BIOSTATISTICS CASEBOOK, VOLUME THREE

By

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FLOW CHARACTERISTICS OF MICROSPHERES IN THE BLOOD VESSELS OF HAMSTERS

Charles Berry*, Byron Wm. Brown, Jr., and Jerry Halpern

Medical Problem: To determine whether microspheres injected into the blood stream distribute themselves symmetrically about the center line of the vessel (axial symmetry), and whether the microspheres tend to follow each other as they travel down the vessel (entrainment).

Medical Investigators: George Harell and Mark Taylor, Stanford University.

Statistical Procedures: Chi-squared test for a type of symmetry; chi-squared and Mantel-Haenszel tests for independence in a sequence.

1. Biomedical Problem

The use of intravascular injections of microspheres into experimental animals to measure the flow of blood to parts of the body has become a commonly used technique in recent years. A bolus of spheres is introduced into the blood stream and, after a short time, the animal is sacrificed. The spheres impact in the microvessels, and their relative frequency in a given volume of organ is taken to be a measure of the relative amount of blood delivered to that volume of organ. The relative frequency of the spheres is usually measured by labeling the spheres with a radioactive tag and comparing the radioactivity (in counts/time) with that of the injected bolus.

*At the University of California, San Diego.
The validity of this technique depends on the characteristics of the microsphere circulation. The investigators were curious about these characteristics and developed a technique for observing the spheres in the pouch of a live hamster as the spheres traveled through a vessel of the pouch shortly after introduction into the blood stream.

The investigators cinefilmed their observations on about 40 animals and then summarized their results with the purpose of asking two questions about the travel of the microspheres through the hamster cheek-pouch vessels. First, do the spheres seem to be symmetrically distributed across the vessel lumen or inside cross-section, or do they tend to flow along one side more than another? Symmetrical flow will be referred to as axial symmetry. Second, do the microspheres tend to follow one another down certain "channels" within the vessel, a hypothesis that will be called entrainment?

2. The Data

Forty-one studies were done, in which enough microspheres were observed to provide information on the two questions. The investigators divided the cinefilmed vessel into 8 channels ("top view") and recorded sequentially the channels (-4, -3, -2, -1, +1, +2, +3, +4) traveled by the individual microspheres, as they passed an observation point in the vessel. Then the investigators summarized these data in 8 x 8 tables (Tables 1-41 in the Appendix) showing the number of times a microsphere in a particular channel was followed by a microsphere in any of the eight channels. In the tables, row and column totals are provided.
For example, in Table 1, ten microspheres were observed to come past the observation point in channel +2, and among these ten instances, the microsphere immediately following was never in channels -4 or -3, but was in channel +2 twice and in channel +3 twice, channel +1 once, channel -1 four times and channel -2 once.

3. Statistical Analysis

1) Axial Symmetry

To test for axial symmetry, we used a chi-squared test for each of the 41 tables, and also pooled the results together, to obtain an overall test. For each of the tables we have:

$$E(f_i) = E(f_{-i}) = \frac{f_i + f_{-i}}{2}$$

where \( f_i \) = the observed frequency in the \( i \)th channel.

For Table 1, the data and calculations are as follows:

<table>
<thead>
<tr>
<th>Channel</th>
<th>Observed (O)</th>
<th>Expected (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>-3</td>
<td>7</td>
<td>5.5</td>
</tr>
<tr>
<td>-2</td>
<td>6</td>
<td>8.0</td>
</tr>
<tr>
<td>-1</td>
<td>11</td>
<td>10.0</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\[ N=52 \quad N=52 \]

\[ X^2 = \sum_{-4}^{4} \frac{(O-E)^2}{E} = \sum_{-4}^{4} \frac{0^2}{E} - N = 2.2182 \]

degrees of freedom = 4.
Note that this test uses the column totals in Table 1, thus omitting the first microsphere. This was done for simplicity, although comparison of row and column totals in the tables would usually reveal the channel of the first microsphere also and this could have been added in. In Table 1 it can be seen that the first microsphere must have been observed in channel -2.

The data for Table 1 are quite consistent with the hypothesis of axial symmetry. The results for all 41 tables are given in the following tabulation.

<table>
<thead>
<tr>
<th>Table</th>
<th>$\chi^2$</th>
<th>Table</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.22</td>
<td>21</td>
<td>6.70</td>
</tr>
<tr>
<td>2</td>
<td>7.66</td>
<td>22</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>9.19</td>
<td>23</td>
<td>9.21</td>
</tr>
<tr>
<td>4</td>
<td>2.73</td>
<td>24</td>
<td>0.96</td>
</tr>
<tr>
<td>5</td>
<td>5.01</td>
<td>25</td>
<td>4.97</td>
</tr>
<tr>
<td>6</td>
<td>8.55</td>
<td>26</td>
<td>9.18</td>
</tr>
<tr>
<td>7</td>
<td>5.98</td>
<td>27</td>
<td>10.33</td>
</tr>
<tr>
<td>8</td>
<td>4.42</td>
<td>28</td>
<td>5.77</td>
</tr>
<tr>
<td>9</td>
<td>7.22</td>
<td>29</td>
<td>5.68</td>
</tr>
<tr>
<td>10</td>
<td>5.57</td>
<td>30</td>
<td>11.34</td>
</tr>
<tr>
<td>11</td>
<td>9.57</td>
<td>31</td>
<td>7.36</td>
</tr>
<tr>
<td>12</td>
<td>20.11</td>
<td>32</td>
<td>2.68</td>
</tr>
<tr>
<td>13</td>
<td>5.59</td>
<td>33</td>
<td>9.20</td>
</tr>
<tr>
<td>14</td>
<td>1.98</td>
<td>34</td>
<td>7.76</td>
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<tr>
<td>15</td>
<td>1.89</td>
<td>35</td>
<td>6.20</td>
</tr>
<tr>
<td>16</td>
<td>8.35</td>
<td>36</td>
<td>5.70</td>
</tr>
<tr>
<td>17</td>
<td>0.67</td>
<td>37</td>
<td>2.13</td>
</tr>
<tr>
<td>18</td>
<td>0.44</td>
<td>38</td>
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</tr>
<tr>
<td>20</td>
<td>5.19</td>
<td>40</td>
<td>12.28</td>
</tr>
</tbody>
</table>

41
The probabilities that a \( \chi^2 \) distributed random variable with four degrees of freedom will exceed 8, 10, and 15 are 9.2%, 4.0%, and 0.5% respectively. Since 12 of the 41 \( \chi^2 \) values exceed 8, 5 of them exceed 10, and one exceeds 15, it seems clear that, overall, the data are not consistent with the hypothesis of axial symmetry for all vessels. Furthermore, the sum of the \( \chi^2 \) values is 255.68, highly significant for \( 41 \times 4 = 164 \) degrees of freedom (\( P < .001 \)).

Some questions might be raised concerning the preceding analysis. First, under the hypothesis of independence and axial symmetry, it is clear that the asymptotic distribution of the test statistic is chi-squared with four degrees of freedom. This follows from the fact that the data are a sample from a multinomial distribution with eight cells (7 d.f.) in which three independent parameters, \( \theta_1, \theta_2, \theta_3 \) are estimated in the eight multinomial parameters, \( \pi_i \), as follows:

\[
\pi_{+i} = \frac{1}{2} \theta_i, \; i = 1, 2, 3 \; \text{and} \; \pi_{+4} = \frac{1}{2}(1-\theta_1-\theta_2-\theta_3).
\]

However, when the estimated expected values are small, this asymptotic approximation may not be very good. For example, in Table 1, two of the expectations are less than 5, the limit usually quoted for a good approximation. For non-significant results this is not a problem, but for significant results the \( \chi^2 \) value might yield an underestimate of the exact probability value for the test. To check this, tables with \( P \) values below 10% were checked by grouping channels with small expected values. The results were not altered enough to matter. For example, among the 12 tables with \( \chi^2_4 \) values greater than 8 (i.e., \( P \) values
less than 9.2%), all but one of them had grouped $\chi^2$ values with $P$ less than 10%. The exception, Table 23, had a $P$ value of 11.9%.

Another concern is the chi-squared approach itself. The channel observations are taken to be statistically independent for the series of microspheres observed in a given vessel. This implies that the microspheres do not entrain. Therefore, we should withhold judgement on the $\chi^2$ tests for axial symmetry, pending a test of the hypothesis of entrainment.

2) Entrainment

A simple test for entrainment compares the frequencies on the diagonal, i.e., the number of times one sphere "follows" another in the same channel, with frequencies predicted from the marginal totals in the eight channels. A simple approach to the problem is as follows. For the $n_i$ microspheres observed as first members of pairs in the $i$th channel (i.e., $n_i$ a row total), the probability that the second or following microsphere is in the same channel is $m_i$, the corresponding column total, divided by $N$, the total number of microspheres. Therefore, if $f_{ii}$ is the observed diagonal element for the $i$th channel, we have

$$E(f_{ii}) = \frac{n_i m_i}{N},$$

$$\text{Var}(f_{ii}) = n_i \left( \frac{m_i}{N} \right) \left( \frac{N-m_i}{N} \right).$$

The test statistic would be
\[
T = \frac{\sum_{-4}^{4} f_{11} - \sum_{-4}^{4} E(f_{11})}{\sqrt{\sum_{-4}^{4} \text{Var}(f_{11})}}.
\]

For Table 24, for example, the results are as follows:

<table>
<thead>
<tr>
<th>Channel</th>
<th>Observed</th>
<th>Expected</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4</td>
<td>9</td>
<td>2.56</td>
<td>2.2509</td>
</tr>
<tr>
<td>-3</td>
<td>5</td>
<td>2.69</td>
<td>2.3425</td>
</tr>
<tr>
<td>-2</td>
<td>10</td>
<td>3.35</td>
<td>3.0763</td>
</tr>
<tr>
<td>-1</td>
<td>10</td>
<td>3.08</td>
<td>2.6643</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>3.08</td>
<td>2.6643</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1.88</td>
<td>1.6866</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2.81</td>
<td>2.4540</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>2.21</td>
<td>1.9396</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>21.66</td>
<td>19.0785</td>
</tr>
</tbody>
</table>

\[
T = \frac{64 - 21.66}{\sqrt{19.0785}} = 9.6935
\]

\[
P = < .0001 \quad \text{(from the normal table)}
\]

This analysis, for Table 24, suggests very strong evidence against the null hypothesis, and in favor of entrainment.

Before examining the results from the other tables, however, the approximate analysis used here needs justification. Another approach is the following conditional analysis. Table 24A (following Table 41 in the Appendix) shows the actual sequence of 173 channels observed. These are the basic data for Table 24. Denote these channels as \( C_i \), \( i = 1, 2, \ldots, K \). Then construct the following set of binary observations:
\[ Z_i = \begin{cases} 
1 & \text{if } C_i = C_{i-1} \\
0 & \text{if } C_i \neq C_{i-1} 
\end{cases}, \]

for \( i = 1, 2, \ldots, N = K-1 \). Thus, the \( Z_i \) will tend to be ones if there is entrainment. One can calculate the expectation and variance for \( Z_i \), conditional on all preceding \( C_j, j = 1, 2, \ldots, i-1 \), and assuming random permutation of the spheres to follow. For example, in Table 24A, \( C_{15} = -2 \) and \( C_{16} = -2 \), so \( Z_{16} = 1 \). There remain \( 173 - 16 = 157 \) microspheres to be observed, and a count reveals that \( 21 \) of them were observed in channel \(-2\). Under the null hypothesis of no entrainment, the probability that \( Z_{17} \) will be one is then \( 21/157 \), and its variance is \( (21/157)(1 - 21/157) \). Using this conditional, exact method, one obtains the test statistic

\[ T = \frac{\sum_{i=1}^{N} Z_i - \sum_{i=1}^{N} E(Z_i)}{\sqrt{\sum_{i=1}^{N} \text{Var}(Z_i)}}. \]

This is a Mantel-Haenszel test statistic and will be asymptotically standard normal.

When this method is applied to Table 24A, the result is:

\[ \Sigma Z_i = 64 \quad \text{(as before)}, \]

\[ \Sigma E(Z_i) = 20.92, \]

\[ \Sigma \text{Var}(Z_i) = 17.9390. \]

Thus, the results for expectation and variance of the number of
"successes" (i.e., number on the diagonal of Table 24) under the null hypothesis are very nearly the same as obtained under the less conditional, approximate binomial approach obtained above.

As a further check on the unconditional binomial approach, Table 34 was analyzed both ways. Table 34A presents the data in detailed form. The following results were obtained.

<table>
<thead>
<tr>
<th></th>
<th>Binomial</th>
<th>Conditional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Successes</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Expectation</td>
<td>30.33</td>
<td>29.37</td>
</tr>
<tr>
<td>Variance</td>
<td>25.12</td>
<td>24.16</td>
</tr>
<tr>
<td>Test Statistic</td>
<td>0.53</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Again the results are very close. Because the data were not readily available in the detailed form of Tables 24A and 34A, and the conditional approach is tedious to carry out, the simpler method was used.

Note that the analysis showed the data in Table 34 to be quite consistent with the null hypothesis, i.e., no entrainment, whereas the data in Table 24A support the hypothesis of entrainment. Following is a tabulation of the results for the 41 tables of data.
<table>
<thead>
<tr>
<th>Table Number</th>
<th>Test Statistic</th>
<th>Table Number</th>
<th>Test Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.57</td>
<td>21</td>
<td>-0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.43</td>
<td>22</td>
<td>-0.96</td>
</tr>
<tr>
<td>3*</td>
<td>0.24</td>
<td>23*</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>0.34</td>
<td>24</td>
<td>9.74</td>
</tr>
<tr>
<td>5</td>
<td>1.17</td>
<td>25</td>
<td>-0.06</td>
</tr>
<tr>
<td>6*</td>
<td>0.74</td>
<td>26*</td>
<td>-0.71</td>
</tr>
<tr>
<td>7</td>
<td>0.10</td>
<td>27*</td>
<td>-1.34</td>
</tr>
<tr>
<td>8</td>
<td>0.74</td>
<td>28</td>
<td>-0.11</td>
</tr>
<tr>
<td>9</td>
<td>-1.32</td>
<td>29</td>
<td>-1.13</td>
</tr>
<tr>
<td>10</td>
<td>-0.40</td>
<td>30*</td>
<td>-0.71</td>
</tr>
<tr>
<td>11*</td>
<td>-0.26</td>
<td>31</td>
<td>-0.57</td>
</tr>
<tr>
<td>12*</td>
<td>-2.36</td>
<td>32</td>
<td>-1.03</td>
</tr>
<tr>
<td>13</td>
<td>0.81</td>
<td>33*</td>
<td>0.85</td>
</tr>
<tr>
<td>14</td>
<td>0.71</td>
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<td>0.53</td>
</tr>
<tr>
<td>15</td>
<td>-0.92</td>
<td>35</td>
<td>0.62</td>
</tr>
<tr>
<td>16*</td>
<td>0.21</td>
<td>36</td>
<td>0.20</td>
</tr>
<tr>
<td>17</td>
<td>-0.24</td>
<td>37</td>
<td>0.10</td>
</tr>
<tr>
<td>18</td>
<td>-0.26</td>
<td>38*</td>
<td>0.48</td>
</tr>
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<td>19</td>
<td>-0.43</td>
<td>39</td>
<td>-1.35</td>
</tr>
<tr>
<td>20</td>
<td>-3.12</td>
<td>40*</td>
<td>-0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41</td>
<td>3.00</td>
</tr>
</tbody>
</table>

*Denotes a table for which the test for axial symmetry was rejected at the 9.2% level in the previous section.

The results of these tests for entrainment are puzzling. Note that the signs are about evenly split between positives and negatives, suggesting that there is no entrainment. However, two tables show strong evidence of entrainment, namely, Tables 24 (P<.0001) and 41 (p<.003). Most puzzling, though, are the results in the negative direction. There
are six tables with relatively low $P$ values and a deficiency of microspheres following one on the other, namely, Table 1 ($P = 5.8\%$), Table 9 ($P = 9.3\%$), Table 12 ($P = 0.9\%$), Table 20 ($P = 0.08\%$), Table 27 ($P = 9.0\%$), and Table 39 ($P = 8.9\%$). These data suggest that, at least in some studies, the microspheres act as though they repel each other, an unexpected finding. If true, this may be due to some characteristic of the microspheres themselves (e.g., a charge they carry, though this has not been found) or it may be due to transient qualities of the vessel or vascular physiology.

These heterogeneous results do not seem to alter the previous rejection of the hypothesis of axial symmetry, however. Note in the preceding tabulation that in 12 of the 13 studies where the hypothesis was rejected (tables with asterisks) the data seemed consistent with the hypothesis of independence and would seem to allow a chi-squared test for axial symmetry that presupposes independence.

4. Conclusion

It seems clear that there is an asymmetry in the flow of microspheres in the pouch vessels of the hamster, though this may vary from one vessel to another and does not seem to be strong. It is also clear that the paths of successive spheres are not independent. However, the results for various vessels suggest that the spheres can exhibit either entrainment or some sort of "negative entrainment". Further explanation and investigation are needed.
Appendix

Tables 1-41: Frequencies with which a microsphere appears in one channel (indicated by rows 1-8 corresponding to channels -4, -3, ..., +4, respectively) followed by a microsphere in channel -4, -3, ..., or +4 (indicated by columns 1, 2, ..., 8, respectively).

Table 1

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>2</th>
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</table>

| 9 7 4 11 10 8 1 1 51 |

**Table 24A: Sequential Channels of Micro-spheres in Vessel #8**

```
start-->4  4  3  3  2  1  -1 -2 -3  4  3  2  1  1 -2 -2
           -2 -3 -4 -4 -3  3  2  2  1  1 -1 -2 -2 -3 -4  4
           3  2  2  1  1 -1 -1 -3 -3 -3 -4 -4  2  2  1 -4
           -4 -3 -3 -1  1  2  4  4  4  3  3  1  1 -1 -1 -3
           -3 -4 -3  4  4  3  1 -1 -1 -1 -2 -2  4 -3 -4 -4
           -2 -4 -3 -2  2  4  4  4  4  3  1 -2 -1 -1 -2
           -3  1 -2  4  3  2  1  1 -1 -1 -2 -4  4  3  3  2
           1 -1 -1 -2 -2 -2  3 -3 -3 -4 -4 -4  4  4  3  3
           3  2  2  1  1 -1 -2 -2 -3 -4 -4 -4  3  3  1  2
           1 -1 -1 -2 -2 -2 -4  4  3  3  3 -1 -1 -3  2
           -3  2  2  1  1 -1 -1 -2 -2 -3 -4 -4 -3 --> end
```
Table 34A: Sequential Channels of Micro-spheres in Vessel #29

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<td>1 -1 -2 2 -1 -4 -1 -2 --&gt; end</td>
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</tbody>
</table>
PERCEPTION OF SHORT DURATION SOUNDS

Ray Faith

Medical Problem: Finding the relationship between the perceived loudness of a signal and its time duration under various experimental conditions.

Medical Investigator: Joyce Marvin, Columbia University.

Statistical Procedures: Two-phase regression; bootstrap hypothesis testing.

1. Background

The human ear does not instantaneously perceive the loudness of a constant sound presented to it. Over a short time interval after a sound begins its perceived loudness is a time integral of the actual loudness, and thus is proportional to the sound's duration. After a critical time duration, however, the perceived loudness levels off to a constant value. This phenomenon, which has a counterpart in visual perception, has been observed and studied by several investigators. One goal of these studies is to develop diagnostic tools for finding the location of pathological conditions in the inner ear by observing the differential response of the ear to duration of sound for different types of sound, since different parts of the auditory nerve respond to different frequencies.

