The causation of splenomegaly in schistosomiasis in mice

ADEL A. F. MAHMOUD* AND A. W. WOODRUFF
Department of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine, London

(Received 26 July 1976; accepted 24 October 1977)

Summary
1. Mice were infected with fertile bisexual Schistosoma mansoni and compared with similar animals infected with unisexual worms or sterile bisexual worms.
2. A significant increase in splenic weight occurred in all infected animals.
3. Administration of well-tolerated doses of 6-mercaptopurine abolished the increase in relative splenic weight in animals infected with ordinary S. mansoni.
4. In splenectomized uninfected mice leucocytosis but no other haematological changes developed.
5. In splenectomized mice lower values for packed cell volume were observed 8 weeks after, but not 12 weeks after, infection with S. mansoni.
7. It is concluded that anaemia in schistosomiasis depends to a significant extent on immunity developed to adult schistosomal worms and can develop in the absence of schistosomal ova.
8. The anaemia resulting from such an immune response may be suppressed by administration of 6-mercaptopurine.
9. Such anaemia occurs even in splenectomized mice; thus hypertrophy is not necessary for its development although splenectomy slightly prolongs the erythrocyte life-span.

Key words: mercaptopurine, schistosomiasis, splenomegaly.

Introduction
The common occurrence throughout the tropics of splenomegaly, sometimes of gross degree, raises many questions as to the nature of the underlying aetiological mechanisms. 'Egyptian splenomegaly', in which the spleen may be enormous, is usually associated with schistosomiasis, and also in some patients with cirrhosis of the liver (Hashem, 1957). The usual liver lesion is the presinusoidal block, with the subsequent portal hypertension, but splenomegaly may precede liver disease. In experimental infections with Schistosoma mansoni, ova taken to the liver, visualized by microscopy in vivo, have been shown to induce granulomas that are responsible for obstruction of portal blood flow (Bloch, Adel Wahab & Warren, 1972). Furthermore, Maghaeles & Coutinho (1961) have demonstrated that lymphatic and reticular hyperplasias leading to enlargement of the spleen start 3 days after experimental S. mansoni infection. Splenomegaly was studied during the course of experimental S. mansoni infection in mice, as well as the effects of unisexual infections with sterile worms, thus eliminating egg deposition and its sequelae in the host tissues. During the course of these studies splenic enlargement was found to precede oviposition, so that an immunological mechanism may underly the enlargement of the spleen, and this was explored by immuno-suppression with 6-mercaptopurine.

Hypertrophy of the spleen might increase destruction of erythrocytes (Woodruff, Shafei, Awad, Pettitt & Abaza, 1966; Sabour, Osman &
El Mazny, 1967), and splenectomy can improve the anaemia seen in some patients with schistosomiasis (Sabour et al., 1967). In animals infected with *S. mansoni* a close correlation was found between the reduction of erythrocyte mass and the increase in the relative splenic weight (Mahmoud, 1971), and haemolysis was shown to contribute to the anaemia (Mahmoud & Woodruff, 1972). The relation between the spleen as a site of erythrocyte destruction and the shortening of erythrocyte survival in experimental schistosomiasis was therefore examined.

**Materials and methods**

Male *Tisilius originalis* Swiss albino mice (weight 18–22 g) were used. Animals were exposed percutaneously to cercariae of an Egyptian–Wellcome strain of *Schistosoma mansoni*.

Experimental groups included: 1, mice exposed to 100 cercariae, obtained from a pool of infected snails to ensure bisexual infections; 2, mice exposed to 1000 X-irradiated cercariae (this has been shown to produce infections with approximately 18 sterile worms per mouse; Mahmoud & Woodruff, 1973); 3, mice infected with cercariae from single snails each previously exposed to one miracidium, thus causing unisexual infection (Mahmoud & Woodruff, 1973); 4, normal control mice.

At 2 week intervals after infection, batches of 10 mice each from the different experimental groups were anaesthetized with ether, weighed, and splenectomized (see below), the spleen weight being recorded immediately and expressed as the relative splenic weight, which is the percentage of the body weight.

