiation hitting it. The counters were mounted with their axes collinear, and the sample inserted between them with its axis on the same line.

The output pulses from the scintillation counters passed through preamplifiers and amplifiers into analogue to digital converters, where they were digitized and fed into a PDP-15 computer. A typical scintillation counter spectrum is shown in Fig. 3. A computer program integrated the counts under the 2.31 MeV peak. The ^{14}N content in the sample is then proportional to the ratio R, given by

$$R = \frac{\text{Number of } \gamma\text{-ray counts under the 2.31 MeV peak}}{\text{Total charge collected in the Faraday cup}}$$

RESULTS

Since the method is nondestructive, its reproducibility could be tested by repeated runs on the same sample. Figure 4 gives the value obtained for the ratio
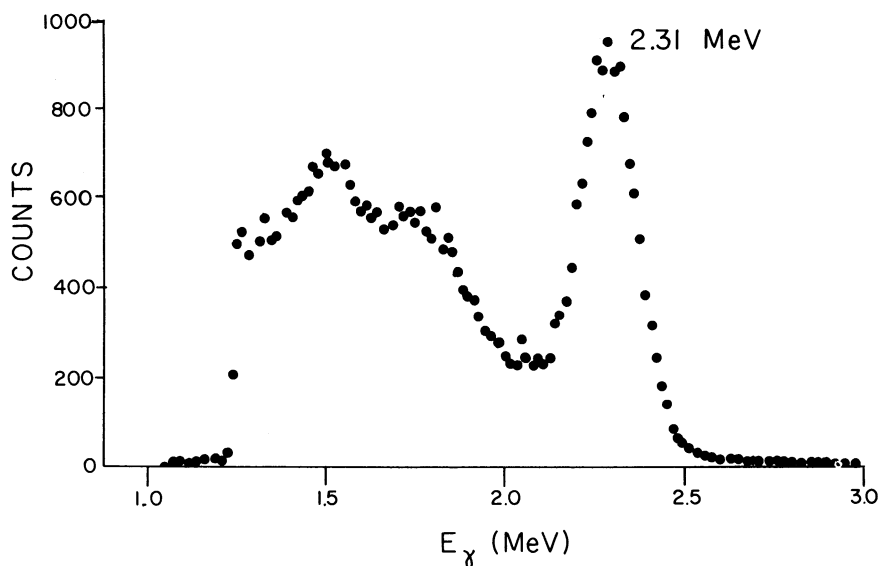

Fig. 3. Scintillation counter spectrum of wheat (above 1 MeV), following irradiation by 16 MeV protons.

R on successive repetitions of the measurement with three wheat samples. In each case, the standard deviation in R is ≈ 0.05, giving a coefficient of variability $\sim$ 1.0%. This figure is consistent with the value expected for statistical counting errors alone, since the total number of counts for each point was $\sim 10^4$.

Figures 5 and 6 show a comparison of the results obtained by the present method with the Kjeldahl analyses for the same samples. The samples of barley, common wheat, durum wheat, rye, and triticale were measured for both whole and ground grain by the new technique; the Kjeldahl results were obtained with ground grain. The straight line in Figs. 5 and 6 is a least squares fit to the experimental points. The RMS deviation of the experimental values of R from the line is 0.138, corresponding to a coefficient of variability of 2.4%. There was no measurable difference between the samples of whole and ground grain.

Corrections

The data points plotted in Figs. 5 and 6 have been corrected for various effects. Although some of the corrections are significant, ranging up to 0.3 at $R = 5$, they can be estimated or measured with reasonable accuracy.

The counting rate correction (pileup) was monitored continuously by superimposing pulses of amplitude somewhat greater than the 2.31 MeV γ-rays. The pulses were put in at a constant rate of 60 Hz so the decrease in the number of such pulses observed at the output was a direct measure of the pileup correction, which ranged from 0.1 to 0.3 at $R = 5$.

