The zones can be measured by the fluorescence intensity emitted at an angle of 45°. The height and area of the peaks over the different zones corresponding to the fluorescence emission of that zone may be measured as relative percentages of the total fluorescence. The method uses unconcentrated early morning urine: 20 μl are applied to paper and electrophoresed for four hours at 200 volts in Michaelis buffer (barbital/acetate), pH = 8.6, μ = 0.075. Measurements were made on a Zeiss spectrophotometer PQ2 with the Sterzl additions of fluorescence equipment, scanner, and recorder. The irradiating light is passed through filter M 365, fluorescence being measured at 460 nm (maximum of fluorescence emission after excitation at 365 nm).

With this method the urine of healthy persons usually shows a characteristic pattern, with a maximum of 5 zones. Another finding is the occasional presence of an additional band. Whereas only 12% of normal people were found to have these, among a group of patients later proved to have carcinoma of the body of the uterus some 70% showed extra bands. Furthermore, extra bands are a common finding in many patients with other proved carcinomas.

Of 58 patients with proved myelomatosis with paraproteins, 47% showed extra bands. Of 14 patients with proved soft-tissue plasmacytoma, 42% of the preoperative urines showed extra bands. In addition, in cases of paraproteinaemia the ratio of fluorescence intensity from the slowest migrating zone (zone A) to the middle zone (zone B) is obviously altered compared with normal cases; indeed, a reversed ratio was seen in 96% of the patients. In five patients with proved myelomatosis, without paraprotein, the reversed ratio was found in all five, though no extra bands were seen.

The compounds detected by fluorescence emission at 365 nm remain as yet uncharacterized. They are unrelated to any chemotherapy used, and also are distinct from the urinary excretion products of pain-relieving drugs such as aspirin, etc. Possibly they are related to isodoline. It is too early to be sure but we believe that this simple test of urine may prove of value for detecting the presence of occult cancers.

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Treatment of Myelomatosis—M.R.C. Trial

D. A. G. GALTON

Until the mid-1950s myelomatosis was treated with little success. Pain could be relieved by local x-irradiation and a minority of patients were improved by treatment with steroids or urethane. In the late 1950s Russian workers reported some success with sarcolysin, the unresolved Δ, form of phenylalamine nitrogen mustard. Earlier, Bergel and Stock had purified the Δ and the L isomers, and Haddow and others had shown that the L isomer melphalan was usually much more active than the Δ isomer. It was therefore decided to start therapeutic trials in myelomatosis with the L-form. The good results reported by the Russians for sarcolysin were amply confirmed for melphalan, and it seemed probable that the intermittent method of administration was less damaging to the bone marrow, and was more often accompanied by sustained increase in the haemoglobin concentration. Meanwhile, apparently equally good results were reported for cyclophosphamide, but no direct comparison had been made. In September 1964 the M.R.C.'s working party on therapeutic trials in leukaemia started a comparative trial of melphalan; patients were entered until 31 July 1968 and surviving patients were followed up to 31 May 1970 for the present analysis.

In the first comparative trial both drugs were administered continuously at low daily dosage. All new patients with the prospect of continuous follow-up were eligible, provided they had received no previous chemotherapy, other than prednisone for the treatment of hypercalcaemia. Radiotherapy for the relief of local pain did not preclude entry. The minimum diagnostic criteria for eligibility were two of the following: firstly, abnormal plasma cells in the bone marrow; secondly, a monoclonal M-component in the serum, urine, or both; and, lastly, radiological findings compatible with myelomatosis.

There were 276 cases for analysis—142 men and 134 women; only 7% of the patients were below 50, but compared with the national distribution patients over 70 were somewhat underrepresented. In respect of all the main clinical features the two treatment groups were similar, while equal proportions had received local radiotherapy before they started chemotherapy, and during the course of the disease, as well as being treated with prednisone for hypercalcaemia.

