Letters to the Editor

Corrected calcium conflict continues

Walker and Payne\(^1\) recently reported no significant interindividual differences between factors used to correct calcium for abnormal albumin concentrations in 15 patients with myocardial infarction. These data conflict. Our data on interindividual variation in correction factors is based on 25 patients with a wide variety of diseases\(^2,3\) as well as on 17 healthy subjects undergoing a tourniquet test.\(^4\) Both groups showed clinically and statistically significant (\(p < 0.001\) and \(< 0.05\), respectively) interindividual differences. Because we have noted a high prevalence of hypercalcaemia in patients with a myocardial infarction (11 of 100 consecutive cases) we wondered if the different results of Walker and Payne had occurred because they had studied a special patient group. We therefore reviewed 22 patients who presented with myocardial infarction at the same time as our original study. Calcium and albumin measurements had been performed by dye-binding methods on the Technicon SMA 12/60 with typical between-batch coefficients of variation of 1.8% and 2% respectively. The mean spontaneous change in albumin concentration was 5.8 ± 0.52 (SEM) g/l. Interestingly, there was no significant interindividual variation in correction factors in these patients (\(p > 0.35\)).\(^5\) We believe that myocardial infarction may affect calcium metabolism, and this deserves further study. However, in patients with other diseases, calcium correction factors vary. The conflict continues!

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We have confirmed the observations of Phillips et al.\(^1\) that normal individuals differ significantly in the relations between the increases in total calcium and albumin after application of a tourniquet.\(^3\) We are pleased that they now confirm our recent observations that individual patients do not differ significantly in the relations between the slow falls in total calcium and albumin which take place during the days after a myocardial infarction.\(^6\)

Apart from some early data,\(^2\) the significance of which has been disputed elsewhere,\(^4,6\) the only difficulty remaining is their claim that patients with myocardial infarction have a surprisingly high incidence of hypercalcaemia and therefore differ from other patients in whom calcium might be adjusted for a low albumin. This claim is without precedent in the literature and is outside our experience, provided specimens are obtained without venous stasis. We await the publication of detailed confirmation with interest.

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The authors have commented as follows:

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Comparison of electron microscopy and immunofluorescence in cell culture for rotavirus detection

Recently, Birch et al.\(^1\) reported in these columns that immunofluorescence (IF) in LLC-MK2 cells was less sensitive than electron microscopy (EM) for the detection of rotaviruses in faeces. My results suggest that the reverse is probably correct.

During the year August 1978 to July 1979, rotaviruses were detected in 112 specimens of faeces submitted to this laboratory for routine viral examination. They were examined by immunofluorescence\(^3\) and EM,\(^2\) as published without modification. Of the 112 specimens, 76 (68%) were positive by both IF and EM, 23 (21%) by IF alone, and 13 (12%) by EM alone. An analysis by age of patient was made of the two groups positive by only one of the methods. It was found that IF was probably superior in diagnosing rotavirus infection in children under 1 year but EM was possibly superior when the child was more than 3 years of age (Table 1). With these older children, it may be that they were suffering from a second or subsequent rotavirus infection, and previously existing antibody in the gut, albeit to a different serotype, was interfering with the IF test but not conferring any protection on the patient. In the same two groups, when the interval between onset of symptoms and collection of specimen was considered (Table 2), IF appears to be superior to EM when the specimen was collected within seven days of onset although EM may well be better for testing samples taken later than this.

Further support for the belief that IF is superior to EM for diagnosing rotavirus infection comes from a study of babies in special care at Sorrento Maternity Hospital, Birmingham in 1977 (to be

References


Table 1  Specimens positive by one method only, according to age of patient

<table>
<thead>
<tr>
<th>Method</th>
<th>Age (years)</th>
<th>Total</th>
<th>&lt; 1</th>
<th>1</th>
<th>2</th>
<th>≥ 3</th>
<th>NS</th>
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</thead>
<tbody>
<tr>
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<td>23</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>EM only</td>
<td>13</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

NS = not stated

Table 2  Specimens positive by one method only, according to number of days post-onset of symptoms when collected

<table>
<thead>
<tr>
<th>Method</th>
<th>Days post-onset</th>
<th>Total</th>
<th>&lt; 7</th>
<th>≥ 7</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF only</td>
<td>23</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>EM only</td>
<td>13</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

NS = not stated

published). Of 28 specimens IF positive, only 17 (61%) were positive by EM, and during the study there was no case of a specimen being EM positive but IF negative. In those very young babies, all less than 1 month old, there is no doubt that IF was the more sensitive test.

Birch and his co-workers rightly suggest that strain variation and cell mutation could affect the sensitivity of the IF test. During the winter of 1978/79, which was probably the poorest for rotavirus since their discovery, the titres of virus present in the faeces of infected children as measured by IF were certainly much lower than in previous years, a point of view held by workers in other parts of the UK (personal communications). By EM, however, the concentration of virus was also apparently much lower hence any strain variation may be a question of reduced pathogenicity in susceptible infants and not of ability to infect LLC-MK2 cells. While I agree that cell variation with passage is highly probable I doubt if there has been any significant alteration in the cells used in this laboratory as stocks of rotaviruses—from other species as well as human strains collected in previous years—show no decrease in their ability to infect cultures.

Finally, the type of serum used for the growth and maintenance of the cells is important as this may affect their susceptibility to virus. In their study, Birch and his colleagues have deviated from the original method by substituting newborn calf serum for fetal calf serum in the growth medium. In a series of tests, as yet unpublished, I have shown that different batches of fetal calf serum, used at 2% in the maintenance medium, may reduce rotavirus titres by anything up to 1 log, although the incorporation of such serum is to be recommended as it helps to reduce toxicity. Newborn and older calf sera are certainly no better, and one batch of older calf serum was almost totally inhibitory to rotavirus growth. No antibody could be demonstrated in this serum by IF, and the inhibition may have been caused by non-specific factors. I feel, therefore, that this change of serum in the growth medium by Birch and his collaborators could reduce the sensitivity of the IF technique, resulting in an unfair comparison with EM.

A reappraisal of both techniques at their optimum sensitivity is required, giving due attention to the points discussed above regarding age of patient and time elapsing between onset of symptoms and collection of specimen.

I thank Dr B A Wharton for allowing a study of infants in special care at Sorrento Maternity Hospital, Birmingham, to be undertaken.

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References


The authors comment as follows:

Our comments on the relative sensitivity of electron microscopy (EM) and immunofluorescence (IF) for the detection of human rotavirus (HRV) were based on the results obtained in this laboratory and those previously reported by Bryden et al. (J Med Microbiol 1977;10:121-125). At that time, they reported that, of 25 specimens examined by both methods, 12 were positive by EM and 11 by IF and that no EM-negative/IF positive specimens were detected.

The previously unpublished results reported above by Mr Bryden, showing that IF is more sensitive than EM (and the occurrence of EM negative/IF positive specimens), suggests that the sensitivity of his test has altered or perhaps that the groups he tested differed in some way. There are numerous reasons for differences in the sensitivity of the IF test performed by different laboratories, and in our paper we suggested several technical reasons for this, including differences in the 'g' forces obtained during centrifugation, varying sensitivities of the cell line used, and virus strain differences. (It would certainly be of interest to analyse HRV strains circulating in Britain and Australia during the period in question.)

We have been unable to demonstrate differences in the sensitivity of the two techniques among differing age groups (Table) and, as practically all our speci-
Comparison of electron microscopy and immunofluorescence in cell culture for rotavirus detection.

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