Most past studies have dealt with sound perception at threshold levels, where the sound is barely audible. The few studies conducted at louder sound levels have used differing experimental methodologies and have come to contradictory conclusions concerning the relationship
between time duration and perceived loudness in normal humans at various sound levels and frequencies. Joyce Marvin, a doctoral candidate at Columbia University, undertook as her dissertation project a comprehensive experiment to use a uniform methodology to measure the response function at threshold and at three loudness levels, for two pure frequencies as well as a white noise signal.

In each experiment the subject is presented with an experimental signal consisting of a series of short bursts of sound separated from each other by one-half seconds of silence. He is told to adjust the volume of this signal, using a volume control device, until it matches a given target loudness. In the threshold experiment the target value is the specification that the sound be "barely audible". In the supra-threshold experiments the target is presented to him as a series of one-half second bursts of sound of a specified level and of the same type as the experimental signal. This target signal is presented immediately prior to his adjustment of the experimental signal. (It has been established that the critical duration is about one-fifth second, so a one-half second burst is comparable to a constant target sound.) In each case the response variable is the actual volume of the experimental signal after the subject's adjustment, as measured electrically.

For a given target signal the response will be large for short durations, to make \( \text{response} \times \text{duration} \) match the target energy, and smaller for longer durations, until the critical duration, at which point the target and response signals should coincide. Mathematically, the expected theoretical relation is

\[
\text{response} \times \text{duration} = \text{constant} \quad \text{for short durations}
\]
and

response = constant for long durations.

Taking logarithms and measuring the response on a logarithmic scale (in decibels) implies that the response should be a two part linear function of log duration.

Joyce Marvin carried out the experiment and subsequently asked me to examine the data for her in the light of the above considerations. The following sections form part of the report I prepared to assist her in writing the analysis section of her thesis.

2. The Data

There are four completely crossed predictor variables:

Subject - 3 Levels ,
Level - 4 Levels (threshold, 20db, 50db, 80db) ,
Hertz - 3 Levels, (two pure frequencies and white noise) ,
Duration - 9 Levels .

Note: The predictors are not completely crossed in that the (white noise, 20 db level) combination is actually delivered at 30 db. This may have affected the tests of (Hz x level) interactions, but was ignored in the analysis.

At each combination of the above there are 6 replications for a total of $32^2 \times 6 = 1944$ observations. There is one response variable, which is measured on the same scale as the level variable.

Figures 1a,b display the data for two different combinations of level and Hertz.
Figure 1a. Response in decibels vs. logarithm of duration for a target signal of 80 db at 1000 Hz. Displayed numbers indicate the multiplicities of points.

Figure 1b. Response in decibels vs. logarithm of duration for a threshold target signal at 1000 Hz. Displayed numbers indicate the multiplicities of points.
3. Models

There are several models of interest. They are all generalizations of the analysis of covariance model, where subject, level, and Hertz are regarded as discrete categorical factors while duration is treated as a continuous scale predictor. Primary interest is on the functional relationship of response to the logarithm of duration, with a view to comparing a linear relationship to a continuous two-part linear relationship.

The linear model can be expressed, for a given combination of the other categorical predictors as

\[ y = \alpha + \beta \ln(d) + e, \]

where \( y \) is a response at duration \( d \) with random error \( e \).

The two-part linear model can, similarly, be expressed for a given combination of the other categorical predictors as

\[ y = \begin{cases} 
\alpha + \beta_1(\ln d - \ln c) + e & \text{if } d \leq c, \\
\alpha + \beta_0(\ln d - \ln c) + e & \text{if } d > c.
\end{cases} \]

This function is linear for \( d \leq c \) as well as for \( d > c \) and is continuous at \( d = c \), equalling \( \alpha \) at this point.

We wish to compare the linear model to the two-part linear model, as well as to compare the two-part linear model, with \( \beta_0 = 0 \) to the two-part linear model with \( \beta_0 \) free. We also wish to determine how \( c \), if it is significant, depends on the other categorical factors.

4. Methods

A unifying element in the analysis is the use of the overall sum of squared errors both for the estimation of parameters and for the comparison of models. The use of this criterion is not called for in
some situations, namely, those where there are occasional wild responses. However, after looking at the residuals from the fitted models here, I would say that they appear very well behaved, and there does not seem to be any reason to fear wild values or even a thick tailed error distribution. The use of least squares looks very suitable here.

To use the sum of squared errors means to choose, for any given model with parameters \( \hat{\beta} \), that value \( \hat{\beta} \) which minimizes

\[
SS_e(\hat{\beta}) = \sum_{i=1}^{1944} (y_i - \mu(\hat{\beta}, \mathbf{x}_i))^2
\]

where \( \mathbf{x}_i \) is the 4-tuple expressing the values of the 4 predictors for the ith observation, \( \mu(\hat{\beta}, \mathbf{x}_i) \) is the predicted expected response for this set of predictors when \( \hat{\beta} \) is the set of parameters, and \( y_i \) is the actual observed response. Note that there are at least 6 different values of \( y_i \) for each value of \( \mu(\cdot) \).

These extra values of \( y \) are used to estimate the error variance and thereby to evaluate the relative improvement in fit between two different models. In going from a given model \( H_0 \) to another more complex one \( H_1 \), which includes the first as a special case, it, of necessity, happens that the best fitting \( SS_e \) either decreases or stays the same. Hence the difference between the models in \( SS_e \), namely, \( SS_{e_0} - SS_{e_1} \), is a nonnegative number. This difference is larger, the greater is the true error variance, and larger, the greater is the number of additional parameters fit in the more complex model. A common estimate of the error variance is provided by the residual \( SS_{e_1} \) for
the more complex model. In the standard analysis of variance, which applies when the expected mean vector \( \mu( ) \) under the simpler model lies in a linear subspace of the possible mean vectors under the more complex model, and assumes that the errors are independent and normally distributed with common mean 0 and common variance \( \sigma^2 \), leads naturally to the use of the F-statistic

\[
F = \frac{SS_{e_0} - SS_{e_1}}{SS_{e_1}} \cdot \frac{df_1}{df_1 - df_0},
\]

which under \( H_0 \) has an F distribution with \( df_1 - df_0 \) degrees of freedom in the numerator and \( df_1 \) degrees of freedom in the denominator.

Here \( df_0 = \# \text{ obs} - \# \text{ parameters in the simpler model } H_0 \) and \( df_1 = \# \text{ obs} - \# \text{ parameters in the more complex model } H_1 \). Hence \( df_1 - df_0 = \# \text{ of additional parameters fit in model } H_1 \).

Unfortunately, the F-statistic is not appropriate in the present case in the sense that the models considered are not in the form of nested linear subspaces of each other, but rather nested non-linear subsets. This means that we cannot be certain if the tabled values of the F distribution give the actual distribution of the test statistic when the null hypothesis is true. There is good reason to expect that the results will be approximately correct (see Feder, 1975a,b), but we can expect there to be some discrepancy.

In order to overcome this problem I have employed a method called the bootstrap. This was developed by Brad Efron (1979). It can be applied to estimating the accuracy of parameter estimators, but the use it was put to here was the calculation of the true distribution of the F-statistic under the null hypothesis. The method works as follows:
1. The two models, $H_0$ and $H_1$, are both fit to the data using least squares. The parameter estimates $\hat{\beta}_0$ and $\hat{\beta}_1$ for the two models, respectively, are obtained, as well as the sums of squared errors $SS_{e_0}$ and $SS_{e_1}$ and the $F$-statistic.

2. Using the fitted values $\hat{\beta}_0$ of the parameters for model $H_0$, the $1944$ residuals are obtained

$$\hat{e}_i = y_i - \mu(\hat{\beta}_0, x_i).$$

3. For each combination of subject, level, and Hertz there are $54$ residuals $\hat{e}_i$ (6 replications for each of the 9 durations). These $54$ residuals are treated as the population of errors for the given combination of subject, level, and Hertz. A random sample of $54$ values $e_i^*$ is selected with replacement from this population, using a pseudo-random number generator to specify the indices. This is repeated for every combination of subject, level, and Hertz, so that a new residual $e_i^*$ is created for each of the $1944$ observations.

4. A pseudo-data set is constructed

$$y_i^* = \mu(\hat{\beta}_0, x_i) + e_i^*, \quad i = 1, \ldots, 1944.$$

5. Step 1 is repeated using the pseudo-data; i.e., we generate

$$\hat{\beta}_0^*, \hat{\beta}_1^*, SS_{e_0}^*, SS_{e_1}^*, \text{ and } F^*.$$

By construction, the pseudo-data $y_i^*$ is an observation of the response when the model $H_0$ is valid. Hence the value $F^*$ is an observation of $F$ under the null hypothesis $H_0$. 

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6. Steps 3-5 are repeated as often as is feasible, always using the original $\hat{e}_i$'s to generate the $e_i^*$'s. When enough values of $F^*$ are generated, the empirical distribution of $F^*$ is a close approximation to the distribution of $F$ under the null hypothesis. We compare $F$ with this.

**Note:** The reason in step 3 for sampling errors within each subject, level, and Hertz combination is in order to eliminate any affect that differing error distributions in the different subject, level, Hertz combinations might have on the analysis. I did, for comparison, one run in which all of the errors were lumped together for sampling, and obtained precisely the same distribution of $F^*$.

Any pair of nested models may be evaluated by bootstrapping. A disadvantage is that one is not able to do a single bootstrap of a whole sequence of nested models. In the usual analysis of variance setup several $F$ statistics are obtained, all using the same denominator mean squared error. Here we must do a separate bootstrap for every pair of models to be compared, since the residuals used are determined by the model used as the null hypothesis.

**The models considered:**

In the following, subscript $s$ denotes subject, $l$ level, $h$ Hertz, $d$ duration, and $i$ replication. $E(y)$ means the expected value of $y$.

I. Linear regression (no cutoff).

$$E(y_{lsdi}) = \alpha_{lhs} + \beta_{lhs} \ln(d).$$
II. Two part linear models.

a) Single cutoff.

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{lhs}} + \beta_{\text{lhs}}^{1}(\ln d - \ln c), & d \leq c, \\
\alpha_{\text{lhs}} + \beta_{\text{lhs}}^{0}(\ln d - \ln c), & d > c.
\end{cases} \]

b) Cutoff depending on level.

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{lhs}} + \beta_{\text{lhs}}^{1}(\ln d - \ln c_{\ell}), & d \leq c_{\ell}, \\
\alpha_{\text{lhs}} + \beta_{\text{lhs}}^{0}(\ln d - \ln c_{\ell}), & d > c_{\ell}.
\end{cases} \]

c) Cutoff depending on Hertz.

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{ lhs}} + \beta_{\text{lhs}}^{1}(\ln d - \ln c_{h}), & d \leq c_{h}, \\
\alpha_{\text{ lhs}} + \beta_{\text{lhs}}^{0}(\ln d - \ln c_{h}), & d > c_{h}.
\end{cases} \]

d) Cutoff depending additively on level and Hertz.

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{ lhs}} + \beta_{\text{lhs}}^{1}(\ln d - \ln c_{\ell+h}), & d \leq c_{\ell+h}, \\
\alpha_{\text{ lhs}} + \beta_{\text{lhs}}^{0}(\ln d - \ln c_{\ell+h}), & d > c_{\ell+h}.
\end{cases} \]

\[ \ln c_{\ell+h} = \ln c_{0}^{\ell} + \ln c_{1}^{\ell} + \ln c_{2}^{h}, \quad \Sigma_{\ell} \ln c_{1}^{\ell} = 0 = \Sigma_{h} \ln c_{2}^{h}. \]

e) Cutoff depending on level and Hertz combination.

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{ lhs}} + \beta_{\text{lhs}}^{1}(\ln d - \ln c_{\ell h}), & d \leq c_{\ell h}, \\
\alpha_{\text{ lhs}} + \beta_{\text{lhs}}^{0}(\ln d - \ln c_{\ell h}), & d > c_{\ell h}.
\end{cases} \]
f) Cutoff depending on subject.

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{lhs}} + \beta^{1}_{\text{lhs}} (\ln d - \ln c_s) , & d \leq c_s , \\
\alpha_{\text{lhs}} + \beta^{0}_{\text{lhs}} (\ln d - \ln c_s) , & d > c_s . 
\end{cases} \]

\[ g) \text{ Cutoff depending on level, Hertz, and subject.} \]

\[ E(y_{\text{lhsdi}}) = \begin{cases} 
\alpha_{\text{lhs}} + \beta^{1}_{\text{lhs}} (\ln d - \ln c_s) , & d \leq c_{\text{lhs}} , \\
\alpha_{\text{lhs}} + \beta^{0}_{\text{lhs}} (\ln d - \ln c_{\text{lhs}}) , & d > c_{\text{lhs}} . 
\end{cases} \]

III(a)-(g): The same as models II(a)-(g) except that \( \beta^{0}_{\text{lhs}} \) is constrained to equal zero.

Method of fitting the models:

Linear regression is standard and requires no explanation. The two-part functions are fit by searching on the cutoff.

For any given value of \( c \), the model

\[ (1) \quad E(y_i) = \alpha + \begin{cases} 
\beta_1 (\ln x_i - \ln c) , & x \leq c , \\
\beta_0 (\ln x_i - \ln c) , & x > c , 
\end{cases} \]

is equivalent to

\[ (2) \quad E(y_i) = \alpha + \beta_1 u_i + \beta_0 v_i , \]

where

\[ u_i = \begin{cases} 
(\ln x_i - \ln c) & \text{if } x_i \leq c , \\
0 & \text{if } x_i > c , 
\end{cases} \]

and

\[ v_i = \begin{cases} 
0 & \text{if } x_i \leq c , \\
(\ln x_i - \ln c) & \text{if } x_i > c . 
\end{cases} \]
This model (2) is in the form of a linear regression with two known predictor variables \((u_i\) and \(v_i\)) and is easily solved. With the exception of models IIId and IIId, \(c\) can be found by a straightforward one-dimensional search. For models IIId and IIId it is necessary to search in two directions (level and hertz). This is done iteratively. For models II the search was restricted to values of \(c\) between 50 ms and 300 ms. For models III it was from 10 ms to 500 ms.

**Nesting of the models:**

The models nest as follows:

I  Lin Reg

\[\downarrow\]

IIa

\[\downarrow\]

IIc \(c_h\)

\[\downarrow\]

IID \(c_{\lambda+h}\)

\[\downarrow\]

IId \(c_{\lambda+h}\)

\[\downarrow\]

IIe \(c_{\lambda h}\)

\[\downarrow\]

IIf \(c_s\)

\[\downarrow\]

IIb \(c_{\lambda}\)

The arrows run in each case from the simpler model to the more complex one.
Note: IIg is nested in IIf. The arrow is missing because I did not apply bootstrapping to this pair. All the arrowed pairs were bootstrapped.

A second figure, identical to the preceding one, but with III replacing II throughout, represents the relations and tests obtained for the models with $\beta_0 = 0$. Each such model is a special case of the corresponding II model so a comparison can be made between them. I did not bootstrap these comparisons, however, except for the comparison of models IIg and IIIg, that is, the models for which $c$ is allowed to vary freely with respect to the combination of level, hertz, and subject.

5. Results

Table 1. Sums of squared errors

<table>
<thead>
<tr>
<th>Model</th>
<th>$SS_e$</th>
<th>Degrees of freedom for error</th>
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<tbody>
<tr>
<td>I</td>
<td>Lin. Reg.</td>
<td>1988.875</td>
</tr>
<tr>
<td>IIa</td>
<td>$c(\beta_0$ free)</td>
<td>1300.375</td>
</tr>
<tr>
<td>IIb</td>
<td>$c_h(\beta_0$ free)</td>
<td>1288.061</td>
</tr>
<tr>
<td>IIc</td>
<td>$c_h(\beta_0$ free)</td>
<td>1294.040</td>
</tr>
<tr>
<td>IIId</td>
<td>$c_{\lambda+h}(\beta_0$ free)</td>
<td>1280.412</td>
</tr>
<tr>
<td>IIe</td>
<td>$c_{\lambda h}(\beta_0$ free)</td>
<td>1272.013</td>
</tr>
<tr>
<td>IIIf</td>
<td>$c_s(\beta_0$ free)</td>
<td>1299.949</td>
</tr>
<tr>
<td>IIg</td>
<td>$c_{\lambda hs}(\beta_0$ free)</td>
<td>1228.072</td>
</tr>
<tr>
<td>IIIa</td>
<td>$c(\beta_0 = 0)$</td>
<td>1367.400</td>
</tr>
<tr>
<td>IIIb</td>
<td>$c_{\lambda}(\beta_0 = 0)$</td>
<td>1357.495</td>
</tr>
<tr>
<td>IIIc</td>
<td>$c_h(\beta_0 = 0)$</td>
<td>1347.177</td>
</tr>
<tr>
<td>IIIId</td>
<td>$c_{\lambda+h}(\beta_0 = 0)$</td>
<td>1337.356</td>
</tr>
<tr>
<td>IIIe</td>
<td>$c_{\lambda h}(\beta_0 = 0)$</td>
<td>1315.692</td>
</tr>
<tr>
<td>IIIIf</td>
<td>$c_s(\beta_0 = 0)$</td>
<td>1366.327</td>
</tr>
<tr>
<td>IIIIf</td>
<td>$c_{\lambda hs}(\beta_0 = 0)$</td>
<td>1268.533</td>
</tr>
</tbody>
</table>
Note that there is a large drop in error when going from linear regression to IIIa (a single constant with $\beta_0 = 0$) in spite of the fact that only one parameter is added to the model. The other comparisons are less dramatic.

