Table 1. Lymphocyte fractions obtained by differential lectin agglutination

<table>
<thead>
<tr>
<th>Fraction</th>
<th>E-R (%)</th>
<th>Slg (%)</th>
<th>OKT3 (%)</th>
<th>OKT4 (%)</th>
<th>OKT8 (%)</th>
<th>Leu-7 (%)</th>
<th>% Specific lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated</td>
<td>73</td>
<td>8</td>
<td>91</td>
<td>54</td>
<td>30</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>Agglutinated-1</td>
<td>(low con.)</td>
<td>56</td>
<td>6</td>
<td>81</td>
<td>38</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Agglutinated-2</td>
<td>(high con.)</td>
<td>68</td>
<td>5</td>
<td>78</td>
<td>56</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>Free cells</td>
<td></td>
<td>80</td>
<td>9</td>
<td>76</td>
<td>60</td>
<td>34</td>
<td>6</td>
</tr>
</tbody>
</table>

Rhesus D/anti-D system (Urbaniak & Greiss, 1980). K-cell activity was greatest among the cells most susceptible to lectin agglutination, and roughly correlated with the Leu-7 marker (Table 1).

There were no precipitation bands visible after Ouchterlony double diffusion of tomato lectin against pooled normal human serum. However, after affinity chromatography of normal human serum on columns of tomato lectin–Sepharose, lectin-binding proteins could be detected. After electrophoresis in the presence of sodium dodecyl sulphate (Weber and Osborn, 1969), bands of apparent M, 110 000, 86 000, 76 000, 50 000 and 24 000 were visualized with Coomassie Blue. The 66 000 band may have been non-specifically bound albumin, since the only band detectable after an otherwise identical control procedure in which unsubstituted Sepharose was used in place of immobilized lectin had a similar apparent M, after electrophoresis in the presence of dodecyl sulphate and a similar mobility to human albumin in a detergent-free system. The bands of apparent M, 24 000 and 50 000 were identical to those of human immunoglobulin used as molecular size markers, and since there was protein staining in the γ region after electrophoresis at pH 8.9 in the absence of detergent, it is possible

tomato lectin can bind to an immunoglobulin population via the carbohydrate moiety of the latter.

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Influence of mast cells on macrophage function

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Mast cells and macrophages are known to play significant roles in hypersensitivity reactions and inflammation. Mast cells, upon stimulation, release a variety of inflammatory mediators, including histamine, leukotrienes, prostaglandins, chemotactic factors, neutral proteases, proteoglycans (Ishizaka & Ishizaka, 1984; Schwartz & Austen, 1984) and superoxide (Henderson & Kaliner, 1978). Activated macrophages release acid hydrolases, proteases and metabolites of arachidonate and undergo marked alteration in oxidative metabolism (Adams, 1982; Cohn, 1982; Johnston & Kitagawa, 1985). The alterations collectively known as the ‘respiratory burst’ include production of O₂⁻ and H₂O₂ (Iyer et al., 1961; Babior et al., 1973). Although the production of reactive oxygen radicals by macrophages is vital for the bacteriocidal activity of the phagocytes, the excessive production of O₂⁻ and its metabolites often results in tissue injury. Recent observations have shown that these oxygen species can also react with nuclear contents and affect the expression of genetic information (Brawn & Fridovich, 1981). The aim of the present study was to determine whether

mast cells or mast cell-derived products could modify O₂⁻ generation by macrophages.

Isolated rat serosal cells were separated into macrophage and mast cell populations using a metrizamide cushion (Winslow et al., 1981). Isolated mast cells alone or in the presence of macrophages were activated by sensitization with monoclonal IgE antibody directed against trinitrophenyl–bovine serum albumin followed by challenge with dinitrophenyl–bovine serum albumin, or by directly treating with compound 48/80 or Ca²⁺ inophore, A23187. Histamine or serotonin release from the mast cells was measured as an index of mast cell activation. Macrophage O₂⁻ generation was measured by the superoxide dismutase-sensitive reduction of ferricytochrome c (McCord & Fridovich, 1969; Babior et al., 1973). Cytochrome c reduction was carried out for 5 min at 37°C after addition of the appropriate stimulus. Opsonized zymosan (0.5 mg/ml) was used to stimulate macrophages when O₂⁻ production was measured.

Mast cells and macrophages either alone or in combination (1:9 ratio) were activated with appropriate stimuli and O₂⁻ production was determined. Little or no O₂⁻ was produced by unstimulated macrophages and mast cells. The production of O₂⁻ by macrophages was markedly increased when the phagocytes were activated with opsonized zymosan (unstimulated cells: 0.05-0.5 nmol of cytochrome c reduced/10⁶ cells,
stimulated cells: 2.0–3.5 nmol/10^6 cells). The O_2^- production was dependent on the presence of oxidative substrates such as glucose and succinate. Mast cells, on the other hand, did not release O_2^- upon activation with IgE antibody or compound 48/80, although both the stimuli caused substantial release of histamine and serotonin. However, both mast cells and macrophages generated significant amounts of O_2^- upon activation by Ca^{2+} ionophore, A23187. In mixed cell experiments, zymosan-induced measurable O_2^- release from macrophages was abolished when mast cells were simultaneously activated with compound 48/80. The abolition of O_2^- generation was also noted when isolated mast cell granules or the granule extract was added to macrophages. The inhibition of O_2^- generation caused by mast cell granules and the granule extract was dose-dependent. The addition of mast cell granules equivalent of 1 x 10^8 mast cells to 5 x 10^6 macrophages completely abolished O_2^- production. This indicated that mast cell granules may contain superoxide dismutase which when released into the medium scavenges O_2^- generated by macrophages. The presence of superoxide dismutase in the mast cell granules was further substantiated by the collagenase perfusion technique of Seglen (1973) and cultured directly in Ham's F-10 medium (Babior, 1981).