The range of a proton has some dependence on the stopping material, although fortunately the dependence is not very large. Since the yield of $^{14}0$ per
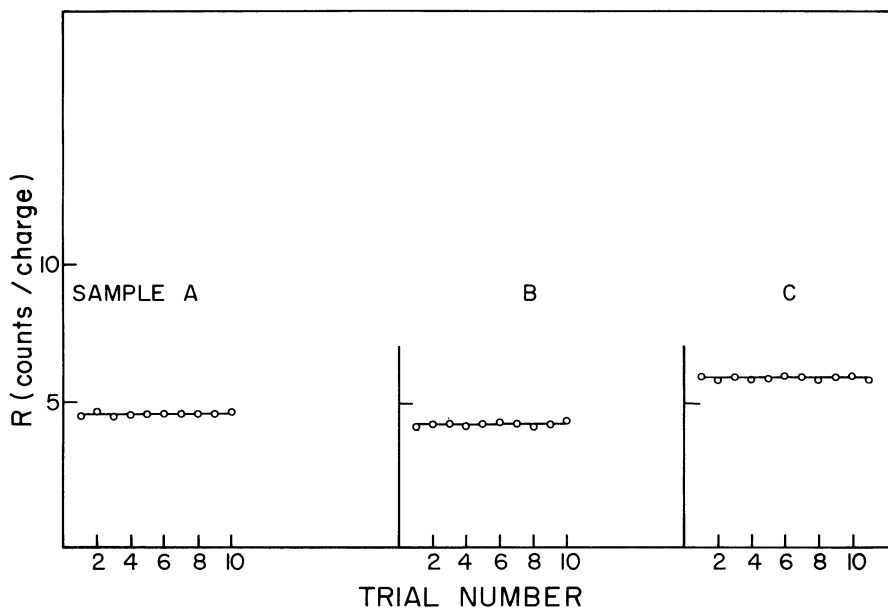

Fig. 4. Reproducibility test for three wheat samples.

MeV energy loss starts to be significant at about 7.5 MeV, and remains constant within $\sim \pm 20\%$ from there to 16 MeV, we have taken Δ, the difference in range between protons of 16 MeV and 7.5 MeV, as the significant parameter. Values of Δ were calculated for materials of interest from the effective ionization potential (20,21). Typical results for Δ were 0.219 g/cm^2 for starch, 0.209 g/cm^2 for water, 0.220 g/cm^2 for glutamic acid, and 0.201 g/cm^2 for triolein. This gave very small corrections to the ratio R for the grain samples (up to 0.03 at R = 5). Because of their oil content, correction for the rapeseed samples was larger, 0.2 at R = 8.

The presence of nitrogen in the air between kernels led to a small correction of 0.07 in R.

The only noticeable background activity came from ^{38}K, which has a γ-ray of energy 2.17 MeV and a half-life $\sim$ 460 sec. It may be formed in the ^{39}K(p, d)^{38}K reaction or the ^{40}Ca(p, ^{3}He)^{38}K reaction, which have thresholds of $\approx$ 11 MeV and $\approx$ 14 MeV. The "tail" of the ^{38}K γ-ray spectrum gave a measurable contribution to the ^{14}0 γ-ray spectrum, a contribution which increased with energy. At 16 MeV, however, the contribution to the ratio R was $\sim$ 0.01 for a typical sample, so no correction was made for this effect.

DISCUSSION

Clearly, more work has to be done to explore the limits of this new technique. However, it already appears from the results of Figs. 4, 5, and 6 that the present method is capable of giving results which are comparable in accuracy to those yielded by the standard Kjeldahl technique. Neither grinding nor weighing of the sample is necessary, so much labor is saved.
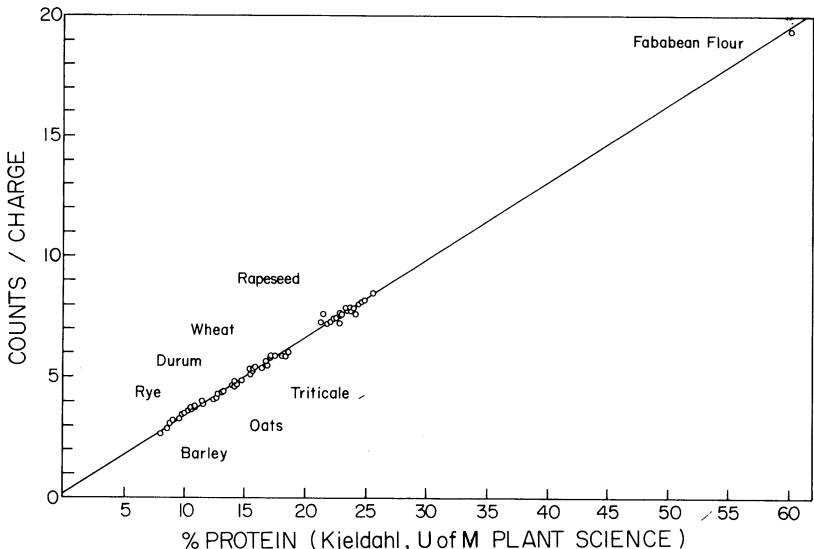