ANOVA tables (as though $F$ distribution were correct):

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff IIA-Lin. Reg.</td>
<td>688.500</td>
<td>37</td>
<td>18.608</td>
<td>27.274</td>
<td>8.91x10^{-8}</td>
</tr>
<tr>
<td>$c_h$ alone IIC-IIa</td>
<td>12.315</td>
<td>3</td>
<td>4.105</td>
<td>6.017</td>
<td>4.47x10^{-4}</td>
</tr>
<tr>
<td>$c_h$ alone IIIC-IIa</td>
<td>6.335</td>
<td>2</td>
<td>3.1675</td>
<td>4.643</td>
<td>9.74x10^{-3}</td>
</tr>
<tr>
<td>$c_s$ alone IIF-IIa</td>
<td>.426</td>
<td>2</td>
<td>.213</td>
<td>.312</td>
<td>.732</td>
</tr>
<tr>
<td>$c_h$ given $c_h$ (add) IId-IIC</td>
<td>13.628</td>
<td>3</td>
<td>4.543</td>
<td>6.658</td>
<td>1.81x10^{-4}</td>
</tr>
<tr>
<td>$c_h$ given $c_h$ (add) IId-IIf</td>
<td>7.649</td>
<td>2</td>
<td>3.8245</td>
<td>5.606</td>
<td>3.74x10^{-3}</td>
</tr>
<tr>
<td>Interaction I*H IIE-IId</td>
<td>8.399</td>
<td>6</td>
<td>1.3998</td>
<td>2.052</td>
<td>.0559</td>
</tr>
<tr>
<td>Interaction I*H*S IIg-IIf</td>
<td>43.941</td>
<td>24</td>
<td>1.8309</td>
<td>2.684</td>
<td>1.90x10^{-5}</td>
</tr>
<tr>
<td>Error</td>
<td>1228.072</td>
<td>1800</td>
<td>.68226</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1988.875</td>
<td>1872</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The p-value is the likelihood, evaluated under the null hypothesis, that the effect would have been as great or greater by chance alone than what was observed. A value less than .01 indicates that the test rejects the simpler model at the 1% level, for example.
Table 3. Table for $\beta_0 = 0$

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff IIIa-Lin. Reg.</td>
<td>621.475</td>
<td>1</td>
<td>621.475</td>
<td>899.486</td>
<td>$2.35 \times 10^{-8}$</td>
</tr>
<tr>
<td>$c_l$ alone IIIb-IIIia</td>
<td>9.905</td>
<td>3</td>
<td>3.301</td>
<td>4.779</td>
<td>$2.54 \times 10^{-3}$</td>
</tr>
<tr>
<td>$c_h$ alone IIIc-IIIa</td>
<td>20.223</td>
<td>2</td>
<td>10.111</td>
<td>14.635</td>
<td>$4.95 \times 10^{-7}$</td>
</tr>
<tr>
<td>$c_s$ alone IIIf-IIIa</td>
<td>1.073</td>
<td>2</td>
<td>.5365</td>
<td>.7765</td>
<td>.4601</td>
</tr>
<tr>
<td>$c_l$ given $c_h$ IIId-IIIc</td>
<td>9.821</td>
<td>3</td>
<td>3.274</td>
<td>4.738</td>
<td>$2.69 \times 10^{-3}$</td>
</tr>
<tr>
<td>$c_h$ given $c_l$ IIId-IIIb</td>
<td>20.139</td>
<td>2</td>
<td>10.0695</td>
<td>14.574</td>
<td>$5.25 \times 10^{-7}$</td>
</tr>
<tr>
<td>Interaction LXH</td>
<td>21.664</td>
<td>6</td>
<td>3.611</td>
<td>5.226</td>
<td>$2.40 \times 10^{-5}$</td>
</tr>
<tr>
<td>Interaction LXHXS</td>
<td>47.159</td>
<td>24</td>
<td>1.965</td>
<td>2.844</td>
<td>$5.34 \times 10^{-6}$</td>
</tr>
<tr>
<td>Error</td>
<td>1268.533</td>
<td>1836</td>
<td>.69092</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1988.875</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bootstrap results:

Each pair of models is compared. The denominator $SS_e$ is calculated using the more complex of the two models in each case; that is, the errors are pooled and include terms involving any models further along in the sequence of models. In each case 100 values of $F^*$ were used.

The number $\#{F^*: F^* > F}$, expressed as a percent, is an estimate of the p-value for the observed effect. A value of 0 can be read as a value of approximately 1% if the maximum $F^*$ is close to $F$, and as $<1\%$ if $F^*$ is much smaller than $F$. 

33
<table>
<thead>
<tr>
<th>Effect tested</th>
<th>F</th>
<th>df-num</th>
<th>df-den</th>
<th>p-value</th>
<th>#{F*: F*&gt;F}</th>
<th>max F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff: IIa-Lin. Reg.</td>
<td>26.258</td>
<td>37</td>
<td>1835</td>
<td>1.07×10⁻⁷</td>
<td>0</td>
<td>1.515</td>
</tr>
<tr>
<td>$c_\xi$: IIb-IIa</td>
<td>5.838</td>
<td>3</td>
<td>1832</td>
<td>5.75×10⁻⁴</td>
<td>0</td>
<td>2.708</td>
</tr>
<tr>
<td>$c_\eta$: IIc-IIa</td>
<td>4.487</td>
<td>2</td>
<td>1833</td>
<td>.0114</td>
<td>0</td>
<td>3.674</td>
</tr>
<tr>
<td>$c_s$: IIIf-IIa</td>
<td>.301</td>
<td>2</td>
<td>1833</td>
<td>.7401</td>
<td>74</td>
<td>4.493</td>
</tr>
<tr>
<td>$c_\xi$: given $c_\eta$ IIid-IIdc</td>
<td>6.492</td>
<td>3</td>
<td>1830</td>
<td>2.28×10⁻⁴</td>
<td>1</td>
<td>8.526</td>
</tr>
<tr>
<td>$c_\eta$: given $c_\xi$ IIid-IIdb</td>
<td>5.408</td>
<td>2</td>
<td>1830</td>
<td>4.55×10⁻³</td>
<td>0</td>
<td>4.926</td>
</tr>
<tr>
<td>Interaction: L×H</td>
<td>2.007</td>
<td>6</td>
<td>1824</td>
<td>.0616</td>
<td>8</td>
<td>3.320</td>
</tr>
<tr>
<td>Interaction: L×H×S</td>
<td>2.683</td>
<td>24</td>
<td>1800</td>
<td>1.91×10⁻⁵</td>
<td>0</td>
<td>2.146</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effect tested</th>
<th>F</th>
<th>df-num</th>
<th>df-den</th>
<th>p-value</th>
<th>#{F*: F*&gt;F}</th>
<th>max F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff: IIIa-Iii. Reg.</td>
<td>850.360</td>
<td>1</td>
<td>1871</td>
<td>9.33×10⁻⁹</td>
<td>0</td>
<td>6.799</td>
</tr>
<tr>
<td>$c_\xi$: IIIb-IIIa</td>
<td>4.538</td>
<td>3</td>
<td>1868</td>
<td>3.56×10⁻³</td>
<td>1</td>
<td>5.117</td>
</tr>
<tr>
<td>$c_\eta$: IIIc-IIIa</td>
<td>14.028</td>
<td>2</td>
<td>1869</td>
<td>8.97×10⁻⁷</td>
<td>0</td>
<td>4.987</td>
</tr>
<tr>
<td>$c_s$: IIIIf-IIIa</td>
<td>.733</td>
<td>2</td>
<td>1869</td>
<td>.4806</td>
<td>45</td>
<td>6.638</td>
</tr>
<tr>
<td>$c_\xi$: given $c_\eta$ IIIid-IIdc</td>
<td>4.568</td>
<td>3</td>
<td>1866</td>
<td>3.41×10⁻³</td>
<td>0</td>
<td>3.635</td>
</tr>
<tr>
<td>$c_\eta$: given $c_\xi$ IIIid-IIdb</td>
<td>14.050</td>
<td>2</td>
<td>1866</td>
<td>8.78×10⁻⁷</td>
<td>0</td>
<td>5.505</td>
</tr>
<tr>
<td>Interaction L×H×S</td>
<td>5.105</td>
<td>6</td>
<td>1860</td>
<td>3.28×10⁻⁵</td>
<td>0</td>
<td>3.450</td>
</tr>
<tr>
<td>Interaction L×H×S×S</td>
<td>2.844</td>
<td>24</td>
<td>1836</td>
<td>5.34×10⁻⁶</td>
<td>0</td>
<td>1.917</td>
</tr>
<tr>
<td>$\beta_0 = 0$ vs. $\beta_0$ free</td>
<td>1.647</td>
<td>36</td>
<td>1800</td>
<td>9.41×10⁻³</td>
<td>0</td>
<td>1.642</td>
</tr>
<tr>
<td>given $c_{\xi S}$ in both models: IIg-IIIg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Each comparison in Table 6 is a test of the hypothesis \( H_0: \beta^0_{\text{chs}} = 0 \) vs. \( H_1: \beta^0_{\text{chs}} \neq 0 \). The tests differ in what other effects are included in both cases; so they are tests adjusted for the other effects.

Table 6. Comparisons of the models with \( \beta^0_0 \) free and \( \beta^0_0 = 0 \).

<table>
<thead>
<tr>
<th>Effect adjusted for</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>c: IIIa-IIa</td>
<td>67.025</td>
<td>36</td>
<td>1.862</td>
<td>2.729</td>
<td>1.94x10^{-7}</td>
</tr>
<tr>
<td>c_k: IIb-IIb</td>
<td>69.434</td>
<td>36</td>
<td>1.929</td>
<td>2.827</td>
<td>6.33x10^{-8}</td>
</tr>
<tr>
<td>c_h: IIc-IIc</td>
<td>53.137</td>
<td>36</td>
<td>1.476</td>
<td>2.163</td>
<td>8.46x10^{-5}</td>
</tr>
<tr>
<td>c_s: IIIf-IIf</td>
<td>66.378</td>
<td>36</td>
<td>1.844</td>
<td>2.703</td>
<td>2.60x10^{-7}</td>
</tr>
<tr>
<td>c_k+h: IIId-IId</td>
<td>56.944</td>
<td>36</td>
<td>1.582</td>
<td>2.318</td>
<td>1.73x10^{-5}</td>
</tr>
<tr>
<td>c_k+h: IIIe-IIe</td>
<td>43.679</td>
<td>36</td>
<td>1.213</td>
<td>1.778</td>
<td>3.74x10^{-3}</td>
</tr>
<tr>
<td>c_k+h: IIIg-IIg</td>
<td>40.461</td>
<td>36</td>
<td>1.124</td>
<td>1.647</td>
<td>9.41x10^{-3}</td>
</tr>
<tr>
<td>Error IIg</td>
<td>1228.072</td>
<td>1800</td>
<td>.68226</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Conclusions

In testing for an effect where the sums of squares are not orthogonal (they are not orthogonal in the present case) there occur, as can be noted in the preceding results, more than one possible test for the given effect. It is the usual and probably the best procedure to use the test which corrects for the other effects in the model, unless there is reason to believe that the other effects are not present. In the present case there appears to be no main effect for subject (Table 2 p-value = .732) so we are justified in failing to adjust for this effect.
There is substantial agreement between the results which use the theoretical distribution of the $F$-statistic and those which use the empirical distribution of the $F^*$ values, with the possible exception of the test for level adjusted for Hertz (Table 4) where the $p$-value is .000228 whereas there was 1 observed $F^*$ greater than $F$, indicating a $p$-value of approximately .01. The theoretical $p$-values are apparently valid.

It is probably best to begin with the question of whether the cutoff exists. Here the evidence is overwhelming. From Table 1 it can be observed that setting $\beta_0 = 0$ and adding a single cutoff $c$ reduces the sum of squared errors by $621.475/1988.875 = 31\%$, using only one parameter. The $p$-value is $2.35 \times 10^{-8}$; that is, an effect of this magnitude would occur by chance only once in 235,000,000 times, assuming that the distribution of $F$ in this case were actually an $F$-distribution. The exact $p$-value is wrong, but the order of magnitude is correct.

None of the rest of the effects reduce the sum of squared errors so dramatically, although most of them are statistically significant. The additional proportion of variance explained in going from the simplest model involving $c$ (IIIa) to the most complex one considered (IIg) is $(1367.400-1228.072)/1988.875 = 7\%$ (at an expense of 71 additional parameters). What this means is that, although the additional effects are strong enough to be statistically significant, they are of relatively little value in improving the predictive ability of the model.

We will look at these effects in order. I will adopt a significance level of 1% for the tests.
For $\beta_{\text{lhs}}^0 \equiv 0$ vs. $\beta_{\text{lhs}}^0 \neq 0$ and adjusting for the other effects, we get a p-value of .00941 (Tables 5 and 6), just barely significant. It appears that $\beta_0 \neq 0$, but is not too different from 0. The parameter estimates $\hat{\beta}_{\text{lhs}}^0$ jump around quite a bit, but are mostly negative, enough so to lend credence to the assertion that the true values are not zero. It seems reasonable that the actual relationship between duration and response is not strictly a two part linear function, but rather reaches an asymptote in a smooth fashion. This would lead to results similar to those observed.

If I were modelling this problem, I would prefer to use the assumption that $\beta_{\text{lhs}}^0 \equiv 0$, even though this is rejected by the data, since it simplifies the interpretation of the remaining results. As is, the noise in the data makes the best-fitting $\beta_{\text{lhs}}^0$ flip about so much that the resulting estimated two-part linear curves would be difficult to justify theoretically.

Assuming $\beta_0 \equiv 0$, the subject effect is not significant by itself. Level adjusted for hertz has a p-value of .0026 whereas hertz adjusted for level has a p-value of .000000525. Hence, while both effects are significant, the effect of hertz on the cutoff value is stronger than that of level. The cutoff is apparently not an additive function of level and hertz the p-value for their interaction is .0000325. Unfortunately the same is true for the interaction between these and subject. Even though there do not seem to be consistent differences between the cutoff durations of the three subjects, they do seem to respond differently when hearing a particular combination of level and hertz. The p-value here is .00000534, and the effect seems real enough.
Allowing $\beta_0$ to be free (see Table 2) the conclusions would be somewhat different in this case. Now Hertz is less significant than level, and the p-value for the interaction between level and Hertz is only .0559. However, the three-way interaction between level, Hertz, and subject is still significant so all three terms must be retained if one is to make decisions on the basis of the statistical significance.

References


Appendix: Raw Data

Each rectangle represents one combination of (level, Hertz, subject), indexed in that order above the rectangle:

Level 1: threshold
   2: 20 dB
   3: 50 dB
   4: 80 dB

Hertz 1: 1000 Hz
   2: 4000 Hz
   3: white noise

Within each rectangle, each row is for one of nine durations

500, 300, 200, 150, 100, 50, 30, 20, 10 milliseconds

in that order. The six columns are the six replications.
MIGRATION OF CELLS ON A PETRI DISH

Lynn Gale and Lincoln Moses

Biological Problem: Estimate the velocity of cell movement.

Biological Investigators: Leslie Brettell and Merton Bernfield, Stanford University.

Statistical Procedures: Brownian motion; least squares; harmonic mean; order statistics.

1. Background

Cell movement has been studied in a number of different ways. These include time lapse photography of a small sample of cells and observation of a "foot race" begun by scraping clear a portion of a field of cells and watching the resulting cell movement across the open plain. The method of interest to Brettell and Bernfield was the radial movement of cells away from a spherical aggregate.

An aggregate was prepared in the following way:

- a field of cells growing in a medium was used throughout the experiment;

- the cells were separated with an enzyme, then put into a rotator which caused them to ball up into a spherical aggregate;

- a number of these spheres were observed falling in suspension and several aggregates of similar size (about a pinhead) were chosen by their rate of fall;
- these were plopped onto one or more petri dishes with possibly different surfaces but covered by the same medium;
- the spheres "flattened out" as the cells began to adhere to the surface and, once touching the surface, the cells migrated outward, away from the aggregate.

To enable comparisons of relative mobility of different types of cells or similar cells on different types of surfaces, a reliable measure of rate of movement was required. This was our goal — to describe the movement of cells away from a given aggregate on a given surface by some measure which would be reproducible from aggregate to aggregate, all observed under the same experimental conditions.

Various biological considerations complicated the problem:
- aggregates contained some 20,000 cells and actual sizes could not be approximated accurately;
- cell movement was possibly affected by "trailing" of cells along the substrate excreted by another cell;
- "contact inhibition" perhaps determined the direction of a cell's movement when other cells were in the way;
- previous studies showed cell division might occur after 27 hours, and a dividing cell would remain stationary for a while;
- rates of movement could vary with time and/or distance from the aggregate (e.g., the jumble of cells in the sphere might have resulted in a "locked" aggregate until a number of cells could free themselves from the periphery and open paths for movement outward).
2. The Data

We were originally presented with data from two series of experiments. The first, referred to as "8/5" (the date), consisted of cell counts outside four fixed radii (measured from the "center" of the aggregate), all taken at 17$\frac{1}{2}$ hours for several separate aggregates. The second, "8/9", had counts outside two fixed radii taken at six different times from many separate aggregates. It was unfortunate that the counting method forced cessation of movement so that a single aggregate could not be observed at multiple times. Subsequent data (4/24, 6/18, 7/7), however, was obtained by photographing the petri dish undisturbed, thus enabling a series of glimpses of the same aggregate over time.

3. Analysis of 8/5 and 8/9 Experiments

Our initial attack on the problem was to consider Brownian motion in the plane and to choose as our "motility parameter" $\kappa$, a constant in the standard two-dimensional diffusion equation,

$$
\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} = \frac{1}{\kappa} \frac{\partial u}{\partial t},
$$

(1)

to be estimated from the data. In (1), $u = u(t, x, y)$ is a function of the plane (petri dish) coordinates $x, y$ and time $t$. Brownian motion is a process in which changes occur continuously in time (rather than by discrete jumps) and the transition probabilities are given by
\begin{equation}
(2) \quad p_t(u,v;x,y) = \frac{1}{4\pi \kappa t} e^{-\frac{(x-u)^2+(y-v)^2}{4kt}} = \text{Pr}\{ \text{moving to } (x,y) \text{ from } (u,v) \text{ in time } t \},
\end{equation}

where \( \kappa \) is a constant, \((u,v)\) is the initial point, and \(x,y\) are variables. Motion in nonoverlapping time increments is assumed independent, and in two dimensions we need rotational symmetry for this particular model. Whether or not the physical situation strictly obeyed these assumptions (and it clearly did not), we hoped there might be enough association between the actual process and the model to warrant the use of the estimated \( \kappa \) as a measure of motility.

The diffusion equation (1) is satisfied by

\begin{equation}
(3) \quad u(t,x,y) = \iint p_t(u,v;x,y) u_0(u,v) \, du \, dv \propto \text{number of cells at } (x,y) \text{ at time } t,
\end{equation}

where \( u_0(u,v) \) is a bounded continuous "initial function" which we took to be a constant \( A \) times a bivariate normal with mean \( (0,0) \) and covariance matrix

\begin{equation}
\left( \begin{array}{cc}
\sigma^2 & 0 \\
0 & \sigma^2
\end{array} \right),
\end{equation}

\begin{equation}
(4) \quad u_0(u,v) = \frac{A}{2\pi \sigma^2} e^{-\frac{u^2+v^2}{2\sigma^2}}.
\end{equation}

Then by separating the integrals in (3), and writing them as convolutions of normals, we obtain,

\begin{equation}
(5) \quad u(t,x,y) = \frac{A}{2\pi(\sigma^2+2\kappa t)} e^{-\frac{1}{2} \left( \frac{x^2+y^2}{\sigma^2+2\kappa t} \right)}.
\end{equation}
If we let $A$ denote the total (unknown) quantity in the aggregate at time $0$, the density (5) has mass $A$, i.e.,

$$A = \iint u(t,x,y) \, dx \, dy.$$  

We write $N(r,t)$ for the number of cells outside a circle of radius $r$ at time $t$, and this is given by

$$N(r,t) = \iint_{x^2+y^2>r^2} \frac{A}{2\pi(\sigma^2+2\kappa t)} e^{-\frac{1}{2}\left(\frac{x^2 + y^2}{\sigma^2+2\kappa t}\right)} \, dx \, dy.$$  

By transforming to polar coordinates we finally obtain the result

$$N(r,t) = Ae^{-\frac{1}{2}\frac{r^2}{\sigma^2+2\kappa t}},$$

(8)

or equivalently,

$$\ln N(r,t) = \ln A - \frac{r^2}{2(\sigma^2+2\kappa t)}.$$  

(9)

Using this model we were then able to estimate the parameters $A$, $\sigma^2$ and, most importantly, $\kappa$, using two methods for the two different types of data. (Refer to Handout I.) For the fixed time data $(8/5)$, we calculated $\ln N(r_i,t) \triangleq y_i$ for four radii $(r_i \triangleq x_i)$ at time $t = 17\frac{1}{2}$ hours. Then using standard least squares theory for the model

$$\ln N(r_i,t) = \ln A - \frac{r_i^2}{2(\sigma^2+2\kappa t)},$$

(10)

or $y_i = \alpha + \beta x_i$, where $\alpha = \ln A$ and $\beta = -\frac{1}{2(\sigma^2+2\kappa t)}$, 

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we obtained estimates \( \hat{\beta} = \Sigma(y_1 - \bar{y})(x_1 - \bar{x})/\Sigma(x_1 - \bar{x})^2 \) and \( \hat{\alpha} = \bar{y} - \hat{\beta}\bar{x} \).