The effect of 6-mercaptopurine [Sigma (London) Chemical Co. Ltd.] on the development of splenomegaly was studied in mice infected with *S. mansoni* before the onset of oviposition at about 5 weeks. In a 2 × 2 factorial design of four groups of animals, two groups were infected with 100 *S. mansoni* cercariae, and the other two were kept as uninfected control mice. One uninfected and one infected group received daily subcutaneous injections of 6-mercaptopurine (0.2 mg/mouse) for 30 days from the day the animals were infected. Ten animals from each group were examined 15 and 30 days after infection; body weight, spleen weight and packed cell volume were determined (Mahmoud & Woodruff, 1972) and the relative spleen weight was calculated. Analysis of variance was used to assess the significance of differences between the different groups.

The haematological effects of splenectomy in normal animals were studied for 1 month, including measurement of the erythrocyte life span. The effect of infection with 100 *S. mansoni* cercariae in splenectomized and intact animals was compared by methods previously described (Mahmoud & Woodruff, 1972).

Splenectomy was carried out under ether anaesthesia, through a small incision in the anterior left abdominal wall. The spleen was drawn outside the abdominal cavity, its blood vessels were severed, the adjacent area was inspected for accessory spleens, and the rest of the abdominal contents were replaced. Excessive haemorrhage was never encountered, and ligation of the splenic vessels was not necessary. The wound was closed with Michell clips. Animals were kept under direct observation until they recovered from the anaesthetic. None developed any manifestations of bleeding or wound infection. The completeness of the splenectomy and presence of any accessory spleens were also noted at necropsy of the splenectomized animals. The mean, standard deviation and standard error of relative splenic weight of each experimental group was calculated and the significance of the difference detected was assessed by Student’s *t*-test.

**Results**

*Splenomegaly in fertile bisexual, sterile bisexual and unisexual infections*

Fertile bisexual *S. mansoni* infections induced a significant increase in relative splenic weight (*P* < 0.05) 2 weeks after infection (Table 1). By the fourth week, splenomegaly was slightly more marked, and by weeks 8 and 12 after infection splenic size was very significantly increased (*P* < 0.001).

Comparison of the relative splenic weight of control mice with three groups of animals infected with fertile bisexual worms, sterile bisexual worms or with unisexual worms showed a significant (*P* < 0.001) increase in relative splenic weight in all groups of infected animals (Fig. 1). An increase in the relative splenic weight was manifest up to 24 weeks after infection of animals with X-irradiated cercariae, though the values were not as high as in those with fertile bisexual infections. Similarly, unisexual infection induced less, though still significant (*P* < 0.05), increases in relative splenic weight at all times after infection at which animals were studied.
Splenomegaly in schistosomiasis

Table 1. Comparison of splenic weight as a percentage of body weight ('relative splenic weight') in normal control mice and mice infected with 100 cercariae

Mean values ± SD are shown for two groups, each of 10 mice.

<table>
<thead>
<tr>
<th>Duration after infection (weeks)</th>
<th>Normal mice</th>
<th>Infected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mean splenic weight (%)</td>
<td>0.51 ± 0.09</td>
<td>0.60 ± 0.77</td>
</tr>
<tr>
<td>Relative splenic weight (%)</td>
<td>0.76 ± 0.19</td>
<td>1.43 ± 1.98</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Effect of 6-mercaptopurine in the early stages of splenomegaly

None of the animals receiving the drug died or sustained clinically observable effects. Their packed cell volume was not significantly different from that in the two other untreated groups. The study of the relative splenic weight of the four groups of animals, 15 days after infection, showed that the splenomegaly observed in the infected untreated group was totally inhibited in the group that received the drug (Table 2). Analysis of variance showed that although infection significantly increased the relative splenic weight ($P < 0.01$), 6-mercaptopurine prevented at nearly the same level of significance ($P < 0.01$) the onset of splenomegaly. Even 30 days after infection, 6-mercaptopurine was still significantly ($P < 0.05$) suppressing the onset of splenomegaly.