Fig. 5. Comparison of measurements with the present method and the Kjeldahl method. See Fig. 6 for more detail on the grain samples.
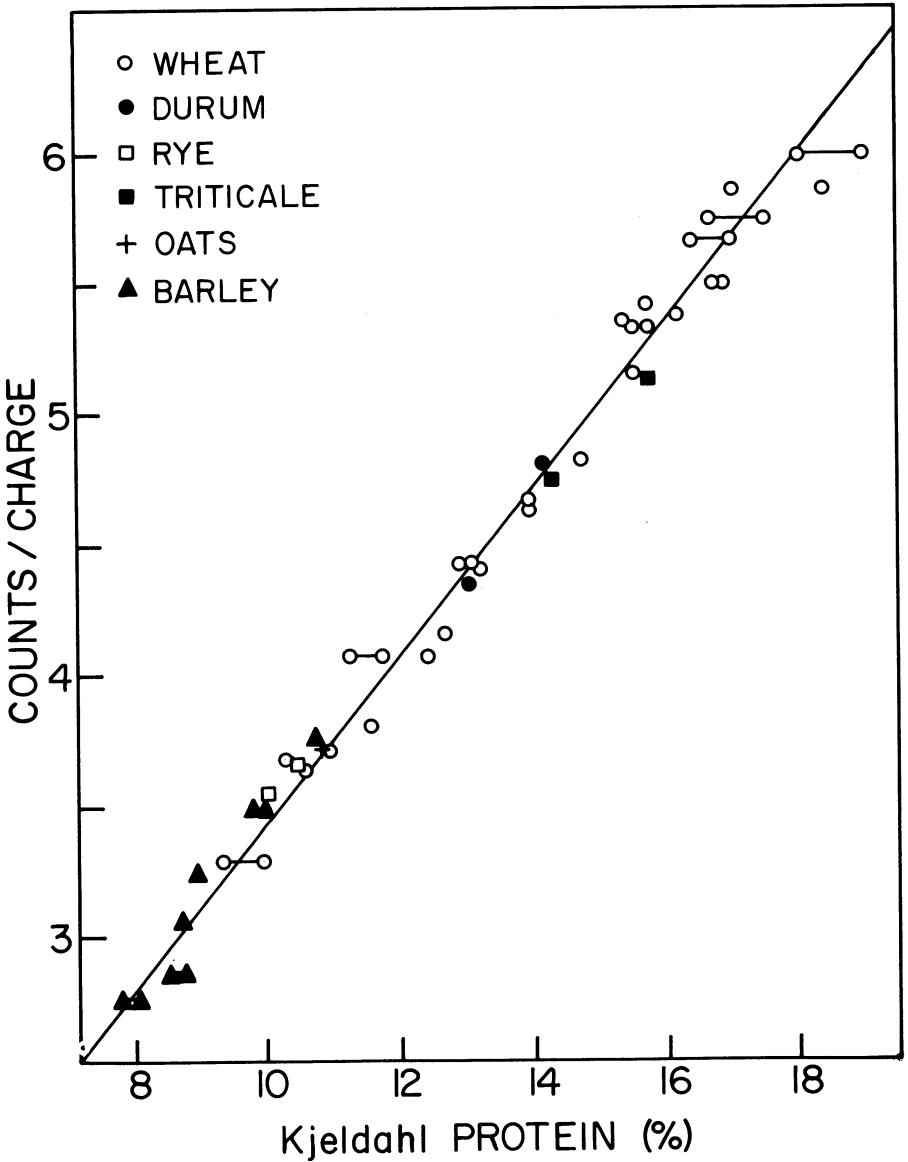
Fig. 6. Comparison of measurements with the present method and the Kjeldahl method. This is an expansion of the part of Fig. 5 which shows the grain samples. Some of the samples received duplicate Kjeldahl analyses; these are indicated by horizontal bars and provide some indication of the error in the Kjeldahl measurements.

Speed

In the rather primitive measurements reported here, the analysis required about 6 min per sample, but it is obvious that this time can be reduced greatly.

The small scintillation counters used for this work were a pair which were available in the laboratory. We have recently acquired new counters specifically for the job — four NaI(Tl) detectors, each 12.7 cm diam $\times$ 7.6 cm long. The measured efficiency of one of the new counters is a factor of eight greater than the efficiency of an old one at 2.31 MeV γ-ray energy. The new counters are expected to yield a production rate of $\sim$ 4 samples per min with other conditions held constant. This may be calculated from the known counting rate with the old counters: 2×10^4 (total number of counts with the old counters),

$\times \dfrac{4}{2}$ (counters) $\times$ 8 (efficiency),

$\times \dfrac{1}{3.3}$ (ratio of irradiation time, corrected for decay),

$\times \dfrac{1}{6.5}$ (ratio of counting time, corrected for decay),

$\approx 1.5 \times 10^4$ counts per sample, which yields a coefficient of variability in the counting statistics better than 1%. This, of course, requires an automated system for sample changing, which is under construction. A further improvement in rate by a factor of two or three is expected to be given by an increase in proton beam and a decrease in the waiting period. Therefore, we estimate a production rate of $\sim$ 10 samples per min when the improvements which are now under way are completed. At a rate of $60 to $120 per hr for cyclotron time, the irradiation cost would then be 10¢ to 20¢ per sample.

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