To obtain an estimate of the motility parameter \( \kappa \), we calculated

\[
\hat{\kappa} = -\frac{1}{2t}\left(\frac{1}{2\hat{\beta}} + \hat{\sigma}^2\right).
\]

Recall that \( \sigma^2 \) represents the initial variance (in area units) of the aggregate. By choosing "reasonable" values for \( \hat{\sigma} \) (values such that the initial diameter equalled approximately \( 5\hat{\sigma} \)), we were able to estimate \( \kappa \) using (11) and determine that the estimate was insensitive to mild changes in \( \hat{\sigma} \).

The second data set afforded us a direct method of estimation rather than resorting to least squares theory (and its implicit normality assumptions). This 8/9 data had counts outside two radii \( r_1 \) and \( r_2 \) at time \( t \), which yielded two numbers:

\[
m_1 \triangleq \text{number between } r_1 \text{ and } r_2, \\
m_2 \triangleq \text{number outside } r_2.
\]

By (9),

\[
(12) \quad \ln N(r_1,t) = \ln A - \frac{r_1^2}{2(\sigma^2 + 2kt)} \quad \text{and} \quad \ln N(r_2,t) = \ln A - \frac{r_2^2}{2(\sigma^2 + 2kt)};
\]

subtraction yields

\[
(13) \quad \frac{\ln N(r_1,t)}{N(r_2,t)} = \frac{r_2^2 - r_1^2}{2(\sigma^2 + 2kt)},
\]

and in the new notation we obtain
\[(14) \quad \ln \frac{m_1 + m_2}{m_2} = \frac{r_2^2 - r_1^2}{2(\sigma^2 + 2\kappa t)} ,\]

or equivalently,

\[(15) \quad \frac{r_2^2 - r_1^2}{2 \ln \left(1 + \frac{m_1}{m_2}\right)} = \sigma^2 + \kappa(2t) ,\]

where \(\kappa\) and \(\sigma^2\) are parameters of interest and \(t, r_1, r_2, m_1, m_2\) are all known. \(\kappa\) was estimated from the 8/9 data by using (15) with the approximation \(D = 5\sigma\) (\(D =\) diameter of aggregate, was given).

The resulting \(\hat{\kappa}\)-values for 8/5 and 8/9 can be seen in Handout I; the 8/5 values are, on the whole, larger than the 8/9 values. These all came from aggregates of one type of cell placed on one particular type of surface. Our goal was to obtain a single \(\kappa\) which would adequately describe the movement in any aggregate of this type on this surface.

Consequently in this first approach, and subsequent ones, we were concerned when the \(\hat{\kappa}\)'s varied (sometimes considerably) and we kept an eye open for systematic variation (e.g., \(\hat{\kappa}\) decreasing with time). We were not so much concerned with the variations we saw in \(\hat{\kappa}\) on this first try, as we were with another problem. Using weighted and unweighted least squares models, we obtained \(\hat{\sigma}^2\) and \(\hat{\kappa}\) from equation (15), with \(2t\) the independent variable and the left hand side the dependent variable. Unweighted least squares on two different segments of data from 8/9 yielded 90\% confidence intervals for \(\hat{\sigma}^2 : (-3.653, .037)\) and \((-4.841, -1.029)\). The weighted model fared no better, yielding a 90\% confidence interval \((-4.078, .004)\). These
signalled a clear-cut problem, since an estimate of \( \sigma^2 \) should not be negative. Recall that the data was taken from different aggregates at different times rather than from one aggregate at various times. Also the times recorded were 14, 16, 17, 18, 19, 22 hours, perhaps too early for the system to "settle in" (lots of 0 counts too), and too closely spaced to see much change from period to period. We waited for the new data (done by photography) before making any decisions.

4. Analysis of Photography Experiments

The remainder of the study was performed on data of the following type. Aggregates were prepared as before, with an attempt to make them approximately the same size. Cell counts were made on a specific aggregate at different hours using polaroid photography (done with magnification), a method which did not disturb the movement on the petri dish. On each picture, rings were drawn at selected radii about the "center" of the aggregate, and one person counted the number of cells between rings on all of the pictures. These data sets are labelled "4/24 - A11, A12" (2 aggregates watched), "6/18 - A21, A22, A23" and "7/7 - A31, A32, A33" in the handouts.

We returned to the model given by (10) and using the 4/24 and 6/18 data, got poor results once again (refer to Handout II, pages 1 and 2). At the suggestion of Joe Keller in the Mathematics Department, we tried another method of approximation still within the framework of the diffusion equation. Joe pointed out that the small counts and discrete nature of the data led to a very crude approximation to the diffusion
equation. He suggested we "smooth" the data (by plotting it and drawing a smooth "eyeballed" curve through the points) and then use values from the smoothed curves to estimate the derivatives in (1) directly, by the method of divided differences. In this way we could get an estimate of the motility parameter $\hat{\kappa}$ without resorting to normality assumptions or least squares theory. Furthermore, we could do a sort of "cross-validation" of the smoothing process by holding radii fixed and smoothing over time, then holding time fixed and smoothing over radii, hoping the two methods resulted in the same $\hat{\kappa}$. The process of smoothing by hand and calculating divided differences was long, tedious, and unfortunately, unsuccessful. $\hat{\kappa}$'s for similar aggregates, smoothed (two ways) and unsmoothed, were inconsistent and sometimes even negative.

At this point we turned our attention to searching for other ways to describe the radial movement away from aggregates. There were many considerations to keep in mind. Was it reasonable to describe the movement in the system by a single number? Were there special relationships between counts and time or distance from the aggregate? Was the movement predominantly away from the aggregate? How much of the data should be used (for example, would early counts confuse the issue)? How should estimates of the same item be combined over time (or distance)? Is it possible to determine the accuracy of the estimate? The photographs afforded us some visual luxuries one would not ordinarily have in data. With pictures taken 2-3 hours apart, it was possible in many instances to follow the path of individual cells (some had easily identifiable characteristics). Thus, for instance, we could assure ourselves that the movement was predominantly outwards (see Chart I).
After a large number of exploratory plots failed to enlighten us to any (quantifiable) systematic relationship between counts, time, distance, etc., we returned to estimating velocities. The estimate "ring steps per cell-hours" was natural in light of the type of data we had, was easy to calculate, and involved only innocuous assumptions about the whole process.

The easiest way to describe this statistic is by example. (Refer to Handout II, page 3.) We considered data from two consecutive times, 19 and 25 hours, and counted cells between various radii each one centimeter apart (on the photograph). So, looking at the photograph, there was a middle circle of radius \( \frac{1}{4} \) cm. where cells were still clustered into an aggregate, and around this there were 8 consecutive rings of radius 1 cm. in which cells were counted, and finally the 9th ring count (denoted \( m_{12,\infty} \)) included all cells outside radius 12 cm. To describe the motion between 19 and 25 hours, we counted the ring steps taken to get from the 19 hour configuration to that at 25 hours. In the example of Handout II, we assumed the cells moved outward; of the 3 cells in ring 6, for example, 2 moved to ring 9 and 1 to ring 8, and the other cell spots were filled in the same manner. A move from ring 6 to ring 9 was counted as 3 "ring steps", staying in the same ring was 0 ring steps and backwards movement would be negative the number of steps. In a closed system (i.e., no new cells entering or leaving), the total number of ring steps taken is the same no matter how you assume the cells got from their 19 hour configuration to the 25 hour configuration (more on this later). Thus, our method of filling in
spaces from the farthest out was simply a matter of convenience in com-
putation. The convention we chose to take care of entering cells was
the following: movement from the center circle to ring 1 was not in-
corporated in the count, but movement from the center to any other ring
counted as ring steps from ring 1 to that ring (e.g., in the example,
17 cells each made 1 ring step from the center to ring 2). The statis-
tic, \( v \), was calculated by finding the total number of ring steps (132)
and dividing it by the number of cells (69) times the number of hours
(25-19 = 6):

\[
(16) \quad v = \frac{\text{total ring steps}}{\# \text{ of cells} \times \text{hours}} = \frac{132}{69 \times 6} = .319.
\]

For a given aggregate, there would be several \( v_j \)'s corresponding
to the different time periods of observation. Since \( v \) is in units
of steps/cell-hour and we're interested in an "average cell" velocity,
we used the harmonic mean to combine the \( v_j \)'s into a single number
for the aggregate:

\[
(17) \quad v = \left( \frac{1}{n} \sum_{j=1}^{n} \frac{1}{v_j} \right)^{-1} \quad \text{where} \quad n \quad \text{is the number of time periods}.
\]

Various \( v \) values are given in Handout II, page 4, Handout III and
Handout IV. The 4/24 results on page 4, Handout II show that there is
some difference in the statistic when it is calculated for ring steps
of .5 cm. rather than 1 cm. One might expect that \( 2 \times \frac{1 \text{ cm. steps}}{\text{cell-hours}} \)
would be the velocity in terms of \( \frac{.5 \text{ cm. steps}}{\text{cell hours}} \).

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Handout III lists eleven \( \nu \)-values, one of which is for an aggregate placed on a different surface (polylysine) which is suspected to slow down cell movement. We looked for patterns in the values with respect to the longest time an aggregate was observed, the largest count for an aggregate and ring size. No obvious patterns were discovered. We noted that the polylysine estimate, \( \nu = .061 \), was indeed smaller than all the rest, but we needed some measure of variation in our estimate before we could determine if it was convincingly smaller than, say, .076 or .084, both estimates made on the regular surface.

Handout IV gives \( \nu_j \) values for all of the aggregates, along with corresponding upper and lower bounds on the standard error. These bounds were obtained by the following construction. For \( N \) cells moving from the \( j \)th configuration to the next one photographed in time \( T \), our statistic is

\[
\nu_j = \frac{\sum_{i=1}^{N} v_i}{N} = \frac{\sum_{i=1}^{N} d_i}{NT}
\]

(18) where \( v_i \) is velocity and \( d_i \) is distance.

Then the corresponding variance is given by

\[
\sigma^2_j = \frac{1}{N} \frac{\sum_{i=1}^{N} (v_i - \overline{v})^2}{N-1} = \frac{1}{NT^2} \frac{\sum_{i=1}^{N} (d_i - \overline{d})^2}{N-1}
\]

(19)

To simplify the calculations, we only considered the movement of the cells observed in the \( j \)th picture; thus, if the \( j+1 \)st configuration had \( N+M \) cells (i.e., \( M \) new ones moved out of the aggregate), the \( M \) cells closest to the aggregate in picture \( j+1 \) did not enter into
the calculations of $v_j$ and $\sigma_j^2$. Let $x_1, \ldots, x_N$ denote the locations of the cells in the $j$th picture (e.g., $x_1 = 2$ means cell 1 is in ring 2). It is important that the rings are spaced at equal intervals. Let $y_1, \ldots, y_N$ denote the locations in the $j+1$st picture. Ideally, $(x_1, y_1)$ would give the two locations of a cell and $y_1-x_1$ would be the distance it moved. However, our data didn't allow us to match pairs like this; for example, the cell at $x_1$ might be the one at $y_5$ in the subsequent picture. So this is where the uncertainty lies. As previously mentioned, the ordering doesn't affect the calculation of $v_j$ since the total distance moved is

\[
\frac{1}{N} \sum_{\alpha \in \{1, 2, \ldots, N\}} \sum_{\beta \in \{1, 2, \ldots, N\}} (y_\beta - x_\alpha) = \sum_{i=1}^{N} y_i - \sum_{i=1}^{N} x_i = \text{constant},
\]

regardless of the choice of pairs $(x_\alpha, y_\beta)$. On the other hand, $\sigma_j^2$ depends on the ordering. From (19),

\[
\sigma_j^2 = \frac{1}{NT^2} \left( \frac{1}{N-1} \sum_{i=1}^{N} (d_i - \bar{d})^2 \right) = \frac{1}{N(N-1)T^2} \sum_{i=1}^{N} \left( y_i - x_i - \frac{(Ey_i - Ex_i)}{N} \right)^2,
\]

\[
\frac{1}{N(N-1)T^2} \left\{ \sum_{i=1}^{N} (y_i - x_i)^2 - \frac{(Ey_i - Ex_i)^2}{N} \right\},
\]

where we let $d_i = y_i - x_i$ as an example. A different pairing, say $(x_1, y_N+1-i)$, would yield a different result, namely,

\[
\sigma_j^2 = \frac{1}{N(N-1)T^2} \left\{ \sum_{i=1}^{N} (y_{N+1-i} - x_1)^2 - \frac{(Ey_i - Ex_i)^2}{N} \right\}.
\]
Denoting the order statistics as $x_{(1)}, \ldots, x_{(N)}$ and $y_{(1)}, \ldots, y_{(N)}$ ($x_{(1)}$ is the smallest observed $x$ value), we can write down the largest possible value of $\sigma_j^2$ (call it $\sigma_U^2$ for upper bound) and the smallest possible value (call it $\sigma_L^2$ for lower bound).

$$
\sigma_U^2 = \frac{1}{N(N-1)T^2} \left\{ \sum_{i=1}^{N} (y_{(N+1-i)} - x_{(1)})^2 - \frac{(\Sigma y_i - \Sigma x_i)^2}{N} \right\},
$$

(23)

$$
\sigma_L^2 = \frac{1}{N(N-1)T^2} \left\{ \sum_{i=1}^{L} (y_{(i)} - x_{(1)})^2 - \frac{(\Sigma y_i - \Sigma x_i)^2}{N} \right\}.
$$

The square roots of these numbers are the standard errors listed in Handout IV. These are indications of the uncertainty of the estimate $v_j$ with respect to cell-to-cell variation. Further uncertainty arises when the $v_j$'s are combined over time periods due to period-to-period variation (within aggregate). Finally, even for aggregates under the same conditions, same cell types and same surfaces, there is between aggregates variation. All of these uncertainties could be estimated, but indications from the first step sufficed to discourage us.

It became apparent that these particular velocity estimates were not achieving the desired goal. We were not obtaining an estimate with reasonably small uncertainty within an aggregate, nor one that was consistent from aggregate to aggregate of the same type. It clearly could not be used for reliable comparisons between experiments.
5. Conclusions

We were unable to obtain a satisfactory summary of these cell movement experiments, either by a complicated model of the situation or by a simple estimate obtained by averaging. Other models and other estimates remain to be tried. And the question arises as to whether the desired velocity number is even attainable, given optimal statistical analysis, under the conditions of this experimental set up. In any case, the problem is complicated by the multiplicity of sources of variation and the biological considerations stated at the beginning of this paper. New ideas which might be explored include restructuring the experiment to control some of the factors of variation and rethinking the prospective form of the summary statistic(s) (e.g., several descriptive numbers rather than a single velocity estimate).
Migration of Cells on a Petri Dish

\[ N(r_i, t_j) = \# \text{ outside radius } r_i \text{ at time } t_j \]
\[ \lambda_{ij} = E(N(r_i, t_j)) \]
\[ A = \text{ initial amount} \quad \sigma^2 = \text{ initial variance} \]
\[ \kappa = \text{ motility parameter} \]

\[ N(r_i, t_j) \sim \text{ Poisson } P(\lambda_{ij}, n_{ij}) \quad \lambda_{ij} = A \exp \left\{ \frac{-r_i^2}{2(\sigma^2 + 2\kappa t_j)} \right\} \]

Two Forms of the Model

I) fixed time \( t \)  \[ \ln N(r_i, t) = \ln A - \frac{1}{2(\sigma^2 + 2\kappa t)} r_i^2 \]
\[ y_i = \alpha + \beta x_1 \]

II) 2 radii fixed \( r_1, r_2 \)  \[ m_1 = \# \text{ between } r_1 \text{ and } r_2 \]
\[ m_2 = \# \text{ outside } r_2 \]
\[ \frac{r_2^2 - r_1^2}{2 \ln \left( 1 + \frac{m_1}{m_2} \right)} = \sigma^2 + \kappa(2t) \]

Table of regression coefficients from (I), using 8/5 Data, fitting 9 lines

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>-.154</td>
<td>-.134</td>
<td>-.138</td>
<td>-.184</td>
<td>-.154</td>
<td>-.185</td>
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<td>-.157</td>
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<td>( \hat{\alpha} )</td>
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<td>( \hat{\sigma}^2_{\text{regr.}} )</td>
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<td>.121</td>
<td>.134</td>
<td>.041</td>
<td>.057</td>
<td>.104</td>
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</table>
Curvature in 8/5 Data?

Eyeball for patterns in curvature, i.e., are the data points "bowed" up or down with respect to the regression lines? Plus (+) indicates point above line, minus (-) below ...

\[
\begin{array}{c|cccc}
\text{regr. line} & 4 & 9 & 16 & 25 \\
\hline
1 & - & + & + & - \\
2 & + & - & - & + \\
3 & + & - & + & + \\
4 & + & + & - & + \\
5 & - & - & + & - \\
6 & + & + & - & + \\
7 & + & + & - & + \\
8 & - & - & + & - \\
9 & + & - & + & + \\
\end{array}
\]

example: from smallest to largest \( r^2: - + + + - \) (bowed down)

Motility parameter - \( \kappa \)

8/5 Data

time \( t \) fixed: \( 2t = 35 \) hours

9 regression lines fitted to 9 sets of data taken at 4 points:

\( r^2_i = 4, 9, 16, \text{ and } 25 \)

\( \hat{\kappa} \) estimated for "reasonable" \( \hat{\sigma}'s \)
HANDOUT I (cont'd)

Table of $\hat{\kappa}$

<table>
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<th>$\sigma$ set</th>
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<td>.070</td>
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<td>.070</td>
<td>.084</td>
<td>.084</td>
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8/9 Data

time $t$ varied: $2t = 28, 32, 3\frac{1}{4}, 36, 38, 44$

radii $r_1$ & $r_2$ fixed: $m_1 =$ number between $r_1$ & $r_2$;
$m_2 =$ number outside $r_2$

$D$ is approximate diameter of aggregate; assume $D = 5\sigma$

<table>
<thead>
<tr>
<th>$2t$</th>
<th>$m_1/m_2$</th>
<th>$D$</th>
<th>$\sigma$</th>
<th>$\hat{\kappa}$</th>
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<td>.055</td>
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Migration of Cells on a Petri Dish

\[ N(r_i, t_j) = \# \text{ outside radius } r_i \text{ at time } t_j \]

\[ \mathbb{E}[N(r_i, t_j)] = A \exp \left( -\frac{r_i^2}{2(\sigma^2 + 2\kappa t_j)} \right) \text{ where } A = \text{ initial amount in aggregate} \]
\[ \sigma^2 = \text{ initial variance (in area units)} \]
\[ \kappa = \text{ motility parameter} \]

\[ \ln N(r_i, t_j) = \ln A - \frac{1}{2(\sigma^2 + 2\kappa t_j)} r_i^2, \text{ or simply } y_i = \alpha + \beta x_i \text{ for given time } t_j. \]

6/18 Data from 3 aggregates

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<thead>
<tr>
<th>t:</th>
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<th>19</th>
<th>25</th>
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<td>-.0312</td>
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<tr>
<td></td>
<td>\frac{1}{2\beta}</td>
<td>5.137</td>
<td>9.416</td>
<td>10.850</td>
<td></td>
</tr>
<tr>
<td>A3:</td>
<td>\hat{\alpha}:</td>
<td>3.767</td>
<td>4.218</td>
<td>5.301</td>
<td></td>
</tr>
<tr>
<td></td>
<td>\hat{\beta}:</td>
<td>-.0763</td>
<td>-.0862</td>
<td>-.0797</td>
<td></td>
</tr>
<tr>
<td></td>
<td>\frac{1}{2\beta}</td>
<td>6.554</td>
<td>5.801</td>
<td>6.273</td>
<td></td>
</tr>
</tbody>
</table>

\[ \frac{1}{2\hat{\beta}} = \sigma^2 + \kappa(2t) \]

Aggregate 1: x
Aggregate 2: o
Aggregate 3: -
### 4/24 Data from 2 aggregates

<table>
<thead>
<tr>
<th>t:</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>23</th>
<th>25</th>
<th>27.5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \hat{\alpha} ):</td>
<td>1.325</td>
<td>1.951</td>
<td>2.581</td>
<td>3.837</td>
<td>4.974</td>
<td>4.613</td>
</tr>
<tr>
<td>( \hat{\beta} ):</td>
<td>-.0411</td>
<td>-.0522</td>
<td>-.0714</td>
<td>-.0919</td>
<td>-.112</td>
<td>-.0909</td>
</tr>
<tr>
<td>( \frac{1}{\alpha} ):</td>
<td>12.165</td>
<td>9.578</td>
<td>7.003</td>
<td>5.441</td>
<td>4.464</td>
<td>5.500</td>
</tr>
<tr>
<td>A2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \hat{\alpha} ):</td>
<td>4.178</td>
<td>4.298</td>
<td>3.771</td>
<td>4.190</td>
<td>4.325</td>
<td>4.536</td>
</tr>
<tr>
<td>( \hat{\beta} ):</td>
<td>-.0836</td>
<td>-.0811</td>
<td>-.0518</td>
<td>-.0617</td>
<td>-.0571</td>
<td>-.0583</td>
</tr>
<tr>
<td>( \frac{1}{\alpha} ):</td>
<td>5.981</td>
<td>6.165</td>
<td>9.652</td>
<td>8.104</td>
<td>8.756</td>
<td>8.576</td>
</tr>
</tbody>
</table>

---

Aggregate 1: \( x \)

Aggregate 2: \( 0 \)
HANDOUT II (cont'd)

Let $m_{ij} = \#$ cells between radii $r_i = i$ and $r_j = j$ (cm.)

