Letter to the Editor

Adaptation of an Automatic Bacterial Colony Counter for Measuring Lung Tumor Growth in Mice

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SUMMARY

Adaptation of an automatic bacterial colony counter proved to be an efficient procedure for detecting and quantitating tumor growth in mouse lungs prepared by the Wexler method of India ink insufflation. After correlation of the size discriminator settings on the automatic counter with the Wexler visual scale, the amount of tumor growth in the lungs of 52 mice was determined by eye and independently by the automatic counter. There was no statistical difference between the two procedures.

When the mouse lungs were grouped according to the number of tumors computed by eye, there was no statistical difference between the two counting procedures in any of the groups. The standard deviation was independent of the number of tumors in the lungs. This caused the precision of the automatic counter to be poor in lungs with few tumors because the error was a greater percentage of the total. In lungs with a large number of tumors, which were difficult to count by eye, close agreement between the two methods of counting was demonstrated.

INTRODUCTION

An experimental method for evaluating pulmonary tumor growth in mice was described by Wexler (2). The technique includes an efficient method for excising, staining, and fixing tumor-bearing lungs. The procedure for evaluating tumor growth by counting and sizing tumor foci is easy if the number of tumor nodules is low, but it becomes tedious and eye straining if the number is high. Evaluation of 1 mouse heavily laden with tumors may require as much as 1 hr of counting. Since experimental trials involving 1 or 2 treatments may require the use of 48 to 72 mice, the counting process in these experiments is a limiting factor in the number of trials that can be performed.

We have adapted an automatic bacterial colony counter (Model 870; Artek Systems Corp., Farmingdale, N. Y.) so that this task might be performed more efficiently. Evaluation of the automatic counter included tests of its ability to discriminate tumor sizes, optical sensitivity, and counting reproducibility. Comparisons were made of tumor counts and task time between the automatic counter and the Wexler method of assessing pulmonary tumor growth by eye.

MATERIALS AND METHODS

Six- to 15-week-old inbred male C3H/HeJ mice were used. All mice were caged 4/box with Iso-caps (Lab Products, Inc., Garfield, N. J.). Wayne Lab Bbox laboratory animal diet (Allied Mills, Inc., Chicago, Ill.) and tap water were administered to mice ad libitum. Cages and bedding were changed twice a week.

Methylcholanthrene-induced sarcoma MC43 was maintained by serial s.c. transplantation in the thigh every 10 to 15 days. Tumors were excised in a sterile field by the method described by Vaage (1), yielding a viable single-cell suspension. A concentration of $5 \times 10^5$ viable tumor cells in 0.2 ml Roswell Park Memorial Institute Medium 1640 was injected into the lateral tail vein with a 25-gauge tuberculin syringe.

Mouse lungs were excised, stained, and fixed, and tumor foci were counted and sized by the method described by Wexler. The Wexler technique for staining involves the injection of dilute India ink into the lungs via the trachea, followed by fixation of lungs in Fekete's solution (100 ml of 70% ethanol, 10 ml of formalin, and 5 ml of glacial acetic acid). The tumor foci appear white against the black, normal lung tissue after this treatment (Fig. 1).

Quantification of tumor growth is accomplished by matching the size of each tumor focus on the lung surface to a graduated scale of circles, each with a numerical value. The sum of the matched values is the amount of tumor growth per lung.

The automatic bacterial colony counter, manufactured by Artek and available in most hospital laboratories, consists of a high-speed scanning television camera that detects and quantitates differences in absorbance and a television monitor that displays the field being observed (Fig. 2). The counting component superimposes a bright dot on the video display over each point counted. The difference in absorbance that the machine detects can be regulated. We found that a setting of 50 reliably identified lung tumors of the smallest size without erroneous detection of artifacts on the surface of the lungs or Petri dish.

Glare artifacts can be minimized by spraying the inside of the Petri dish with flat-black paint and by placing a sheet of

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flat-black paper below the dish, which increases the contrast between the white tumor foci and the black-stained lung tissue. The lobes of the lung are separated, removing as much of the bronchial stump as possible, and submerged in the painted Petri dish containing Fekete's solution (2) for counting. The lungs are submerged to minimize glare from their curved surfaces.

The automatic counter is equipped with a size discriminator that eliminates progressively larger foci from the counting circuit as its control knob is rotated clockwise. Size discriminator settings that we correlated with the Wexler visual scale for counting tumors by eye are listed in Table 1. These settings are determined by selecting several typical tumor foci of a given "Wexler" size and by finding the threshold at which the counter would not include them in the tally.

Counting reproducibility of the automatic counter was tested by counting the same group of 5 lobes 12 times in 1 position and 12 times in randomly rotated positions of the Petri dish in the counter. The accuracy of automatic counts compared to the accuracy of counts by eye was tested with the lungs from 52 mice. The results were analyzed with a paired t test.

**RESULTS AND DISCUSSION**

Counting reproducibility tests demonstrated that counts of the 5 lobes, which had an average tumor load of 334 on the Wexler scale, showed a standard error of less than 1.0% whether the Petri dish was in the same position or in randomly rotated positions. This difference was not of sufficient magnitude to affect the statistical analysis of an experiment.

We compared the accuracy of the automatic counter with that of counts performed by eye. Groups of 25 and 27 mouse lungs were counted by eye and then counted independently by the machine. A paired t statistic was computed for each group and for both groups combined. In each case there was no statistical difference in the 2 procedures. The standard deviation of the errors was the same in both groups. This is probably explained by the fact that the number of counts recorded by machine that resulted from bronchial stumps and other artifacts in the Petri dish was independent of the number of tumors. Consequently, the precision of the machine count was not as good in lungs with only a small number of tumors. Graphic representation of the data is given in Charts 1 and 2.

**Table 1**

<table>
<thead>
<tr>
<th>Wexler size</th>
<th>Size discriminator</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0–350</td>
</tr>
<tr>
<td>5</td>
<td>350–525</td>
</tr>
<tr>
<td>7</td>
<td>525–999</td>
</tr>
</tbody>
</table>

Counts from all 52 lungs, demonstrating the accuracy of the automatic counter over a wide range (multiple $r^2 = 0.96$). Chart 2 is a plot of the counts of the lungs with fewer than 75 tumors. In this range, the automatic counter was not as accurate (multiple $r^2 = 0.32$). The automatic counter required about 1 min for counting both sides of a lung. Counts by eye may take as little as 10 min for a lung with less than 50 tumor counts to 1 hr or more for lungs with at least 150 tumor counts.

Since the automatic counter is not as accurate for tumor counts in the low ranges, we recommend that lungs with fewer tumors be counted by eye. Lungs with more tumors,
which are more tedious and time consuming to tally by eye, can quickly be counted by the automatic counter with sufficient accuracy. The automatic counter seems to us to be a valuable time- and labor-saving device in this application.

REFERENCES

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