These problems have made it almost impossible to study this work was supported by Carey Arbthritis Funds and BRSG S07-RR0573, NIH.

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The maintenance of adult rat hepatocytes in primary culture with the concomitant preservation of specific hepatic functions has proved difficult. This has been particularly so for the metabolism of drugs and steroids (Stenberg et al., 1978), and has involved the use of enzyme inhibitors and technically demanding co-culture techniques as well as hormone supplements (Hockin and Paine, 1973; Begue et al., 1984). These problems have made it almost impossible to study the physiological regulation of the drug- and steroid-metabolizing enzymes by this method.

In this study, hepatocytes were prepared from adult male and female rats by the collagenase perfusion technique of Seglen (1973) and cultured directly in Ham's F-10 medium supplemented with 2% Ultroser (LKB Products, Sweden). Medium was changed after 3 days. Directly after a density of 3 x 10^6 cells/cm^2 in 6 cm or 9 cm diameter Petri dishes. Medium was changed after 3 days. Directly after scraping from the plate, washed with incubation medium (Hank's balanced salt solution supplemented with 1 g of glucose/l) and resuspended in the same medium at a concentration of approx. 3 x 10^6 cells/ml. 4-[4,4-C]Androstene-3,17-dione (10^6 c.p.m., 500 mg) was added to 3 ml of cell suspension and incubated in a shaking waterbath for 30 min. Metabolites and unchanged substrate were separated and analysed as described previously (Berg & Gustafsson, 1973). Freshly prepared hepatocytes were obtained at approx. 10^6 cells/g of liver and were over 90% viable as assayed by Trypan Blue exclusion. Cell number fell by up to 50% over the period of the study. The hepatocytes were found to be attached to the dish but few adopted the flat, hexagonal shape reported by other workers (Guguen-Guillouzo et al., 1983; Chessebeuf & Padieu, 1984). The activities of the 17-oxosteroid oxidoreductase, 6β-hydroxylase and 16α-hydroxylase could be analysed and it was found that activities of all of these enzymes decreases (in the male-derived cells) from 0 to 3 days after plating but were increased again at 6 days (Table 1). In the female-derived cells, there was little change in enzyme activity over the period studied; all activities started at a lower level than in the male-derived cells and remained that way throughout the study. Enzyme activities of the freshly prepared cells were comparable with those found in microsomal suspensions in the presence of excess cofactor indicating that NADPH production by the cells is not the rate-limiting factor in the metabolism measured. All of the enzymes studied are found in higher activities in male than in female microsomal preparations (Berg & Gustafsson, 1973) and are maintained in the intact animal by various hormonal influences (Skett & Gustafsson, 1979). It is not surprising, therefore, that the removal of the cells from these influences causes a decrease in the male-specific enzyme activities. The lack of effect of culturing on the female-derived cells confirms this interpretation. This study suggests that the serum-free culture of adult rat hepatocytes is feasible and can maintain the basal activities.

Table 1. Metabolism of 4-[4,4-C]androsten-5,17-dione by isolated rat hepatocytes derived from adult male and female animals cultured in a defined serum-free medium

<table>
<thead>
<tr>
<th>17-Oxosteroid</th>
<th>Male-derived cells</th>
<th>Freshly prepared</th>
<th>3-day culture</th>
<th>6-day culture</th>
<th>Female-derived cells</th>
<th>Freshly prepared</th>
<th>3-day culture</th>
<th>6-day culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>6β-Hydroxylase</td>
<td>0.67 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.44 ± 0.02</td>
<td>0.05 ± 0.03</td>
<td>0.03 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td>16α-Hydroxylase</td>
<td>0.58 ± 0.04</td>
<td>0.23 ± 0.01</td>
<td>0.44 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>16β-Hydroxylase oxidoreductase</td>
<td>1.16 ± 0.14</td>
<td>0.23 ± 0.01</td>
<td>0.44 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

of the above-activities of the freshly prepared cells were comparable with those found in microsomal suspensions in the presence of excess cofactor indicating that NADPH production by the cells is not the rate-limiting factor in the metabolism measured. All of the enzymes studied are found in higher activities in male than in female microsomal preparations (Berg & Gustafsson, 1973) and are maintained in the intact animal by various hormonal influences (Skett & Gustafsson, 1979). It is not surprising, therefore, that the removal of the cells from these influences causes a decrease in the male-specific enzyme activities. The lack of effect of culturing on the female-derived cells confirms this interpretation. This study suggests that the serum-free culture of adult rat hepatocytes is feasible and can maintain the basal activities.