<table>
<thead>
<tr>
<th></th>
<th>14</th>
<th>16</th>
<th>19</th>
<th>25</th>
<th>39 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_{4,5}$</td>
<td>8</td>
<td>17</td>
<td>22</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>$m_{5,6}$</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>18</td>
<td>54</td>
</tr>
<tr>
<td>$m_{6,7}$</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>13</td>
<td>76</td>
</tr>
<tr>
<td>$m_{7,8}$</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>16</td>
<td>69</td>
</tr>
<tr>
<td>$m_{8,9}$</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>$m_{9,10}$</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>$m_{10,11}$</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>$m_{11,12}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>$m_{12,\infty}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>39</td>
<td>52</td>
<td>96</td>
<td>318</td>
</tr>
</tbody>
</table>

Calculating "ring steps per cell-hours"

Example: movement from 19 to 25 hours

- Step size 1: $\#$ cells $= [17+1+2] = 20$
- Step size 2: $[13+8+9+3+1+1] = 35$
- Step size 3: $[8+3+1+2] = 14$

Total $\#$ cells $= 69$

total steps $= 20+2(35)+3(14) = 132$

69 cells moved 132 ring steps in 6 hours

$$\frac{132}{6 \times 69} = .319 = \frac{\text{ring steps}}{\text{cell-hours}}$$
**HANDOUT II (cont'd)**

### 6/18 Data from 3 aggregates

<table>
<thead>
<tr>
<th>measured from:</th>
<th>ring steps/cell-hours</th>
<th>ring &quot;radius&quot; = 1 cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14-16 hrs.</td>
<td>16-19 hrs.</td>
</tr>
<tr>
<td>A1:</td>
<td>.346</td>
<td>.214</td>
</tr>
<tr>
<td>A2:</td>
<td></td>
<td>.333</td>
</tr>
<tr>
<td>A3:</td>
<td></td>
<td>.051</td>
</tr>
</tbody>
</table>

### 4/24 Data from 2nd aggregate

<table>
<thead>
<tr>
<th>ring steps/cell-hours</th>
<th>ring radius = .5 cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-18 hrs.</td>
<td>18-20 hrs.</td>
</tr>
<tr>
<td>.200</td>
<td>.206</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ring radius = 1 cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>.133</td>
</tr>
</tbody>
</table>
### HANDOUT III

**Migration of Cells on a Petri Dish**

<table>
<thead>
<tr>
<th>Data from:</th>
<th>ring radius (cm.)</th>
<th>cm. cell-hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/24</td>
<td>Aggregate 1</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>.5</td>
<td>.104</td>
</tr>
<tr>
<td>2</td>
<td>.5</td>
<td>.091</td>
</tr>
<tr>
<td>1.0</td>
<td>.082</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>directional data</td>
<td>.075</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>.251</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>.209</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>.082</td>
</tr>
<tr>
<td>7/7</td>
<td>Aggregate 1</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>.4</td>
<td>.084</td>
</tr>
<tr>
<td>2</td>
<td>.4</td>
<td>.076</td>
</tr>
<tr>
<td>(Polylysine) 3</td>
<td>.4</td>
<td>.061</td>
</tr>
</tbody>
</table>

- **longest time**: 42 hrs. | 0.084, 0.076, 0.061
- **longest time**: 39 | 0.251
- **longest time**: 27.5 | 0.132, 0.082, 0.075
- **longest time**: 25 | 0.209, 0.082

- **largest count**: 318 cells | 0.251
- **largest count**: 152 | 0.084
- **largest count**: 142 | 0.076
- **largest count**: 69 | 0.061
- **largest count**: 55 | 0.209
- **largest count**: 49 | 0.082
- **largest count**: 37 | 0.082, 0.091
- **largest count**: 22 | 0.132, 0.104

- **ring size**: 1 cm. | 0.132, 0.082, 0.251, 0.209, 0.082
- **ring size**: 0.5 | 0.104, 0.091
- **ring size**: 0.4 | 0.084, 0.076, 0.061
# HANDOUT IV

Migration of Cells on a Petri Dish

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hours</th>
<th># Cells</th>
<th>Total Steps</th>
<th>Harmonic Mean</th>
<th>Harmonic steps/cell-hr.</th>
<th>(upper and lower bound on standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/24 A11</td>
<td>16-18</td>
<td>2</td>
<td>1</td>
<td></td>
<td>.250</td>
<td>.750, .250</td>
</tr>
<tr>
<td></td>
<td>18-20</td>
<td>3</td>
<td>0</td>
<td></td>
<td>0</td>
<td>.577, 0</td>
</tr>
<tr>
<td></td>
<td>20-23</td>
<td>6</td>
<td>5</td>
<td>$v = .132$</td>
<td>.278</td>
<td>.181, .056</td>
</tr>
<tr>
<td></td>
<td>23-25</td>
<td>15</td>
<td>5</td>
<td></td>
<td>.167</td>
<td>.187, .080</td>
</tr>
<tr>
<td></td>
<td>25-27.5</td>
<td>20</td>
<td>3</td>
<td></td>
<td>.060</td>
<td>.137, .033</td>
</tr>
<tr>
<td>A12</td>
<td>16-18</td>
<td>15</td>
<td>4</td>
<td></td>
<td>.133</td>
<td>.241, .059</td>
</tr>
<tr>
<td></td>
<td>18-20</td>
<td>17</td>
<td>7</td>
<td></td>
<td>.205</td>
<td>.254, .062</td>
</tr>
<tr>
<td></td>
<td>20-23</td>
<td>19</td>
<td>2</td>
<td>$v = .082$</td>
<td>.035</td>
<td>.169, .024</td>
</tr>
<tr>
<td></td>
<td>23-25</td>
<td>26</td>
<td>8</td>
<td></td>
<td>.154</td>
<td>.203, .046</td>
</tr>
<tr>
<td></td>
<td>25-27.5</td>
<td>32</td>
<td>6</td>
<td></td>
<td>.075</td>
<td>.143, .028</td>
</tr>
<tr>
<td>6/18 A21</td>
<td>14-16</td>
<td>26</td>
<td>16</td>
<td></td>
<td>.308</td>
<td>.227, .049</td>
</tr>
<tr>
<td></td>
<td>16-19</td>
<td>39</td>
<td>23</td>
<td>$v = .268$</td>
<td>.196</td>
<td>.143, .032</td>
</tr>
<tr>
<td></td>
<td>19-25</td>
<td>52</td>
<td>115</td>
<td></td>
<td>.368</td>
<td>.065, .012</td>
</tr>
<tr>
<td></td>
<td>25-39</td>
<td>96</td>
<td>348</td>
<td></td>
<td>.259</td>
<td>.025, .006</td>
</tr>
<tr>
<td>A22</td>
<td>16-19</td>
<td>22</td>
<td>22</td>
<td>$v = .204$</td>
<td>.333</td>
<td>.146, .044</td>
</tr>
<tr>
<td></td>
<td>19-25</td>
<td>26</td>
<td>23</td>
<td></td>
<td>.117</td>
<td>.086, .014</td>
</tr>
<tr>
<td>A23</td>
<td>16-19</td>
<td>13</td>
<td>2</td>
<td>$v = .083$</td>
<td>.051</td>
<td>.196, .035</td>
</tr>
<tr>
<td></td>
<td>19-25</td>
<td>17</td>
<td>23</td>
<td></td>
<td>.225</td>
<td>.073, .024</td>
</tr>
<tr>
<td>7/7 A31</td>
<td>12-15</td>
<td>1</td>
<td>1</td>
<td></td>
<td>.333</td>
<td>0, 0</td>
</tr>
<tr>
<td></td>
<td>15-18</td>
<td>6</td>
<td>4</td>
<td></td>
<td>.222</td>
<td>.222, .111</td>
</tr>
<tr>
<td></td>
<td>18-24</td>
<td>14</td>
<td>22</td>
<td>$v = .217$</td>
<td>.262</td>
<td>.080, .023</td>
</tr>
<tr>
<td></td>
<td>24-36</td>
<td>55</td>
<td>117</td>
<td></td>
<td>.177</td>
<td>.020, .007</td>
</tr>
<tr>
<td></td>
<td>36-38</td>
<td>99</td>
<td>50</td>
<td></td>
<td>.252</td>
<td>.098, .025</td>
</tr>
<tr>
<td></td>
<td>38-42</td>
<td>127</td>
<td>76</td>
<td></td>
<td>.150</td>
<td>.030, .011</td>
</tr>
<tr>
<td>Experiment</td>
<td>Hours</td>
<td># Cells</td>
<td>Total Steps</td>
<td>Harmonic Mean</td>
<td>steps cell-hr.</td>
<td>$\sigma_U$</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>---------</td>
<td>-------------</td>
<td>---------------</td>
<td>----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>7/7 A32 12-15</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-18</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-24</td>
<td>4</td>
<td>4</td>
<td>v=.208</td>
<td></td>
<td>.167</td>
<td>0</td>
</tr>
<tr>
<td>24-36</td>
<td>19</td>
<td>60</td>
<td>.263</td>
<td></td>
<td>.016</td>
<td>.007</td>
</tr>
<tr>
<td>36-38</td>
<td>80</td>
<td>35</td>
<td>.219</td>
<td></td>
<td>.108</td>
<td>.028</td>
</tr>
<tr>
<td>38-42</td>
<td>98</td>
<td>81</td>
<td>.207</td>
<td></td>
<td>.033</td>
<td>.010</td>
</tr>
<tr>
<td>A33 (Polylysine) 12-15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-24</td>
<td>0</td>
<td>0</td>
<td>v=.162</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24-36</td>
<td>11</td>
<td>26</td>
<td>.197</td>
<td></td>
<td>.044</td>
<td>.023</td>
</tr>
<tr>
<td>36-38</td>
<td>46</td>
<td>17</td>
<td>.185</td>
<td></td>
<td>.188</td>
<td>.036</td>
</tr>
<tr>
<td>38-42</td>
<td>58</td>
<td>29</td>
<td>.125</td>
<td></td>
<td>.060</td>
<td>.016</td>
</tr>
</tbody>
</table>
Chart I

Analysis of Cell Movement - Magnitude and Direction

Magnitude (cm.)

3

2

1

16-18  18-20  20-23  23-25  25-27.5

# obs.  9    13    19    23    22

# directional obs.  3    8    15    16    12

69
DO HODGKIN'S DISEASE PATIENTS WITH DNBC SENSITIVITY SURVIVE LONGER?

Gail Gong

Medical Problem: Observing the relationship of DNBC sensitivity to time until relapse in Hodgkin's disease patients.

Medical Investigator: Richard Hoppe, Stanford University.

Statistical Procedures: Survival functions; Kaplan-Meier estimator; Cox model.

1. Medical Background

If the man-made compound 2-4-dinitrochlorobenzene, which we will hereafter call DNBC, is initially applied to the skin of a human, no reaction occurs; we call this first application of DNBC sensitization. A second application of DNBC, called the primary challenge, results in inflammation in 90 to 100 percent of normal individuals, whereas in patients with Hodgkin's disease, the proportion resulting in inflammation is much lower. Furthermore, as the seriousness of the disease increases, the proportion of inflammation decreases. For example, in Display 1, of those patients in stages I and II, 36 percent had positive reaction, whereas only 25 percent of stages III and IV had positive reaction.

Begun in 1969, the study by Dr. Hoppe consists of sensitizing and continually challenging each Hodgkin's disease patient until inflammation, i.e., positive reaction, occurs. At that time we say the patient has migrated from the (-) population into the (+) population. The stage

70
of the patient and the time until relapse of the disease are also recorded. Of course, there will be patients who become lost to the study before their times of relapse and others who will not have relapsed by the time this analysis is performed. Therefore, we will have censored data. (The data are included in the Appendix.)

The question we want to answer is: Does being in the (+) population improve the prognosis of the patient? Equivalently, is the rate of relapse of the (+) population lower than that of the (-) population. If the answer is yes, DNBC testing might be used as an indicator of high risk patients.

2. Statistical Analysis

The statistical tools that we will use are the Cox model (Cox 1972) and the Kaplan-Meier estimate of the survival function (Kaplan and Meier 1958). Here, the survival time of a patient is the time until his relapse. For the i-th patient, define

\[ t_i = \text{"event time" (time until relapse or loss to observation)} \]

of the i-th patient

\[ Z_i(t) = \begin{cases} 
1 & \text{if patient } i \text{ is in (+) at time } t \\
0 & \text{if patient } i \text{ is in (-) at time } t 
\end{cases} \]

Notice that we have two populations, (+) and (-), and we have the added complication that the patients can migrate between populations. Under the Cox model, the hazard rate of the i-th patient is assumed to be
\[ \lambda_1(t) = \lambda(t) e^{\beta Z_1(t)} \]

where \( \lambda(t) \) is an unknown, and \( \beta \) is the unknown parameter of interest.

If a patient is in (+) at time \( t \), his hazard is

\[ \lambda^{(+)}(t) = \lambda(t) e^{\beta} \equiv \lambda(t) \gamma, \]

and if a patient is in (-) at time \( t \), his hazard is

\[ \lambda^{(-)}(t) = \lambda(t). \]

We want to test

\[ H_0 : \beta = 0 \text{ vs. } H_1 : \beta < 0. \]

We need to decide if the Cox model is appropriate. Define

\[ P^+(t) \equiv \exp\left[ -\int_0^t \lambda^{(+)}(s)ds \right] \]

\[ P^-(t) \equiv \exp\left[ -\int_0^t \lambda^{(-)}(s)ds \right]. \]

The Cox model implies

\[ \frac{\log P^{(+)}(t)}{\log P^{(-)}(t)} = e^\beta \equiv \gamma \]

so if we had estimates of \( P^{(+)}(t) \) and \( P^{(-)}(t) \), a plot would reveal whether the ratio remains constant.
By renumbering the patients, assume that
\[ t_1 < t_2 < \ldots < t_n \]
are the ordered event times of all the patients, and let \( t_0 = 0 \). Considering only the (+) population at the moment, let the "risk set of the (+) population at time \( t \)" be those patients who at time (immediately before) \( t \):

1. have not relapsed
2. have not become lost to the study
3. are in the (+) population.

Define

\[ n_j^{(+)} = \# \mathcal{R}^{(+)}(t_j) \]

\[ d_j^{(+)} = \# \text{ relapses during } [t_j, t_{j+1}) \text{ of those patients in } \mathcal{R}^{(+)}(t_j). \]

Notice that \( d_j^{(+)} \) takes on values 0 or 1. Then the Kaplan–Meier estimate of \( P^{(+)}(t) \) is

\[ \hat{P}^{(+)}(t) = \prod_{j=0}^{J} \frac{n_j^{(+)} - d_j^{(+)}}{n_j^{(+)}} \text{ if } t \in [t_j, t_{j+1}), \]

and Greenwood's formula

\[ \text{Var} \, \hat{P}^{(+)}(t) = \hat{P}^{(+)}(t)^2 \sum_{j=0}^{J} \frac{1}{n_j^{(+)}} \left( \frac{d_j^{(+)}}{n_j^{(+)} - d_j^{(+)}} \right) \]

is an estimate of the variance of \( \hat{P}^{(+)}(t) \). Analogous formulas hold for the (-) population.

We still need to make inferences on \( \beta \). Let \( t_{j_1}, \ldots, t_{j_K} \) be the times of relapse; that is, \( j_1, \ldots, j_K \) are the indices of the
patients who have relapsed. Define the partial likelihood to be

\[ L(\beta) = \prod_{k=1}^{K} \frac{\beta^{Z_j(t_j)} e^{\beta Z_j(t_j)}}{\sum_{j \in \mathcal{R}(t_j)} e^{\beta Z_j(t_j)}}. \]

where \( \mathcal{R}(t) = \mathcal{R}^{(+)}(t) \cup \mathcal{R}^{(-)}(t) \) is the risk set of populations (+) and (-) combined. If we treat \( L(\beta) \) as a true likelihood, and define the log likelihood

\[ \ell(\beta) = \log L(\beta), \]

then the locally most powerful test rejects

\[ H_0 : \beta = 0 \quad \text{vs.} \quad H_1 : \beta < 0 \]

whenever \( \frac{\partial \ell(\beta)}{\partial \beta} \bigg|_{\beta=0} \) is small. Define

\[ \mathcal{J}(\beta) = -\frac{\partial^2 \ell(\beta)}{\partial \beta^2}; \]

then \( \frac{\partial \ell(\beta)}{\partial \beta} \bigg|_{\beta=0} \) has the asymptotic distribution \( \mathcal{N}(0, \mathcal{J}(0)) \):

\[ \frac{\partial \ell(\beta)}{\partial \beta} \bigg|_{\beta=0} \sim \mathcal{N}(0, \mathcal{J}(0)). \]

Also, the maximum likelihood estimate \( \hat{\beta} \) is the solution of \( \frac{\partial \ell(\beta)}{\partial \beta} = 0 \), and

\[ \hat{\beta} \sim \mathcal{N}(\beta, \mathcal{J}(\beta)^{-1}). \]

For details the reader is referred to Cox (1972, 1975).
3. Results

Our data consist of 489 patients. Display 2 gives plots of \( \hat{P}^{(+)}(t) \) and \( \hat{P}^{(-)}(t) \). Display 3 gives plots of \( \log \hat{P}^{(+)}(t) \) and \( \log \hat{P}^{(-)}(t) \); one should be a constant multiple of the other if the Cox model is appropriate. Display 4 gives a plot of

\[
\frac{\log \hat{P}^{(+)}(t)}{\log \hat{P}^{(-)}(t)},
\]

and this should be a constant function of \( t \) if the Cox model is appropriate. In view of these plots the Cox model seems reasonable.