Haematological indices in splenectomized healthy and infected mice

Apart from leucocytosis noticed after splenectomy in normal animals, no other haematological abnormality was detected (Table 3). Animals that were infected after splenectomy developed significantly ($P < 0.05$) lower values for packed cell volume 8 weeks after infection (Table 4), and their total leucocyte counts were higher. However, 12 weeks after infection, no significant difference was detected between the two groups.

The survival of isogenic $^{51}$Cr-labelled erythrocytes in the intact and splenectomized infected animals (Fig. 2) shows the $t_{0.5}$ $^{51}$Cr of the intact infected group to be 4–5 days, whereas in the splenectomized infected group it was 6 days. The survival in their own circulation of $^{51}$Cr-labelled erythrocytes of splenectomized infected animals was compared with that of similarly labelled cells from non-splenectomized infected animals. Four days after injection 63% of labelled cells were still in the circulation of splenectomized infected animals, significantly greater ($P < 0.05$) than the 56% present in the circulation of the intact infected control mice.

Discussion

The mechanisms responsible for schistosomal splenomegaly are thought to be due to the parasite as well as the host reaction to infection (Hashem, 1957). The present study suggests that splenomegaly is produced through the contribution of each of the parasitic stages in the host. Thus the cercarial penetration and the development of schistosomules induced a significant increase in relative splenic weight, although the worms were still immature and no eggs were produced. Later, with maturation, eggs are laid and a sharp rise in relative splenic weight occurs.

Only in infections in which fertile adult schistosomes developed was splenomegaly marked. In animals infected with irradiated cercariae, where bisexual sterile infection was produced, and in unisexual infections, low to moderate splenomegaly was observed. The only discernible difference between these groups was that no ova were

Table 2. Relative splenic weight 15 and 30 days after infection in animals treated or untreated with daily subcutaneous injection of 6-mercaptopurine

Mean values are shown; 10 animals were examined in each group at each time interval. Analysis of variance showed a difference significant at $^*P = 1\%$ or $^{**}P = 5\%$.

<table>
<thead>
<tr>
<th>Duration of 6-mercaptopurine therapy (days)</th>
<th>Relative splenic weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected animals</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
</tr>
</tbody>
</table>

Table 3. Relative splenic weight 15 and 30 days after infection in animals treated or untreated with daily subcutaneous injection of 6-mercaptopurine

Mean values are shown; 10 animals were examined in each group at each time interval. Analysis of variance showed a difference significant at $^*P = 1\%$ or $^{**}P = 5\%$.
A. A. F. Mahmoud and A. W. Woodruff

FIG. 1. Comparison of the relative spleen weights of mice infected with *Schistosoma mansoni* cercariae and normal control mice. ♦, Mice infected with 100 *S. mansoni* cercariae; △, mice infected with 100 *S. mansoni* γ-irradiated cercariae; ●, mice infected with 50 *S. mansoni* male cercariae; O, normal uninfected mice.

deposited in the tissue of animals infected with unsexual or sterile worms.

These studies attempted to separate the splenic reaction in experimental schistosomiasis into its components. The early stage of enlargement of the spleen represented part of the host's reaction towards the infection. It occurred before any detectable oviposition and the subsequent liver pathology. Hashem (1957) considered early splenomegaly to be due to hypertrophy of the reticular tissues. 6-Mercaptopurine, a powerful immunosuppressant, abolished the early stages of splenomegaly. The host immunological response to schistosomiasis and its pathological role has been the subject of several studies (Warren, 1972). Immunosuppression of the host either by cholera toxin or by Hodgkin's-like tumours induced marked amelioration of the disease in experimental schistosomiasis (Warren, Mahmoud, Boros, Rall, Mandel & Carpenter, 1974; Warren, 1969). The animals in these studies were patently infected and developed less splenic enlargement than the untreated control mice.

