Technical method

The assay of tritium-labelled folate in faeces by the oxygen flask combustion technique followed by liquid scintillation counting

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The assay of tritium-labelled substances in faeces requires conversion of tritium into either tritium gas (Payne and Done, 1958) or tritium-labelled water. Belcher (1961) and Kremenchuzky, Musso, Hoffbrand, and Viola (1967) used wet oxidation of faeces followed by liquid scintillation counting, while Burns and Glass (1963) used dry oxidation in a bomb calorimeter.

There is a need for a reproducible and relatively sensitive means of measuring tritium activity in tissues and materials such as faeces. This method was developed in order to measure intestinal absorption of tritium-labelled compounds such as folic acid and its analogues and is a modification of the oxygen-flask combustion technique developed by Schöninger (1955).

Method

Subjects were given an oral dose of tritium-labelled folate analogue in 20 ml water containing about 50 μCi. Faeces were collected for four days. The 96-hour faecal collection was weighed, homogenized with water, and the homogenate weighed again. Two one-hundredth aliquots of homogenate (about 10 ml) were placed in previously weighed porcelain crucibles. To one was added 0·1 ml of the oral folate dose given to the patient to serve as an internal standard. The contents of both crucibles were evaporated to dryness in a sand bath at 200-250°C for two hours. The crucibles were weighed again and the dried faeces ground into a fine powder. One-tenth of the total dry weight (about 100 mg) was placed in Visking tubing, carefully folded and tied with a piece of string into a small pellet. Enough string was left at one end for use in ignition. The sample was then ignited in an atmosphere of oxygen in the flask illustrated in the diagram (McFarlane, 1973). This consists of a spherical flask of 1 litre capacity with a ‘finger’ at the base. It was fitted with a ground glass stopper, stopcock, and a hook from which a platinum gauze boat was suspended. Before use the platinum boat was thoroughly dried with heat. It was attached to the glass hook, the length of the platinum wire being such that the sample was situated near the centre of the flask during combustion. The mesh of the platinum gauze (36 gauge) should be coarse enough to allow free access of oxygen to the sample but so fine that no unburned particles can drop through it. The apparatus is shown in figure 1.

To prepare the flask for use, distilled water, 0·25 ml, in the ‘finger’ was frozen in dry ice/ethanol. The flask was evacuated to about 5 mm of mercury, the stopcock closed, and the water vapourized by submerging the finger in hot water, thus producing
a film of condensation over the inner surface of the flask (the 'shell'). When all the water had evaporated, the stopcock was opened and the flask allowed to fill with air. The flask was then immersed in dry ice/ethanol mixture at $-72^\circ\text{C}$ to freeze the shell of condensate. It is this shell which adsors the combustion water from the tritium. While still immersed in dry ice/ethanol the flask was flushed out with cylinder oxygen for about two to three minutes. The pellet was placed in the platinum basket, the piece of string ignited and immediately plunged into the flask, where it should burn completely within a few seconds. The stopper and the flask were held firmly together during the process since the pressure in the flask rises rapidly during combustion. After three or four minutes, when the cloud of combustion products had disappeared, the stopcock was opened and the flask left at room temperature for the shell to thaw. The procedure was best carried out in a fume cupboard with a safety screen. The combustion procedure was quite safe, even in inexperienced hands, despite the spectacular reaction.

The tritiated water in the flask was then rinsed out with three 5-ml amounts of scintillator fluid consisting of:

\[
\begin{align*}
200 \text{ g Naphthalene} \\
10 \text{ g 2-5-diphenyloxazole} \\
0.25 \text{ g 2-p-phenylene-bis (5-phenyloxazole)} \\
\end{align*}
\]

\[
\quad \text{Added to 1 litre dioxane and 100 ml toluene}
\]

The radioactivity was counted using a LKB-Wallac liquid scintillator spectrometer.

**Results**

**Effect of Drying on Tritium Activity**

Four aliquots of faeces from a patient who had had an oral dose of tritium-labelled folate were dried in a sand bath for 30, 60, 90, and 180 minutes respectively at 200 to 250°C. Figure 2 shows the effect of drying on identical samples. After one hour a plateau in the counting rate was reached when most of the water was driven off by heat. To maintain uniformity, all specimens were heated up to two hours.

**Relationship between Counts and Size of Sample**

Twenty-five to 200 mg of dried faecal material from a 96-hour stool collection of a patient who received 50 μCi tritium-labelled folate by mouth was ignited and counted. Figure 3 shows that there is a linear increase in counts with increased weight of sample.

![Graph showing relationship between counts and size of sample](http://example.com/graph.png)

**Recovery of Tritium Added to Faeces In Vitro**

To aliquots of normal stool were added increased concentrations of tritium-labelled folate which were dried, set alight, and counted. Figure 4 shows a linear increase in counts with increasing amounts of added folate.

**Comparison of Expected and Actual Counts**

Known amounts of tritium-labelled folic acid were also added to aliquots of normal faeces and subjected to dry oxidation, followed by scintillation counting. Using the quench curve for the particular
scintillator fluid, the disintegrations per minute were calculated. An unseeded sample was run simultaneously to correct for background counting. The expected nuclear transformations per minute from the actual amount of radioactive folate added were calculated. Table I indicates the difference between the number of disintegrations per minute found in identical samples but different in radioactivity to disintegrations per minute calculated for the samples, the actual counts experimentally found being about 30% higher than the expected counts.