Under the Cox model, if \( \beta < 0 \), then \( \hat{P}^{(+)}(t) \) should be greater than \( \hat{P}^{(-)}(t) \). This is the case in Display 2. To test

\[
H_0 : \beta = 0 \quad \text{vs.} \quad H_1 : \beta < 0,
\]

we need

\[
\frac{\partial}{\partial \beta} \ell(\beta) \bigg|_{\beta=0} = -2.61,
\]

which can be regarded under \( H_0 \) as having an approximate standard normal distribution. This gives

\[
p = 0.005.
\]

\( n = 489; \# \text{ of uncensored observations} = 139).\]

To construct confidence intervals, we use the maximum likelihood estimate
\[ \hat{\beta} = -0.450 \]

\[ J(\hat{\beta}) = 33.127 . \]

The relative risk of relapse, \( \gamma = e^\beta \) (i.e., the risk of relapse of (+) relative to (-)) is estimated to be \( \hat{\gamma} = .64 \). A two-sided, 95 percent confidence interval for \( \beta \) is

\[ (\hat{\beta} - 1.96 \sqrt{J(\hat{\beta})^{-1}}, \hat{\beta} + 1.96 \sqrt{J(\hat{\beta})^{-1}}) = (-0.79, -0.11) \]

The corresponding interval for relative risk is (0.45, 0.90).

From the above, the Cox model seems reasonable, and we believe that being in the (+) population does improve the prognosis. But it can be argued that it is not unexpected that DNCB testing does so well; being in the (+) population is correlated with lower stage, as we saw in Display 1, and being in a lower stage is correlated with better prognosis. We need to take out the effect due to stage. To this end, we divide the population by stages and perform the above analysis on each subpopulation.

Displays 6, 7, and 8 for the Stage I subpopulation are analogous to Displays 2, 3, and 4 for the entire population. Because of the small number of observations in this subpopulation it is hard to say if

\[ \frac{\log \hat{P}(+) (t)}{\log \hat{P}(\cdot)(t)} \]

is nearly constant and thus if the Cox model is reasonable. But looking at this ratio for the other subpopulations (Displays 11, 14, and 17) we feel the Cox model is reasonably satisfied for those subpopulations, and there is no reason to believe that the Stage I subpopulation should follow a different model. In Display 6, we see \( \hat{P}(+) (t) > \hat{P}(\cdot)(t) \)
so that we would expect $\beta < 0$ but not with much statistical significance since at, say $t = 1$ year, $\hat{P}^{(+)}(t)$ lies below the upper 95 percent confidence bound of $\hat{P}^{(-)}(t)$. Looking at Display 5, we see that $\hat{\beta} = -1.07$, $\hat{\gamma} = .34$, a 95 percent confidence interval for $\beta$ is $(-2.35, 0.22)$, a 95 percent confidence interval for $\gamma$ is $(.10, 1.24)$, and the p-value for the test $H_0: \beta = 0$ vs. $H_1: \beta < 0$ is $p = 0.05$.

Looking at the other subpopulations in the same way, we see that after removing the effect due to stage, DNBCB testing is a weaker indicator of high risk patients, and the value of DNBCB testing seems to decrease with stage.

4. Conclusion

We have seen that Hodgkin's disease patients with positive DNBCB reactions have a lower rate of relapse than those with negative reactions. Analysis of the data by stages suggests that DNBCB testing is an indicator of relapse for patients of any stage, but that the value of DNBCB testing is greater for those patients in earlier stages of Hodgkin's disease.

References

Display 1

Results of the primary challenge

<table>
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<tr>
<th>Stages</th>
<th>Number of Patients with Positive Reaction on First Challenge</th>
<th>Total Patients</th>
<th>Proportion with Positive Reaction</th>
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<td>Stages I &amp; II</td>
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<td>311</td>
<td>113/311 = 36%</td>
</tr>
<tr>
<td>Stages III &amp; IV</td>
<td>56</td>
<td>220</td>
<td>56/220 = 25%</td>
</tr>
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</table>
Display 2

\( \hat{p}^{(+)}(t) \) and \( \hat{p}^{(-)}(t) \) (based on all patients)

\[
\sqrt{\text{Var} \, \hat{p}^{(+)}(1)} = \sqrt{\text{Var} \, \hat{p}^{(-)}(1)} = 0.015
\]

\[
\sqrt{\text{Var} \, \hat{p}^{(+)}(7)} = \sqrt{\text{Var} \, \hat{p}^{(-)}(7)} = 0.037
\]
Display 3

\[ \log \hat{P}(+) (t) \text{ and } \log \hat{P}(-) (t) \] (based on all patients)
Display 4

\[ \log \frac{\hat{p}^{(+)}(t)}{\log \hat{p}^{(-)}(t)} \] (based on all patients)
### Testing $H_0 : \beta = 0$ vs. $H_1 : \beta < 0$

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<th>$\beta$</th>
<th>$\hat{\gamma}$</th>
<th>$\gamma$</th>
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</table>

**Results after dividing into subpopulations by stage**
Display 6

\( \hat{p}^{(+)}(t) \) and \( \hat{p}^{(-)}(t) \) (based on stage I patients)

\[
\sqrt{\text{Var} \, \hat{p}^{(+)}(1)} = 0.031 \quad \sqrt{\text{Var} \, \hat{p}^{(-)}(1)} = 0.086
\]

\[
\sqrt{\text{Var} \, \hat{p}^{(+)}(5)} = 0.067 \quad \sqrt{\text{Var} \, \hat{p}^{(-)}(5)} = 0.134
\]
Display 7

$\log \hat{p}^{(+)}(t)$ and $\log \hat{p}^{(-)}(t)$ (based on stage I patients)
Display 8

\[
\frac{\log \hat{p}^{(+)}(t)}{\log \hat{p}^{(-)}(t)} \quad \text{(based on stage I patients)}
\]
Display 9

\(\hat{p}^{(+)}(t)\) and \(\hat{p}^{(-)}(t)\) (based on stage II patients)

\[\sqrt{\text{Var} \ \hat{p}^{(+)}(1)} = 0.026 \quad \sqrt{\text{Var} \ \hat{p}^{(-)}(1)} = 0.029\]

\[\sqrt{\text{Var} \ \hat{p}^{(+)}(5)} = 0.045 \quad \sqrt{\text{Var} \ \hat{p}^{(-)}(5)} = 0.051\]
Display 10

$log \hat{p}^{(+)}(t)$ and $log \hat{p}^{(-)}(t)$ (based on stage II patients)
Display 11

\[ \frac{\log \hat{p}(+) (t)}{\log \hat{p}(-) (t)} \]
(based on stage II patients)
Display 12

$\hat{p}^+(t)$ and $\hat{p}^-(t)$ (based on stage III patients)

$\sqrt{\text{Var} \hat{p}^+(1)} = 0.042 \quad \sqrt{\text{Var} \hat{p}^-(1)} = 0.040$

$\sqrt{\text{Var} \hat{p}^+(5)} = 0.085 \quad \sqrt{\text{Var} \hat{p}^-(5)} = 0.068$
Display 13

\[ \log \hat{P}^{(+)}(t) \] and \[ \log \hat{P}^{(-)}(t) \] (based on stage III patients)
Display 14

\[ \frac{\log \hat{p}^{(+)}(t)}{\log \hat{p}^{(-)}(t)} \]  (based on stage III patients)
\( \hat{P}^+(t) \) and \( \hat{P}^-(t) \) (based on stage IV patients)

\[
\sqrt{\text{Var} \hat{P}^+(1)} = 0.104 \quad \sqrt{\text{Var} \hat{P}^-(1)} = 0.115
\]

\[
\sqrt{\text{Var} \hat{P}^+(5)} = 0.145 \quad \sqrt{\text{Var} \hat{P}^-(5)} = 0.133
\]
Display 16

$log \hat{p}^+(t)$ and $log \hat{p}^-(t)$ (based on stage IV patients)
Display 17

\[ \frac{\log \hat{P}^{(+)}(t)}{\log \hat{P}^{(-)}(t)} \] (based on stage IV patients)
### Appendix 1: Stage I patients

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<th>Change-time ((\text{days}))</th>
<th>Lifetime ((\text{days}))</th>
<th>Uncensored (^2)</th>
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1. Each patient is in the (-) population during the time interval \([0, \text{Changetime})\) and in the (+) population during \([\text{Changetime, Lifetime})\).

2. Uncensored \(= 0\) implies the patient was censored at time Lifetime; Uncensored \(= 1\) implies the patient had a recurrence at time Lifetime.
### Appendix 2: Stage 2 patients

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<th>Lifetime (days)</th>
<th>Uncensored</th>
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DOES ADOPTION AFFECT FERTILITY? A PROPORTIONAL HAZARDS MODEL

Sue Leurgans

Medical Problem: Evaluate whether adoption of a child increases the fertility of a couple experiencing infertility problems.

Medical Investigator: Emmett Lamb, Stanford University.

Statistical Procedures: Proportional hazards model; Cox's conditional likelihood analysis; Mantel-Haenszel statistic.

1. Background

Whenever a couple who have adopted a child subsequently conceive, they are often reminded of an old wives' tale that adoption of a child increases fertility. Dr. Emmett Lamb of the Stanford University Department of Gynecology and Obstetrics wished to combine the data in the files of the Stanford Infertility Clinic with the most appropriate statistical techniques to determine if any evidence of this increased fertility could be observed. This question has been of interest for gynecologists. (A recent article is Arronet, Bergquist, and Purekh (1974).) This report describes the statistical methods which were used to answer this question. Lamb and Leurgans (1979) is a less technical discussion of the conclusions.

The next section of this report describes the information which was obtained from the clinic's files. The third section describes the statistical techniques which were applied and explains why they were chosen. The fourth section consists of the results and conclusions. Some limitations of the techniques and some additional questions are formulated in the last section.
2. Information Obtained from the Clinic

Each woman's file was transferred onto one computer card. Each woman is identified by name, medical record number, and referring physician. The date of registration at the clinic is recorded, as is the date of conception or of her last contact with the clinic (whether by visit or by letter). If the couple reported adopting a child, the date at which the child was adopted is given. (Subsequent adoptions are not recorded.) The date of adoption is the date at which the child enters the home. Each record is given a termination code, which indicates whether or not the couple was observed to adopt a child before pregnancy. The termination codes also classify the couples into one of the four following categories: pregnant, censored, lost or withdrawn. A couple is classified as withdrawn if they cease to be at risk of pregnancy due to divorce, sterilization, use of contraceptives, or death of one partner. Those few couples whom Dr. Lamb deemed to be completely sterile for medical reasons (such as both Fallopian tubes completely blocked, or no sperm cells present in the husband's semen) were coded as having been withdrawn at registration. A couple is coded as lost if they have not responded to two letters. (If a couple appears to have moved, the referring physician (if any) is contacted for further information.) A couple is classified as censored if they are still under observation (neither lost nor withdrawn) and have not conceived by the closing date of the study (December 31, 1976).

The rest of the card gives medical information recorded on each woman. At registration, each woman supplies her age and parity and estimates the length of time the couple has been infertile. Menstrual abnormalities, certain medical findings (such as endometriosis or pelvic adhesions) or
previous events (such as previous pelvic inflammatory disease) are also recorded. If any tests were run on husband or wife, the results are coded. Specific therapies (such as surgery or "fertility drugs") are also recorded. See Appendix 1 for a detailed description of the data available.

Initially, there were 1221 cards. Of these cards, 58 corresponded to women who were pregnant when they registered at the clinic. (Dates of conception were recorded as two weeks after the initial day of the last menstrual period.) For these 58 women, the median number of days pregnant at registration was 26, the maximum number 123. These women were omitted from the study. Additional cards (274) were omitted for a variety of technical reasons. Most of these were women who only visited the clinic once, and who could not influence the study. Some of the other cards were duplicates. Table 1 displays the number of women retained in each termination code.

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**Table 1.** Women in the Study Classified by Termination Code.
3. Statistical Techniques

Since different couples are under observation for vastly different amounts of time, it is important to use a technique which incorporates the length of time under study. One of the covariates is adoption status. This is a covariate which can change once with time, when and if the couple does adopt a child. Until a couple does adopt a child, that couple is indistinguishable from those couples who never adopt children. The rest of the covariates are based on the medical history information. The age of the wife and the length of prior infertility are the only medical variables which change with time — and these both change in a deterministic manner. The age of the wife and the duration of prior infertility are also the only variables recorded on a continuous scale; all the other medical covariates are categorical.

This problem is closely related to that of the Stanford Heart Transplant Study. The parallels between the terminology of heart transplants (see Crowley and Hu (1977)) and the language here are given in Table 2.

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<td>Receive a heart for transplant</td>
<td>Adopt a child</td>
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<td>Deselected</td>
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*Table 2:* Correspondences Between the Heart Transplant Problem and the Adoption Problem
Crowley and Hu (1977) found it instructive to use a proportional hazards model for censored life-table data. Clearly, this model can account for the vastly different times of observation. In the form suggested by Cox (1972), the model assumes that a couple whose adoption status and medical covariates at time $t$ are represented by the vector $z(t)$ has hazard rate

\begin{equation}
    e^{\beta'z(t)} \lambda_0(t),
\end{equation}

where $\lambda_0(t)$ is an unknown underlying hazard rate and $\beta$ is an unknown vector of parameters. Asking whether the hazard rate (and hence the cumulative probabilities of conception) depends on a particular coordinate of $z$ is equivalent to asking whether the corresponding coordinate of $\beta$ is nonzero. Cox (1972) advocates a hypothesis test and an estimation procedure based on a factor of the likelihood which depends on $\beta$ and not on the nuisance parameter $\lambda_0$. This approach is supported by the arguments of Cox (1975) and of Efron (1977). The model (1) is suitable for very general covariates. As we shall see, the simple nature of the covariates considered here leads to relatively simple calculations.

In Cox's notation, the covariate vector for the $i$th person at time $t$ is denoted $z_i(t)$. The $\xi$th coordinate of this vector is $z_{\xi i}(t)$. Assuming for the moment that all the event times are distinct and that the subjects are numbered such that the first $k$ subjects are those with whom events (rather than censored observations) are associated, it is natural to denote the event-time of the $i$th subject by $t_i$. With this convention, Cox's partial likelihood equation is
\[ U(\hat{\beta}) = 0, \]

where

\[ U_{\xi}(\hat{\beta}) = \sum_{i=1}^{k} \left[ z_{\xi i}(t_i) - A_{\xi i}(\hat{\beta}) \right]. \]

The risk set at time \( t_i \) (denoted \( R(t_i) \)) is the set of individuals still under observation just before \( t_i \). \( A_{\xi i}(\hat{\beta}) \) is the weighted average of the \( z_{\xi} \)'s in the \( i \)th risk set, with weights proportional to the relative hazard:

\[ A_{\xi i}(\hat{\beta}) = \frac{\sum_{j \in R(t_i)} z_{\xi j} e^{z'_{j}(t_i) \hat{\beta}}}{\sum_{h \in R(t_i)} e^{z'_{h}(t_i) \hat{\beta}}}. \]

Now let us reduce to the case in which the covariate is a category. Note that several categorical variables can be recoded to give one categorical variables. A general parametrization uses one coordinate of \( z \) as an indicator variable for each of \( p \) mutually exclusive categories. If the categories are exhaustive, the covariate vector of each subject is a vector which consists entirely of zeroes, except for a single one. Therefore, for each subject, each time, and each vector \( \beta \), \( e^{z'_{j} \beta} \) must assume one of the \( k \) values \( e^{\beta_{j}} \). Denote these values by \( \gamma_{j} \).

\[ \gamma_{j} = e^{\beta_{j}}, \quad j = 1(1)p. \]

The reader is warned that the notation will be abused below – functions which were defined as functions of \( \beta \) will be written as functions of
\( \gamma \) and vice versa. Since \( \beta \) is transformed to \( \gamma \) and \( \gamma \) to \( \beta \) via 1-1 mappings, pedantic precision is being sacrificed to the clarity inherent in a less cumbersome notation.

For each distinct death time \( t_i \), let \( \eta(i) \) be the vector such that \( n_\xi(i) \) is the number of subjects under observation in category \( \xi \) just before time \( t_i \). In this new notation, (3) becomes

\[
A_{i\xi}(\beta) = \frac{n_\xi(i) \gamma_\xi}{\sum_{\eta=1}^{p} \gamma_{\eta} n_{\eta}(i)}.
\]

Thus \( A_{i\xi}(\beta) \) is the weighted proportion of subjects under observation at time \( t_i \), if the \( \eta \)th category receives weight \( \gamma_{\eta} \). From (4), it is clear that \( (A_{i1}(\beta), \ldots, A_{ip}(\beta))' \) is a probability vector. It will be convenient to denote this vector by

\[
\pi[\gamma,i] = \begin{pmatrix} A_{i1}(\beta) \\ \vdots \\ A_{ip}(\beta) \end{pmatrix}.
\]

Let \( d \) be the \( p \)-vector, whose \( j \)th component is the number of events observed in the \( j \)th category. Using this notation, (2) can be rewritten as the following vector equation:

\[
U(\beta) = d - \sum_{i=1}^{k} \pi[\gamma,i].
\]

Thus, solving (2) for \( \hat{\beta} \) (or \( \hat{\gamma} \)) is equivalent to choosing weights \( \gamma \) such that the observed number of deaths in each category is equal to the sum of that category's weighted proportion of subjects.
\( \gamma \) and \( \beta \) are an overparametrization of the problem. From (5) and the assumption that the \( k \) event times are distinct, \( U(\beta) \) can be shown to be a singular matrix:

\[
\sum_{j=1}^{p} \prod_{j=1}^{k} \frac{d_j}{\pi_j[\gamma, i]} = k - k = 0.
\]

This suggests an ad hoc modification of \( U \) to allow for ties. If \( m_i \) (for multiplicity) is the number of events at the \( i \)th distinct time of event, \( U \) is redefined by

\[
U(\beta) = d - \sum_{i=1}^{k} m_i \pi[\gamma, i].
\]

This modification was the one used in the computations presented in the third section. See Section 5 for a further discussion of ties.

Although there does not exist a closed-form solution for (1), because \( U(\beta) \) is a monotone function of each coordinate of \( \beta \), equation (1) can be readily solved numerically, using the Newton-Raphson method of iteration.

The Newton-Raphson method uses two approximations to compute the \((k+1)\)st-stage estimator \( \hat{\beta}_{k+1} \) from the \( k \)th stage estimator \( \hat{\beta}_k \). The first idea is that if the \( \hat{\beta}_k \) and \( \hat{\beta}_{k+1} \) are close together, and \( D(\beta) \) is the \( p \times p \) matrix of the derivatives of \( U(\beta) \) with respect to the coordinates of \( \beta \), (7) must be nearly true.

\[
D(\hat{\beta}_{k})(\hat{\beta}_{k+1} - \hat{\beta}_k) = U(\hat{\beta}_{k+1}) - U(\hat{\beta}_k).
\]

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Note that since $D$ is the matrix of second derivatives of the partial likelihood, $-D(0)$ can be thought of as an empirical partial information matrix if $\hat{\beta}$ is zero. The second approximation involves an optimistic declaration that the next iteration will nearly result in a solution, and therefore that $U(\hat{\beta}_{k+1})$ can be replaced by zero and (8) is nearly true.

\[(8) \quad D(\hat{\beta}_k)(\hat{\beta}_{k+1} - \hat{\beta}_k) = -U(\hat{\beta}_k).\]

Equation (8) can be used to solve for $\hat{\beta}_{k+1}$ in terms of $\hat{\beta}_k$. In categorical problems with a full parametrization, $D(\hat{\beta}_k)$ will turn out to be singular. Therefore the computations will be done with $\hat{\beta}^*$, the first \((p-1)\) coordinates of $\hat{\beta}$ and $D^*$ the upper \((p-1) \times (p-1)\) major of $D$. This is equivalent to setting $\hat{\beta}_p$ equal to zero. Equation (8) can be used to obtain (9):

\[(9) \quad \hat{\beta}^*_{k+1} = \hat{\beta}^*_k - (D^*(\hat{\beta}^*))^{-1} U^*(\hat{\beta}_k).\]

Now let us turn to the evaluation of $D(\beta)$. It can be shown that, in Cox's notation,

\[(10) \quad D_{\xi \eta}(\beta) = -\sum_{i=1}^{k} m_i \left[ \sum_{l \in G(t_i)} z_{\xi l} z_{\eta l} e^{B^' z_l} - \sum_{l \in G(t_i)} e^{B^' z_l} \right] .