Though the main purpose of the trial was the comparison of the effects of melphalan and cyclophosphamide, no significant difference emerged in their therapeutic efficacy, or in their effect on survival, so the following account is based on the total series of patients. Many of the clinical features at presentation were correlated with survival when considered separately, but two features—namely, the blood urea and the serum albumin concentrations—were strongly and independently correlated with survival. When the correlations with survival of all the other factors were adjusted for the effect of the blood urea and the serum albumin concentrations, their effects largely or entirely disappeared. The explanation of renal damage is that the tubules are damaged by long-continued passage of Bence Jones protein and by its precipitation within the tubules. In our series the prognostic influence of Bence Jones proteinuria was strongly correlated with the presence of uremia, and disappeared when adjusted for it. This again correlates with the clinical impression that if Bence Jones proteinuria can be reduced by treatment before renal function has become irreversibly impaired the process can be arrested and the patient may survive. It is interesting that none of the radiological findings had any prognostic significance. Indeed, 24% of all the patients had no radiologically detectable lesions.

Type of Protein Abnormality

The possible effect of the type of M-component has been of interest since Bergsagel's original observation of a difference in therapeutic response to melphalan of patients with kappa (K) or lambda (L) type light chains. In our series the survival was rather worse in L-type cases, but these included a higher proportion of patients with a high blood urea level, and when this was allowed for the adverse influence disappeared. The relationship perhaps depends on the duration of exposure of the renal epithelium to the serum albumin, and our data provide some evidence of this: twice as many of our IgGL patients as IgGK patients had more than 100 mg/100 ml of Bence Jones protein in their urine, the mean concentration being 291 mg compared with 64 mg/100 ml for the IgGK cases.

In our series the initial level of the serum albumin was strongly correlated with survival, and the influence was independent of the blood urea concentration. We do not know whether the fall in serum albumin level was a result of a reduced rate of synthesis or of an increase in the catabolic rate, but it was not a result of loss in the urine. The survival curve of the 276 patients shows an
initial steep fall, and then the curve declines less steeply; the median survival was 18 months, and the 20% survival four years. For the 125 patients whose blood urea was less than 40 mg/100 ml the median survival was 33 months, and nearly 40% of them were alive at four years. For the 96 patients whose blood urea was between 41 and 79 mg/100 ml the median survival was 20 months, and for the 55 patients whose blood urea was 80 mg/100 ml or higher was only two months.

Of the 35 patients who survived four years there were relatively rather more men than in the whole series; there were also relatively more IgG patients, and fewer IgA patients, and relatively a few more K and a few less L cases. Only 23% of the long survivors had an initial haemoglobin concentration below 10 g/100 ml, compared with 48% in the whole series. Only 13% of the 30 IgG and IgA survivors had urinary Bence Jones protein above 100 mg/100 ml, compared with 32% in the IgG and IgA cases in the whole series. As expected, the proportion of cases with normal blood levels of urea was more than three times higher than in the whole series, and there were more in the high urea group. The proportion of cases with high serum albumin levels was higher and of cases with low levels was lower than in the whole series. Of the 12 patients with moderately raised blood urea levels, four had Bence Jones protein disease (urinary concentrations of 180, 180, 200, and 560 mg/100 ml); the remaining eight did not have Bence Jones proteinuria, but three were hypercalcaemic. Thus the presenting features of the 35 long-survivors were precisely what we should expect from the statistical analysis of the whole series of 276 cases.

## Urea, Albumin, and Response Rates

### R. PETO

The rate at which myeloma responds to a cytotoxic attack has been measured for about half of the series of patients in the first Medical Research Council myeloma trial. The biochemical histories over the first six months of treatment were reviewed for all of the 197 patients who were still alive six months after first admission to estimate the individual percentage paraprotein reduction that had occurred four months after admission. This showed that 79 patients died within six months of admission; 12 survivors had less than 1 g/100 ml of paraprotein initially, which was considered insufficient for the calculation of percentage changes. In 60 survivors the biochemical histories were insufficient for percentage estimations to be possible; while in 36 survivors the paraprotein levels were not finally reduced four months after admission. Finally, in 89 survivors the paraproteins had responded by four months; of these 89, 37 were down by half or more and 52 were down by less than half four months after admission.

On examination of their histories after the sixth month the average death rate among the fast responders was about twice that among the slow responders, confirming Hobbs's suggestion that a myeloma which responds rapidly to cytotoxic therapy is in fact a myeloma with rapidly dividing myeloma cells—and so is dangerous. The Table shows how strongly the death rate depends on two factors—the blood urea and serum albumin levels.