<table>
<thead>
<tr>
<th>µCi ³H-PGA Added</th>
<th>Actual Counts</th>
<th>Expected Counts</th>
<th>DPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>6 140</td>
<td>5 550</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>14 655</td>
<td>11 110</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>28 240</td>
<td>22 200</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>64 971</td>
<td>55 300</td>
<td></td>
</tr>
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</table>

Table I Increasing amounts of ³H-folate added to aliquots of faeces and the aliquots processed

1Results are expressed in disintegrations per minute and show that the actual counts experimentally found are about 30% higher than the expected counts.

**EFFECT OF ‘STORAGE’ OF FAEces ON TRITIum ACTIVITY**

To aliquots of normal stool were added increased concentrations of radioactive folate. One series was processed at once and the other was incubated for four days at 37°C before being dried, set alight, and counted. The results expressed in fig 5 indicate that incubation causes slight loss of radioactivity. This may be explained by the possible conversion of radioactive folate to tritiated water through bacterial action, which is subsequently lost in the drying-out process. Internal standardization cannot be used to correct for loss of activity due to possible bacterial action in the intestinal tract.

**REPRODUCIBILITY**

In two patients consecutive estimations were done on the same specimen. Table II shows that the coefficients of variation were 7.8% and 3.7% respectively, indicating a high reproducibility of results.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sample No.</th>
<th>Counts per Minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>6608</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>7528</td>
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<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7287</td>
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<td>Coefficient of variation</td>
<td>7.8%</td>
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<tr>
<td>2</td>
<td>5</td>
<td>8213</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>8899</td>
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</tr>
<tr>
<td>11</td>
<td>11</td>
<td>9070</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>3.7%</td>
<td></td>
</tr>
</tbody>
</table>

Table II Consecutive estimations done on the same specimen showing a coefficient of variation of less than 8%.

**Comment**

The advantages of the oxygen-flask combustion technique for the assay of tritium-labelled folate in faeces are the ease in preparing samples and the relatively high counting rates obtained. In the drying-out process some tritiated water may be lost and care must be taken to avoid conditions which favour bacterial action before the assay of the specimen.
In our experiments collections were done over a 96-hour period during which time the specimens were kept refrigerated. For the same reason internal standards should be processed immediately after addition. Quenching is compensated for by the use of an internal standard where a two- to three-fold increase in the counting rate is usually found.

One of the main attractions of this method on a routine or research basis is the fact that untrained technicians can obtain excellent results after a little practice. It is also suitable for assaying a large number of samples provided pellets have been prepared in advance. Many estimations can be performed rapidly and simply by this method. It also employs inexpensive and readily available apparatus.

I am indebted to Dr Janet Perry and Dr I. Chanarin, for valuable discussions.

References

Letter to the Editor

The Effect of Bilirubin on the Assay of Gentamicin

The lack of effect of bilirubin on the assay of gentamicin by the large-plate technique in the studies of Renshaw and Cornere (1974) confirms my earlier findings (George, 1973). My own studies showed no difference in serum gentamicin levels in jaundiced patients receiving gentamicin when assayed by the tube and large-plate techniques. No differences from the expected levels of gentamicin were observed when known amounts of gentamicin were added to serum with raised levels of conjugated or unconjugated bilirubin or to which sodium taurocholate had previously been added.

A possible explanation for the original findings of Stratford (1970) has recently been published (George, Bint, and Prangnell, 1974).

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References

Book reviews


This is an account of an international symposium held in Sardinia in April 1972; it was organized by the Wellcome Foundation to gather together the experience gained during the first three years that trimethoprim/sulphamethoxazole (co-trimoxazole) was generally available. There were sessions on the laboratory aspects, pharmacokinetics, urinary tract infections, respiratory tract infections, and paediatric use. A session on miscellaneous infections includes papers on the treatment of brucellosis (75 patients), chronic osteomyelitis (25 patients), and chronic salmonella carriers. There is also a review on the treatment of typhoid fever. The final session was devoted largely to a full account of the various side effects which had been reported to the Wellcome Foundation’s Adverse Reactions Centre. This paper is particularly valuable in showing the incidence of the different side effects seen; it also points out that these are much as would be expected from treatment with or similar dose of a sulphonamide alone. The papers in each session were followed by discussions, which are apparently fully reported.

E. JOAN STOKES


To do this book justice, it must be viewed in the context of the author’s intentions. He states quite clearly in his preface that ‘The principles of pathology’ is intended to help medical and dental practitioners prepare for the examinations in basic medical sciences set by several Royal Colleges and consequently has a strong clinical orientation. Thus the audience for which it is intended has, presumably, already received a grounding in general pathology during their medical training, so that this book and the lectures on which it is based should be, in theory, by way of revision.

However, because it is concise and inexpensive, it will be attractive to medical students, and the danger may be that they will use it instead of, rather than as well as, a standard textbook, though I would doubt the author ever intended that even the particular audience he had in mind should ever use it in this way.

The book itself is well written and very readable, and the inclusion of historical background welcome. The almost total absence of illustrations is a drawback, particularly for those for whom pathology may be a dim memory and those undergraduates who may be lured into thinking that this book provides a short cut to success. Illustrations increase the cost, but one wonders if the author has not been over anxious to keep the cost down in this connexion as well as in the brevity of the text. The latter covers the field of general pathology, but some expansion in
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