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The term inside the square brackets is Cox's \( C(\xi_{i\eta}) \). As he mentions, \( C(\xi_{i\eta}) \) can be thought of as the covariance of the \( \xi \)th and the \( \eta \)th coordinates of \( z \), with weights \( \varepsilon_{z,\xi} \). Equation (10) can be greatly simplified for the special problem of interest here if the covariance matrix of one observation from a \( p \)-category multinomial with probability vector \( \pi \) is denoted by \( V(\pi) \).

\[
V(\pi) = \begin{pmatrix}
\pi_1 & 0 & 0 \\
0 & \ddots & 0 \\
0 & \cdots & \pi_p
\end{pmatrix} - \pi \pi'.
\]

Equation (12) becomes equation (11):

\[
D(\beta) = -\sum_{i=1}^{k} m_{i} V(\pi(\gamma, i)).
\]

Cox suggests testing significance of the \( \beta \)'s by computing the quadratic form statistic (12)

\[
U(0)^{\prime} (-D(0))^{-1} U(0)
\]

whose asymptotic distribution is \( \chi^{2}_{p-1} \). Expanding this statistic as

\[
- (-D(0))^{-1} U(0)^{\prime} D(0) (-D(0))^{-1} U(0)
\]

and noticing that (9) implies that \( (-D(0))^{-1} U(0) \) is the first iteration estimate of \( \beta^{*} \), if the initial estimate is \( 0 \), we see that (12) is the "length" of the first-iteration estimator of \( \beta^{*} \) in a Euclidean space.
with semi-norm determined by $-D_X(0)$. But, as noted above, $-D_X(0)$ can be thought of as a sample information matrix. Thus the test statistic (12) gives weight to the length of each component of $\beta_{-1}$ in any particular direction in proportion to the amount of sample partial information in that direction. Thus if the first iteration from zero results in a good estimate, the statistic (12) is reasonable. Otherwise, a log-likelihood ratio statistic would be preferable, since its justification does not require the assumption that the first iteration estimate is close to the solution. However, the log-likelihood ratio statistic can not be computed until an accurate solution to (6) has been obtained. The quadratic form statistic can be computed without any iteration.

Many of the analyses below focus on all the people with a particular medical condition (such as anovulation). Therefore it will be useful to consider the simplest case of the time-changing proportional hazards model, in which the univariate covariate changes from one of two possible values to the other possible value at most once. This corresponds to a two population problem in which each subject can switch populations once. In this study, one population is the population of couples which have never adopted and the other population is composed of those couples who have adopted a child.

Formally, this restriction corresponds to the categorical case with $p = 2$. Cox's quadratic form to test whether $\gamma = 1$ reduces to

$$
\left( \frac{d_1 - \sum_{i=1}^{k} m_i n_1(i) + n_2(i)}{\sqrt{\sum_{i=1}^{k} m_i (n_1(i) + n_2(i))^2}} \right)^2.
$$

(13)
This statistic is suggestively close to the statistic suggested by Mantel and Haenszel for combining many 2×2 tables. In the notation of this report, (14) is a form of the Mantel–Haenszel statistic derived by Hyde (1977):

\[
\frac{\left( d_1 - \sum_{i=1}^{k} \frac{m_i}{n_1(i) + n_2(i)} \right)^2}{\sum_{i=1}^{k} \frac{m_i}{(n_1(i) + n_2(i))^2} \left[ 1 - \frac{m_i}{n_1(i) + n_2(i)} \right]} \tag{14}
\]

The only difference between (13) and (14) is the presence in the denominator of (13) of a term in brackets, \((1 - m_i/(n_1(i) + n_2(i)))\). Since this term is always strictly less than 1, the denominator of (13) is always greater than that of (14). Therefore (13) is always less than or equal to (14), and the approximate level-α test based on (13) is slightly more conservative than the comparable test based on (14). The expression Cox suggested for handling ties is actually slightly different:

\[
\frac{\left( d_1 - \sum_{i=1}^{k} \frac{m_i}{n_1(i) + n_2(i)} \right)^2}{\sum_{i=1}^{k} \frac{m_i}{(n_1(i) + n_2(i))^2} \left[ \frac{n_1(i) + n_2(i) - m_i}{n_1(i) + n_2(i) - 1} \right]} \tag{15}
\]

The statistic (15) is the Mantel–Haenszel statistic. The numerator can be thought of as the difference between the number of events in the first category and the expected number of events in the first category under a hypergeometric model at each event time. The denominator is the sum of the hypergeometric variances at each death time.
If there are no ties, (15) reduces to (13). In general, (15) leads to a more conservative test than (14), although less conservative than (13). In what follows, (14) is used for the two sample tests. Use of (14) is supported by proofs in Hyde (1977) and Crowley (1974) that this test has the correct asymptotic level in the heart transplant problem. Hyde's proof requires neither absolutely continuous distributions nor identical censoring mechanisms in the two populations. The continuity correction is not applied. Iterative estimation used the form corresponding to (14). The quadratic form statistic suggested by Cox is used when more than one population is considered simultaneously.

4. Results

Dr. Lamb identified those variables which he either knew to be or thought could be related to conception rate (see Table 3). Each of these variables was analyzed separately. First the population was divided into two groups according to the variable under consideration (such as ovulatory or anovulatory). See Appendix 2 for precise definitions of the populations. The square root of the statistic (14) (with the obvious sign convention) was used within each population to test the null hypothesis that adoption does not affect the conception rate. The couples who had not adopted are the first population, those couples who had adopted (before the event time under consideration) are the second. Therefore a positive number in the second column of Table 3 corresponds to an excess number of events in the first population, that is, more conceptions than expected among the couples who had not adopted. If the old-wives'
<table>
<thead>
<tr>
<th>Medical Variable</th>
<th>Mantel-Haenszel $\sqrt{14}$</th>
<th>Estimate of $\hat{\beta}$ (number of iterations)</th>
<th>Estimate of $\hat{\gamma}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years Infertile at Registration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 2.9</td>
<td>1.083</td>
<td>.253</td>
<td>1.287</td>
</tr>
<tr>
<td>3.0 or more</td>
<td>.695</td>
<td>.224 (4)</td>
<td>1.251</td>
</tr>
<tr>
<td>Age at Registration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 - 29</td>
<td>-.323</td>
<td>-.069</td>
<td>.933</td>
</tr>
<tr>
<td>30 - 39</td>
<td>2.045*</td>
<td>1.038 (5)</td>
<td>2.823</td>
</tr>
<tr>
<td>Semen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>.725</td>
<td>.193 (4)</td>
<td>1.213</td>
</tr>
<tr>
<td>Abnormal</td>
<td>.688</td>
<td>.624</td>
<td>1.866</td>
</tr>
<tr>
<td>Tubes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>.132</td>
<td>.043</td>
<td>1.043</td>
</tr>
<tr>
<td>Abnormal</td>
<td>-.220</td>
<td>-.065</td>
<td>.937</td>
</tr>
<tr>
<td>Ovulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulatory</td>
<td>.953</td>
<td>.242 (5)</td>
<td>1.274</td>
</tr>
<tr>
<td>Anovulatory</td>
<td>-1.852</td>
<td>-.052 (3)</td>
<td>.949</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never pregnant</td>
<td>.035</td>
<td>.007 (2)</td>
<td>1.007</td>
</tr>
<tr>
<td>Previous pregnancy to term</td>
<td>1.119</td>
<td>.58 (10)</td>
<td>1.786</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific</td>
<td>.920</td>
<td>.223</td>
<td>1.250</td>
</tr>
<tr>
<td>No specific</td>
<td>-.420</td>
<td>-.124</td>
<td>.883</td>
</tr>
</tbody>
</table>

*1.854 with continuity correction

Table 3
tale is supported by this data set, the numbers in the second column of Table 3 should be significantly negative. This is not the case.

The third column is an estimate of \( \beta \). In some cases, this estimate is the first iteration estimate. In other cases, the number of iterations is given in parentheses. (The termination criterion is that the iteration not change the thousandths place of \( \hat{\beta} \).) In most cases in which an iterative estimate was obtained, it was moderately close to the first iteration estimate. The fourth column of the table is \( \hat{\gamma} \), which is \( e^{\hat{\beta}} \). \( \hat{\gamma} \) is the estimated ratio of the risk of conception in the couples before adoption to the risk of conception in the couples after adoption. The old-wives' tale implies that \( \hat{\beta} \) should be negative and \( \hat{\gamma} \) should be less than one. This does not appear to be the case. Table 3 also suggests what magnitudes of risk ratio might be detected by this method. There does not appear to be a calculation which can be used to support this statement rigorously, but it appears that if the underlying \( \beta \) were either \( \pm 1 \), this would be detected in several of these significance tests. Thus Table 3 does not support the old-wives' tale.

The quadratic form statistic (12) was also applied directly. Each medical variable can be combined with adoption category to give a set of four categories. The statistic (12) can then be used to test that each of these four categories has the same conception rate (as a function of time). Under the null hypothesis, the quadratic form statistic has a \( \chi^2 \) distribution. Table 4 gives the quadratic form statistics.
<table>
<thead>
<tr>
<th>Years Infertile</th>
<th>21.952</th>
<th>Percentage Points:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Registration</td>
<td>8.816</td>
<td>$\chi^2(0.25) = 4.11$</td>
</tr>
<tr>
<td>Semen</td>
<td>6.797</td>
<td>$\chi^2(0.10) = 6.25$</td>
</tr>
<tr>
<td>Tubes</td>
<td>5.793</td>
<td>$\chi^2(0.05) = 7.81$</td>
</tr>
<tr>
<td>Ovulation</td>
<td>1.308</td>
<td>$\chi^2(0.025) = 9.35$</td>
</tr>
<tr>
<td>Parity</td>
<td>1.479</td>
<td>$\chi^2(0.005) = 12.84$</td>
</tr>
<tr>
<td>Therapy</td>
<td>1.102</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.** Quadratic form test statistics.

From Table 4, we see that years infertile and age at registration give strongly significant statistics. (These two variables are necessarily positively correlated.) Since the only significant Mantel-Haenszel statistic associated with these variables is in the direction which contradicts the old-wives' tale, the significance (particularly of years infertile) is more plausibly associated with the medical factor than with adoption. The medical factors semen and tubes approach significance in Table 4. Since neither of these medical factors generates any remotely significant Mantel-Haenszel statistics, it is more plausible to conjecture that the medical factor changes conception rates than that adoption changes the conception rate. Ovulation, parity and therapy do not have even slightly significant test statistics.
Appendix 3 gives the total population sizes for each category used in Table 4. These numbers cannot be used to construct a valid test of the effect of adoption, since these totals do not reflect the lengths of time the women were under observation. However, Appendix 3 can be used to see how large a sample the comparisons are based on.

Thus it appears that there is no variability in conception rate which must be ascribed to adoption. This does not prove that the old-wives' tale is false. However, since we have no evidence of its truth, it can be dismissed as a non-scientific notion. Of course, as with any observational study, those who have faith in the association of adoption with fertility can claim that there are other explanations for our results which leave the old-wives' tale intact. The key to this argument is the fact that adoption was not allocated randomly among the couples who sought Dr. Lamb's help. Not only are adoption agencies unlikely to practice randomization, but Dr. Lamb did not withhold his opinion. He indicates to couples in his clinic roughly how fertile he thinks they are. This presumably influences how energetically the couple attempts to adopt. While controlling for medical factors largely compensates for this, there is no way of eliminating such influences entirely. Human beings are unlikely to consent to an experiment in which they are randomly allowed to adopt. Furthermore, it is not possible to invent a placebo for this situation.

5. Theoretical Considerations

If every idea latent in the papers and minds of contemporary statisticians could be applied to the questions outlined below, this section
would probably not be necessary. Unfortunately, these latent ideas are not developed enough to be applied to current problems. We shall discuss the problem of tied observations and the adequacy of the proportional hazards model.

No energy was expended in the investigations of the treatment of ties. Asymptotically, for the two-sample problem, (14) has been shown to be an adequate test of the null hypothesis of equality. However, in general, there is debate about the appropriate likelihood (and approximations) to be maximized in the case of ties. Vaeth (1977) compared the suggestions of Efron (1979), of Peto (1972), and of Cox (1972). They are similar, but not identical. Their properties do not seem to have been compared, although Efron's suggestion is more exact.

The adequacy of the proportional hazards model is quite difficult to test. In the case of two populations where the classification is independent of time, cumulative hazard plots have become a standard technique. Cumulative hazard plots are based on the fact that if $\Lambda$ is the cumulative hazard function of the event times $T_1, \ldots, T_n$, the random variables $\Lambda(T_{(1)}), \ldots, \Lambda(T_{(n)})$ are distributed as exponential order statistics. These ideas can be used to compare the empirical cumulative hazards and see if they appear to be proportional. The difficulty is that the very idea of order statistics is quite ambiguous if the subjects can shift population. Even the total population sizes are stochastic. Thus this approach is not auspicious, particularly in situations such as this where one of the populations is much smaller than the other, and is indeed, composed entirely of individuals who began in the first population.
Acknowledgements

It is a pleasure to acknowledge the practical suggestions and discerning questions of Dr. Emmet Lamb. Professor Rupert Miller contributed liberally of his statistical insight. The work described in this report was commenced on an NIH Traineeship and completed on an NSF Graduate Fellowship.

References


Appendix 1

Columns

20-24  Medical Record Number
25-26  Age at registration
27     Number of term pregnancies
28     Number of premature pregnancies
29     Number of abortions
30     Number of living children
33-36  Code for referring physician
37-42  Registration date
44-49  Last contact date (estimated conception date if pregnant)

50     Findings
     1  Endometriosis
     2  Myoma
     3  Sclerocystic ovaries
     4  Pelvic adhesions
     5  Atrophic ovaries
     6  Pelvic mass
     7  Ovarian cyst
     9  Two of above
     0  None of above

51     Miscellaneous History
     1  DES exposure
     2  Previous pelvic surgery
     3  Previous pelvic inflammatory disease
     4  Serious medical problem
     5  Previous tubal sterilization
     6  Weight gain (obesity)
     7  Weight loss
     8  Psychiatric problem, severe
     9  Two of above
     0  None of above
52 Miscellaneous History
1 IUD use
2 Hirsutism
3 Virilization
4 Galactorrhea, other breast disease
5 Sexual precocity
6 Congenital defects – hemaphrodite
7 Pelvic gain
8 Pregnancy problem – male
9 Two of above
0 None of above

53 Menses
1 Secondary amenorrhea over 6 months
2 Obligomenorrhea
3 Anovulatory; dysfunctional bleeding
4 Dysmenorrhea, mittleschmerz
5 Menopause
6 Primary amenorrhea
7 Normal (before 1973)
9 Two of above
0 Normal

54 Endometrium
1 Atrophic or insufficient
2 Proliferative
3 Polyp-hyperplasia
4 Secretory
5 Other (biopsy)
6 Hysteroscopy (abnormal)
9 Two of above
0 None of above

55 Testes
1 PKT only – normal
2 PKT only – abnormal
3 Semen analysis – normal
4 Semen analysis – abnormal
5 Duke’s test normal
6 Duke’s test abnormal
7 Tests – biopsy
8 Two of above – both normal
9 Two of above – one abnormal
0 None of above
56 Adrenal
1 17-kosteroids - normal
2 17-kosteroids - abnormal
3 17-OHCS or cortisol - normal
4 17-OHCS or cortisol - abnormal
5 Special tests - P3, KS fract.
6 Special tests - suppression
8 Two of above - both normal
9 Two of above - one abnormal
0 None of above

57 Ovary
1 BBT only - abnormal
2 BBT only - normal
3 Pelvic pneumogram - normal
4 Pelvic pneumogram - abnormal
5 Testost. or Andost.
6 Estrogen (urine or serum)
8 Two of above - both normal
9 Two of above - one abnormal
0 None of above

58 Ovary
1 Estrogen assay
2 Pregnanedial assay
3 Serial Pap or mucus
4 FSH abnormal
5 LH abnormal
6 FSH and LH normal
7 PRL
8 Two of above - both normal
9 Two of above - one abnormal
0 None of above

59 Tube
1 Rubin's only - normal or abnormal
2 HSG - normal [hyspaniogram]
3 HSG - abnormal
4 HSG - inadequate
5 Culdoscopy/Laparoscopy, normal
6 Culdoscopy/Laparoscopy, abnormal
7 Culdoscopy/Laparoscopy, inadequate
8 Two of above - both normal
9 Two of above - one abnormal
0 None of above
Pituitary
1 Urine gonadotropin - normal
2 Urine gonadotropin - high
3 Urine gonadotropin - low
4 Urine gonadotropin - mixed
5 Sella - normal
6 Visual fields - normal
7 Visual fields and/or sella - abnormal
8 Two of above - both normal
9 Two of above - one abnormal
0 None of above

Follow-up Code
1 Pregnant
2 Permanently lost
4 Withdrawn
6 Adopted, then withdrawn
7 Adopted
8 Adopted, then pregnant
9 Adopted, then permanently lost
0 None of above

Primary cause of infertility (not coded for all women)
1 Unknown
2 Male
3 Female
4 Ovulation
5 Uterine - cervical
6 Exposure
7 Combination of any two female and male
8 Combination of any two female only
9 Psychiatric
0 Not coded

Therapy
1 None
2 Non-specific
3 Psychotherapy
4 Laparotomy (Surgery)
5 Gonadotropins
6 Climophene
7 Corticosteroids
8 Other specific prescription
9 Two of above
64  Thyroid, Miscellaneous Tests
1  Thyroid test - normal
2  Thyroid test - abnormal
3  Chromosomes
4  Buccal smear
5  Carotone
6  Hypothalmus test
8  Two of above - both normal
9  Two of above - one abnormal
0  None of above

65-70  Date of adoption (if any)
71-75  Years infertile (to nearest tenth of year)
76-80  Sequence Number
Appendix 2: Definitions of Populations

Semen
Normal: 3, 5, or 8 in column 55
Abnormal: 4 or 7 in column 55

Tubes
Normal: 2, 5, or 8 in column 59
Abnormal: 3, 6, or 9 in column 59

Ovulation:
Yes: 0, 4, 7, in column 53
No: Any other code in column 53

Parity
Never Pregnant: 0000 in columns 27-30
Previous Pregnancy to term: Nonzero code in column 30

Therapy
Specific: 4, 5, 6, 7, or 8 in column 63
Not specific: 0, 1, 2, or 3 in column 63
## Appendix 3

### Population Sizes

<table>
<thead>
<tr>
<th>Medical Factor</th>
<th>Number of Pregnancies Before Adoption</th>
<th>Number Never Pregnant, No Adoption</th>
<th>Number Pregnant after Adoption</th>
<th>Number Adopted, Never Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Years Infertile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 2.9</td>
<td>211</td>
<td>410</td>
<td>27</td>
<td>69</td>
</tr>
<tr>
<td>3.0 or more</td>
<td>118</td>
<td>360</td>
<td>12</td>
<td>53</td>
</tr>
<tr>
<td><strong>Age at Registration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>221</td>
<td>504</td>
<td>35</td>
<td>93</td>
</tr>
<tr>
<td>30-39</td>
<td>96</td>
<td>232</td>
<td>4</td>
<td>28</td>
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<tr>
<td><strong>Semen</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>159</td>
<td>340</td>
<td>19</td>
<td>55</td>
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<tr>
<td>Abnormal</td>
<td>31</td>
<td>104</td>
<td>1</td>
<td>10</td>
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<tr>
<td><strong>Tubes</strong></td>
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<tr>
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<td>88</td>
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<tr>
<td>Abnormal</td>
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<td>64</td>
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<tr>
<td><strong>Ovulation</strong></td>
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<tr>
<td>Ovulating</td>
<td>208</td>
<td>521</td>
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<td>73</td>
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<tr>
<td>Anovulatory</td>
<td>122</td>
<td>248</td>
<td>18</td>
<td>49</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Never Pregnant</td>
<td>199</td>
<td>458</td>
<td>30</td>
<td>90</td>
</tr>
<tr>
<td>Previous Pregnancy to term</td>
<td>74</td>
<td>193</td>
<td>4</td>
<td>15</td>
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<tr>
<td><strong>Therapy</strong></td>
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<tr>
<td>Specific</td>
<td>212</td>
<td>518</td>
<td>21</td>
<td>68</td>
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<tr>
<td>No Specific</td>
<td>117</td>
<td>249</td>
<td>18</td>
<td>54</td>
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</tbody>
</table>
KANAMYCIN LEVELS IN PREMATURE BABIES

Rupert G. Miller, Jr.