<table>
<thead>
<tr>
<th>Chester Beatty Research Institute, London S.W.3</th>
<th>D. A. G. GALTON</th>
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</table>

### Blood Urea

High blood urea is due either to excessive production or to insufficient excretion. Probably neither high urinary urea nor a high urinary urea/creatinine ratio carry a bad prognosis, so that high blood urea must be associated with a poor prognosis simply because it is an index of renal damage.

Over half the patients who presented with severe uraemia (> 80 mg/100 ml) were dead within two months of admission. Two years after admission the death rate among patients who originally presented with mildly raised blood urea levels (40-79 mg/100 ml) remains much higher than among those who presented with normal (10-39 mg/100 ml) blood urea. Renal damage is therefore important and frequently irreversible.

### Serum Albumin

Low serum albumin is due to reduced production (such as liver dysfunction or amino-acid starvation), increased excretion (kidney damage), or increased catabolism (some catabolic myeloma activity, for example). Low serum albumin must therefore be of prognostic significance because it measures one or other of these effects. Kidney damage allowing albumin leakage into the urine is an obvious explanation, but if this was the reason for the prognostic importance of albumin we would expect a strong association between low albumin and high urea (which does not in fact exist; see Table), and we would expect urinary albumin to be a better index of prognosis than serum albumin, and it is not.

Hence probably a myeloma can somehow catabolize circulating albumin or inhibit its synthesis, and low albumin indicates a very active myeloma. Comparing the data in initial urea and albumin levels with those on initial levels of response in the 89 patients surviving in the six months from admission and responding (so any correlations must be treated with care), we find that 9 of these survivors presented with low (< 3 g/100 ml) albumin, and 14 with high (> 60 mg/10 ml) urea. Though only 40% of the whole 89 were fast responders and 60% were slow responders, eight of the nine low-albumin patients and 10 of the 14 high-urea patients were fast responders. This can be taken either to mean that a myeloma catabolizes albumin and low albumin indicates an active myeloma (and hence very sensitive to cytotoxic drugs) or that if you have low albumin or high urea your myeloma has to react fast if you are to survive even six months.

### Estimated Death Rates by Initial Urea and Albumin Levels, Expressed as Proportions of the Overall Average Rate

<table>
<thead>
<tr>
<th></th>
<th>Blood Urea 10-39 mg/100</th>
<th>Blood Urea 40-79 mg/100</th>
<th>Blood Urea &gt; 80 mg/100</th>
<th>All Blood Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>1.40</td>
<td>1.94</td>
<td>2.72</td>
<td>3.89</td>
</tr>
<tr>
<td>&lt;3 g/100</td>
<td>(19)</td>
<td>(18)</td>
<td>(13)</td>
<td>(50)</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.74</td>
<td>0.99</td>
<td>2.45</td>
<td>2.41</td>
</tr>
<tr>
<td>3.0-3.9 g/100</td>
<td>(99)</td>
<td>(90)</td>
<td>(26)</td>
<td>(131)</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.35</td>
<td>0.92</td>
<td>2.00</td>
<td>0.68</td>
</tr>
<tr>
<td>&gt;4 g/100</td>
<td>(46)</td>
<td>(31)</td>
<td>(17)</td>
<td>(94)</td>
</tr>
<tr>
<td>All Albumins</td>
<td>0.64</td>
<td>1.07</td>
<td>3.11</td>
<td>1.00</td>
</tr>
<tr>
<td>(124)</td>
<td>(95)</td>
<td>(56)</td>
<td>(275)</td>
<td></td>
</tr>
</tbody>
</table>

Factors examined and found to be of no statistically significant prognostic importance after adjustment for urea and albumin levels included hypercalcaemia, anaemia, alkaline-phosphatase, age, paraprotein type, paraprotein concentration, all radiological findings (lytic lesions, wedging, osteoporosis, or pathological fractures) and all histological findings (staining properties, number, and shape of myeloma cells).

*The proportion of survivors at time t from first treatment is estimated from a life-table to be P(t), then for a group of patients at risk for times T<sub>1</sub>, T<sub>2</sub>, ..., in which N deaths occur, the relative death rate is estimated by ~N/log(P(T)).

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