Later, in the course of infection and with deposition of eggs in the liver parenchyma, there

### TABLE 3. Comparison between the haematological indices of normal and splenectomized mice

Mean values ± SD are shown. After labelling the erythrocytes with $^{51}$Cr the erythrocyte volume was calculated by the formula

\[
\text{PCV} = \frac{\text{radioactivity of standard} \times \text{dilution of standard} \times \text{volume injected} \times \text{radioactivity of post-injection sample}}{\text{100}}
\]

The total blood volume was calculated from the erythrocyte volume (RCV) by the formula

\[
\text{Total blood volume} = \frac{\text{RCV} \times 100}{\text{corrected PCV}}
\]

The corrected packed cell volume (PCV) was obtained by multiplying the venous packed cell volume by 0.88 (Nachman, James, Moore & Evans, 1950).

<table>
<thead>
<tr>
<th>Mice</th>
<th>Body weight (g)</th>
<th>Packed cell volume (%)</th>
<th>Leucocyte count (10^6 cells/l)</th>
<th>Erythrocyte volume (ml)</th>
<th>Total blood volume (ml)</th>
<th>$^{51}$Cr t_{1/2} (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (control)</td>
<td>33.6</td>
<td>50.2 ± 2.3</td>
<td>7.8</td>
<td>0.98</td>
<td>2.20</td>
<td>13.5</td>
</tr>
<tr>
<td>(10 animals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenectomized</td>
<td>34.6</td>
<td>50.6 ± 3.0</td>
<td>14.7</td>
<td>1.02</td>
<td>2.22</td>
<td>13.0</td>
</tr>
<tr>
<td>(10 animals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 4. Comparison of the haematological findings in infected splenectomized and non-splenectomized animals

Mean values are shown. All animals had an average worm load of 18. N.S., Difference not significant at 5% level.

<table>
<thead>
<tr>
<th>Mice</th>
<th>8 weeks after infection</th>
<th>12 weeks after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haemoglobin (g/dl)</td>
<td>Packed cell volume (%)</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>7.8</td>
<td>22.6</td>
</tr>
<tr>
<td>Infected</td>
<td>2.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Non-splenectomized</td>
<td>10.7</td>
<td>33.6</td>
</tr>
<tr>
<td>Infected</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Splenomegaly in schistosomiasis

was a rapid increase in the splenic weight. Several factors could be contributing: the pooling of blood in the spleen or congestion of the portal circulation due to the presinusoidal block of blood flow. In schistosomiasis, splenomegaly is usually seen along with anaemia, leukopenia or sometimes with pancytopenia (Woodruff et al., 1966). Experimentally infected mice developed splenomegaly that closely correlated with the decrease in erythrocyte mass (Mahmoud, 1971).

The survival studies show a slightly longer \( t_{0.5} \) for \(^{51}\text{Cr} \) in splenectomized infected animals. Furthermore, their circulation maintained the \(^{51}\text{Cr} \)-labelled cells for a relatively longer period than the circulation of intact infected animals. This difference may be assumed to be due to the absence of the spleen in the former group, i.e. measurable numbers of erythrocytes are destroyed in the spleen in schistosomiasis. Excessive trapping and destruction of damaged erythrocytes within the enlarged spleen of schistosomiasis has been demonstrated (Mahmoud & Woodruff, 1972).

These studies indicate that splenomegaly depends not only on parasitic factors, but also on the host response to infection. They provide evidence that immunological mechanisms are in part responsible for the splenomegaly of schistosomiasis, which has been shown to arise without any liver pathology which might be brought about by the presence of schistosomal ova in the liver. The work gives further support to the view that gross splenomegaly in schistosomiasis probably has much in common with that of 'big spleen disease' in Uganda and with that of kala-azar (Woodruff, Topley, Knight & Downie, 1972), which is now generally agreed to have an immunological basis, and to result from phagocytosis in the spleen of erythrocytes damaged in the circulation, and not primarily from portal hypertension.

References