Medical Problem: Can kanamycin be accurately measured from an umbilical catheter rather than from a heel venapuncture?

Medical Investigator: Charles Prober, Stanford University.

Statistical Procedures: Structural relationship; errors in variables; jackknife.

1. Background

Premature babies are extremely susceptible to infections. In the Stanford Medical Center Intensive Care Nursery kanamycin, which is an aminoglycoside, is being used for the treatment of sepsis. Because kanamycin is ineffective at low levels and has potentially harmful side effects at high levels, it is necessary to constantly monitor its level in a premature baby's blood during treatment.

The standard procedure for measuring serum kanamycin levels is to take blood samples from a finger or heel, and a heelstick has been customarily used at Stanford. Unfortunately, due to the necessity of frequently drawing samples, this leaves neonates with badly bruised heels.

Kanamycin is routinely administered through an umbilical catheter. An alternative procedure to a heelstick for measuring the serum kanamycin level is to reverse the flow in the catheter and draw a blood sample from it. However, physicians at Stanford and elsewhere are reluctant
to rely on concentrations measured from blood obtained through the catheter. There is concern that the level in blood drawn from the point of infusion may be elevated above the level at more distant points in the body. Also, the wall of the catheter might have residual amounts of kanamycin attached to it, which would raise the level as the blood is drawn back through the catheter.

2. Experiment and Data

The aim of this experiment was to see if a blood sample carefully drawn from the catheter would give as accurate a reading of serum kanamycin as the standard heelstick method. If true, this would eliminate the unnecessary trauma to the newborn of repeated venapunctures.

A three-way stop cock system was devised for the catheter so that 5cc of blood could be drawn back through the line to flush it. Then, a .5cc sample of blood would be drawn for the kanamycin assay. Once the .5cc sample was drawn, the flow in the catheter would be returned to its normal direction and the 5cc of blood infused back into the baby.

Simultaneous measurements through the just described system and the heelstick method were obtained on twenty babies. Kanamycin was infused over a one hour period every twelve hours, and the samples were drawn immediately at the end of an infusion period.

The twenty pairs of heelstick and catheter values are presented in Table 1. Figure 1 is a graphical display of these twenty pairs.
<table>
<thead>
<tr>
<th>Baby</th>
<th>Heelstick</th>
<th>Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.0</td>
<td>25.2</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>26.8</td>
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<td>18.0</td>
</tr>
<tr>
<td>20</td>
<td>13.8</td>
<td>15.6</td>
</tr>
</tbody>
</table>

Table 1. Serum kanamycin levels in blood samples drawn simultaneously from an umbilical catheter and a heel venapuncture in twenty babies.

3. First Analysis

When Charles Prober initially came to me, he had the first 16 pairs of serum kanamycin levels; the last 4 were added later. As presented to me, the goal was to establish whether or not the catheter value differed from the heelstick value by no more than would be expected.
Figure 1. Plot of the twenty pairs of serum kanamycin levels from Table 1.
from laboratory variability. If this were so, then physicians would be willing to use the catheter value in place of the heelstick value.

The question was phrased according to the way the physicians thought about it. It was necessary to convert this into a more precise statistical problem.

A very naive first step was to hypothesize that the catheter and heelstick values represented duplicate measurements on the same true value. Their squared difference would be an estimate of twice the laboratory variance if indeed the catheter level was no different from the heelstick level. Letting \( x_i \) equal the heelstick value for the \( i \)th baby and \( y_i \) the catheter value, this approach gives

\[
\hat{\sigma}^2 = \frac{1}{16} \sum_{i=1}^{16} (x_i - y_i)^2 ,
\]

(1)

\[= 8.63 .\]

The laboratory had run a control serum through the assay more than 100 times and reported a plus-and-minus two standard deviation interval of 18.4 to 21.6. This is equivalent to a standard deviation of .8, which is not at all commensurate with the estimated standard deviation 2.08 from (1).

The fact that these two estimates do not agree is not surprising, and there are many potential explanations for the disparity. The catheter and heelstick samples are exposed to sources of variation which the carefully maintained laboratory standard is not. Also, the catheter value could systematically differ from the heelstick value, but still could be substituted for it after correction for bias. This latter possibility was explored through a regression analysis.
Regressing the catheter level \( y \) on the heelstick level \( x \) under the model \( y = \alpha + \beta x + e \) gives the least squares estimates

\[
\hat{\alpha} = 5.42, \quad \hat{\beta} = .73, \quad \hat{\sigma}_e^2 = 7.92.
\] (2)

The slope .73 is less than 1, but the difference is not statistically significant. Presumably, the regression relation \( y = \hat{\alpha} + \hat{\beta}x \) could be used to convert a catheter level into a heelstick value.

Interest at this time still centered on the amount of variability. Adjustment for a slope and intercept different from 1 and 0, respectively, reduces the variability between \( x \) and \( y \) from 8.63 to 7.92. The lower and upper limits for a 95% confidence interval for \( \sigma_e^2 \) based on the \( \chi^2 \) distribution are

\[
\frac{\hat{\sigma}_e^2 \times 14}{\chi^2_{14}(.975)} = 4.25 \quad \text{and} \quad \frac{\hat{\sigma}_e^2 \times 14}{\chi^2_{14}(.025)} = 19.71,
\] (3)

where \( \chi^2_{14}(p) \) is the \( p \)-percentile point of a \( \chi^2 \) distribution with 14 degrees of freedom.

A Q-Q plot (i.e., probit plot) of the residuals reveals them to be reasonably normally distributed; see Figure 2. Although by visual inspection of Figure 1 baby no. 2 is a bit of an outlier, it does not have the largest residual because it has substantial influence in pulling the slope below 1. The largest residual (5.52) corresponds to baby no. 12, and this is not particularly an outlier in Figure 2.
Figure 2. Probit or Q-Q plot of the 16 residuals.
Because the $\chi^2$ test for variances is known to be so sensitive to normality I decided to run the jackknife on the variance. For a reference on the jackknife the reader is referred to Miller (1974). The 16 deleted estimates and pseudo-values are given in Table 2. The jackknifing is performed on the logarithms of the variances because this usually produces better results. The jackknifed estimate of $\ln \sigma^2$ is

$$\ln \hat{\sigma}_e^2 = \frac{1}{16} \sum_{i=1}^{16} (16 \ln \hat{\sigma}_e^2 - 15 \ln \hat{\sigma}_e^2 (-i) )^2,$$

$$= 2.19,$$

which gives $\hat{\sigma}_e^2 = 8.90$. The standard deviation of the pseudo-values is 1.47 so the 95% confidence interval for $\ln \sigma^2$ is

$$2.19 \pm (1.96)(1.47)/\sqrt{4},$$

or 1.47 and 2.91. Untransforming these values gives the confidence interval 4.33 to 18.29. This is remarkably close to the $\chi^2$ confidence interval (3).
<table>
<thead>
<tr>
<th>Baby No.</th>
<th>Deleted Estimate $\hat{\sigma}_e^2(-i)$</th>
<th>Pseudo-value $16 \ln \hat{\sigma}_e^2 - 15 \ln \hat{\sigma}_e^2(-i)$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4</td>
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<td>7.67</td>
<td>2.54</td>
</tr>
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<td>6</td>
<td>8.24</td>
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</tr>
<tr>
<td>16</td>
<td>8.37</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Table 2: Estimates of $\sigma_e^2$ from the regression analysis with the $i$th baby deleted and the corresponding pseudo-values for the logarithm of $\sigma_e^2$ for the first 16 babies.

The general conclusion from this analysis was that the variability was too high to convince the physicians to convert to the umbilical catheter measurement as standard procedure. However, while these computations were being run and Charles Prober was gathering data on four more babies, I was coming to the conclusion that the problem was ill-posed by the physicians. I convinced Charles Prober to look at the problem differently and to consider the results of a second analysis.
4. Second Analysis

The heelstick value is not the true serum kanamycin level in the baby as presupposed in the preceding regression analysis. It is subject to sampling and assay errors just as the catheter value is. Thus, it seems more reasonable to admit that there is error in the measurements from both the umbilical catheter and the heel venapuncture, and the size of these errors is not so important. The crucial question is whether the two are measuring the same thing.

This leads into a classical "structural relationship" or "errors in variables" analysis. For reference the reader is referred to Kendall and Stuart (1961), pp. 377-382. The model assumes the correct heelstick and catheter levels in the ith baby are $x_i$ and $y_i$, respectively, but these cannot be observed without error. The observable quantities are $\xi_i$ and $\eta_i$, which are related to $x_i$ and $y_i$ by

$$\xi_i = x_i + \delta_i \quad \text{and} \quad \eta_i = y_i + \epsilon_i.$$  \hspace{1cm} (6)

The errors $\delta_i$ and $\epsilon_i$ are due to sampling, assay, etc. The correct levels $x_i$ and $y_i$ are related through the structural relation

$$y_i = \alpha + \beta x_i.$$ \hspace{1cm} (7)

(In Kendall and Stuart's (loc. cit.) notation $\alpha = \alpha_0$ and $\beta = \alpha_1$.) The basic question of whether the two different methods of measurement are equivalent reduces to whether $\alpha$ equals zero and $\beta$ equals 1.
The underlying probability structure assumes that the pairs \((x_i, y_i)\) are independently, identically distributed with \(x_i\) having mean \(\mu\) and variance \(\sigma_x^2\). The errors \(\delta_i\) and \(\varepsilon_i\) are assumed to be identically distributed, each according to its own distribution with mean 0 and variance \(\sigma_\delta^2\) or \(\sigma_\varepsilon^2\), respectively, and to be independent of each other and the pairs \((x_i, y_i)\).

If the \(\xi_i, \eta_i\) are also assumed to have normal distributions, then the maximum likelihood equations for the unknown parameters are

\[
\hat{\mu} = \bar{\xi}, \quad \hat{\alpha} + \hat{\beta} \hat{\mu} = \bar{\eta},
\]

\[
\hat{\sigma}_x^2 + \hat{\sigma}_\delta^2 = S_{\xi}^2, \quad \hat{\beta}^2 \hat{\sigma}_x^2 + \hat{\sigma}_\varepsilon^2 = S_{\eta}^2,
\]

\[
\hat{\beta} \hat{\sigma}_x^2 = S_{\xi \eta},
\]

where \(\bar{\xi}, \bar{\eta}, S_{\xi}^2, S_{\eta}^2,\) and \(S_{\xi \eta}\) are the sample means, variances, and covariance of the observable \(\xi\) and \(\eta\) variables. For non-normal populations the equations \((8)\) are method of moments equations. Unfortunately, without an additional assumption these equations do not have a unique solution because they are five equations in six unknowns.

Extra information is needed to single out a unique solution to \((8)\). Often some other information is available about \(\sigma_\delta^2, \sigma_\varepsilon^2\), or their ratio, and that is the case here. These variances quantify the amount of error involved in measuring the level of serum kanamycin in the blood at the heel and umbilical artery, respectively. They include
blood sampling error, variability in the handling process, and assay error.

However, since both samples are treated in an identical fashion, it seems
safe to assume that $\sigma_\delta^2 = \sigma_c^2$ or, equivalently, $\lambda = \sigma_c^2/\sigma_\delta^2 = 1$. When
the ratio $\lambda$ is known, the solution to (8) for $\hat{\beta}$ is
\[
\hat{\beta} = \frac{(s_\eta^2 - \lambda s_\delta^2) + \{(s_\eta^2 - \lambda s_c^2)^2 + 4\lambda s_\delta^2\}^{1/2}}{2 s_\delta \eta}, \tag{9}
\]
and the estimates for the other parameters follow from (8).

By the time of the second analysis the data for all 20 babies were
available, and the estimates are
\[
\hat{\alpha} = -1.16, \quad \hat{\beta} = 1.07, \\
\hat{\sigma}_\delta^2 = \hat{\sigma}_c^2 = 4.60, \tag{10}
\]
\[
\hat{\sigma}_x^2 = 21.4, \quad \hat{\sigma}_y^2 = 24.5.
\]
The line with $\hat{\alpha}$ and $\hat{\beta}$ from (10) is drawn in Figure 1.

The estimates for $\alpha$ and $\beta$ in (10) are very close to 0 and 1,
respectively, which seems to indicate that the serum kanamycin levels
are the same whether measured from a heelstick or an umbilical catheter.
In this connection it is interesting to note that the regression esti-
mate of the slope based on 20 babies is .88. In ordinary regression
when the $x$ variable is subject to error the slope estimate converges to
\[
\beta \times \frac{\sigma_x^2}{\sigma_x^2 + \sigma_\delta^2} \tag{11}
\]
Using the estimates from (10), expression (11) equals .88, which offers
an explanation of why the regression slope is less than 1 in the first
analysis.
The original naive estimate of the error in each variable from (1) was 4.32. This is very similar to the 4.60 obtained from the analysis of the structural relationship. It would seem that the measurement error associated with drawing a blood sample in the nursery and having it assayed in the lab is considerably higher than what is reported for the laboratory standard.

The jackknife can be used to assess the variability in the estimates (10); see Brillinger (1966) for related discussion. The jackknifed estimates of the intercept, slope, and logarithms of the variances together with the jackknifed standard errors are presented in Table 3. For each parameter \( \theta \) the jackknifed estimate is \( \tilde{\theta} = \Sigma_1^n \tilde{\theta}_i / n \) where \( \tilde{\theta}_i = n \hat{\theta} - (n-1) \hat{\theta}_{-i} \), \( i = 1, \ldots, n \), and the jackknifed standard error is the square root of \( \frac{n}{\Sigma_1^n (\tilde{\theta}_i - \tilde{\theta})^2 / n(n-1)} \).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jackknifed Estimate</th>
<th>Jackknifed Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>-.40</td>
<td>4.97</td>
</tr>
<tr>
<td>( \beta )</td>
<td>1.03</td>
<td>.26</td>
</tr>
<tr>
<td>( \ln \sigma^2 )</td>
<td>1.80</td>
<td>.59</td>
</tr>
<tr>
<td>( \ln \sigma_c^2 )</td>
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<td>.37</td>
</tr>
<tr>
<td>( \ln \sigma_x^2 )</td>
<td>3.22</td>
<td>.30</td>
</tr>
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</table>

Table 3. Jackknifed estimates and their standard errors.

The jackknife 95% confidence interval for \( \beta \) is (.52, 1.54). By way of contrast it is possible to construct a confidence interval for \( \beta \) based on normal theory assumptions; see Kendall and Stuart (1961), pp. 388-390. The interval is the transform of
\[
\tan^{-1} \beta \in \tan^{-1} \hat{\beta} \pm \frac{1}{2} \sin^{-1} \left\{ 2 t_{n-2}^{\alpha/2} \left\{ \frac{\frac{s^2}{\xi} - \frac{s^2}{\eta} - \frac{s^2}{\xi \eta}}{(n-2)\left(\frac{s^2}{\xi} - \frac{s^2}{\eta}\right)^2 + 4\frac{s^2}{\xi \eta}} \right\}^{1/2} \right\}, \tag{12}
\]

where \( t_{n-2}^{\alpha/2} \) is the \( 1 - (\alpha/2) \) percentile point of the \( t \) distribution with \( n-2 \) degrees of freedom. In this case the 95\% normal confidence interval is \((.76, 1.52)\).

The outcome of this second analysis was that, when I last spoke with Charles Prober, he indicated that the Stanford Intensive Care Nursery was beginning to switch to the routine use of carefully drawn umbilical catheter samples. This use would be monitored, and some heelstick samples would continue to be drawn in the future. A paper for submission to a medical journal was being prepared.

5. Concluding Remarks

(1) Baby no. 2 is troublesome. By eye it is a potential outlier in Figure 1. The Mahalanobis distance between it and the other 19 points is

\[
D^2 = (33.2 - 20.2, 26.0 - 20.9) \left( \begin{array}{cc} 18.56 & 20.69 \\ 20.69 & 29.36 \end{array} \right)^{-1} \left( \begin{array}{c} 33.2 - 20.2 \\ 26.0 - 20.9 \end{array} \right), \tag{13}
\]

\[
= 23.04 ,
\]

which is substantial. However, there was nothing unusual about baby no. 2, and no reason could be found to suspect the validity of its measured levels.
If baby no. 2 is discarded, then the line for the structural relation becomes

\[ \hat{\alpha}_2 = -5.26, \hat{\beta}_2 = 1.29. \quad (14) \]

This suggests slight underestimation by catheter for low values and overestimation for high values. However, this line is heavily influenced by babies nos. 17 and 18 with the two highest catheter levels; they are no longer counterbalanced by baby no. 2 with the highest heelstick level.

It was decided to retain baby no. 2 in the analysis. However, his influence was noted, and the possibility that the umbilical catheter might not yield identical levels to the heel venapuncture is being kept in mind as babies' serum levels are measured in the future.

(2) A suggestion was made in the Biostatistics Workshop to run a weighted jackknife on the data a la Hinkley (1977) for regression analysis. This would dampen the influence of the outlying babies without requiring in-or-out decisions. Unfortunately, the theoretical details of how to do this for structural relations have not been worked out as yet.

(3) Another suggestion was to vary \( \lambda = \sigma^2 / \sigma_\delta^2 \) and see how sensitive the analysis is to the assumption \( \lambda = 1 \). Some argument might be made that due to potential contamination from the catheter there might be more variability in the catheter levels than in the heelstick levels. While this is a worthwhile suggestion the necessary computations have not been carried out to date.
(h) Finally, in the Biostatistics Workshop a proposal was made to run a different experiment. Within the same baby vary the dose of kanamycin to get different readings for known changes in the dose. This should give information on which measurement is better. This idea has never been implemented.

Acknowledgements

Thanks go to Jerry Halpern for programming the jackknife computations and to Peter Gregory for computing the normal theory confidence interval for $\beta$. Bill Brown, Brad Efron, Guy Kraines, and Don Pierce provided the suggestions in Section 5.

References


