Hepatitis in Association with Prolonged 6-Mercaptopurine Therapy

By Samuel K. McIlvanie and John D. MacCarthy

The usual toxic effects of Purinethol* are those of bone marrow depression, nausea, vomiting and diarrhea. There have been, to our knowledge, no published reports of the development of acute hepatitis. Animal studies indicated degenerative changes in the intestinal epithelium and liver which were characterized by impaired function, parenchymal necrosis, cirrhosis and, clinically, with jaundice and frequently severe diarrhea.¹²

Four instances of acute onset of anorexia, diarrhea, jaundice, ascites and generalized toxic states were encountered by one of us (SKM). Detailed case reports are included, with autopsy examination in three. The highlights in the case reports are summarized in figure 1.

Case Reports

Case 1: T. R., white male, aged 13, weight 45 Kg., was first seen on 3/26/56 because of pallor and fatigue. Positive physical findings consisted of petechial hemorrhages, pallor and splenomegaly. The hemogram was as follows: 4,000 leukocytes/cu. mm. with a normal differential, 8.7 Gm. hemoglobin, 13,000 platelets/cu. mm. and 4.6% reticulocyte count. The heterophile test was positive 1:56, A:G ratio normal, van den Bergh, immediate acting, 1.0 mg. and total 1.8. The Coombs' test was negative. Saline fragility was normal. Cold hemolysins and agglutinins were present 1:3,560. Blood or plasma transfusion therapy is shown in figure 2. A diagnosis of acquired hemolytic anemia was made and the patient was started on prednisone; however, in one to two days severe bone pain developed. Upon re-examination the marrow findings were compatible with early aleukemic myeloblastic leukemia. Steroids were discontinued and 6-mercaptopurine was given at 4.4 mg./Kg. per day for 26 days, during which time the patient was critically ill. 6-Mercaptopurine was then discontinued and amethopterin given at 2.5 mg. per day for 2½ weeks. Leukopenia of 3,000 cu. mm. and persistent fever forced hospitalization and amethopterin was discontinued. Bone marrow revealed improvement and after a critical period with leukopenia a complete remission occurred, on 7/1/56, which was maintained for approximately three months. During this period he received 1.1 mg./Kg./day 6-mercaptopurine and 1.25 mg./day of amethopterin.

In late September symptoms of headache, fatigue and splenomegaly returned. Amethopterin was discontinued and 6-mercaptopurine was gradually increased to a maximum dose of 4.4 mg./Kg./day. Seventeen days later remission was heralded by a reduction in spleen size, reticulocytosis and platelet increase. At this time bile appeared in the urine and there was marked anorexia and diarrhea. The patient was hospitalized at Sacred Heart Hospital on 10/30/56.

Physical examination revealed ascites, jaundice, clay-colored stools and the liver, palpable and tender, down four fingerbreadths. The leukocyte count was 400/cu. mm. 6-Mercaptopurine was discontinued on 11/1/56 and continuous intravenous drip of Compound F,

*Purinethol (6-mercaptopurine), Burroughs Wellcome & Co., Tuckahoe, N.Y.
*Methotrexate brand amethopterin, Lederle Laboratories, New York, N.Y.
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Fig. 1.—Medical course of children who developed hepatitis.

Fig. 2.—Relationship of blood transfusions and 6-mercaptopurine therapy to the onset of hepatitis.

120 mg./day, was given for 12 days. Liver function improved (table 1) and hematologic improvement was nearly complete by 12/9/56. A maintenance dose of 1.1 mg./Kg./day of 6-mercaptopurine was resumed and 10 days later right upper quadrant tenderness and anorexia returned, which promptly cleared after stopping 6-mercaptopurine.

A partial remission persisted until bone marrow relapse was noted on 3/1/57. Amethopterin was given for three weeks; even so, a myeloblast crisis of 22,000 leukocytes/cu. mm. occurred. Amethopterin was discontinued. 6-mercaptopurine, 6.6 mg./Kg./day, was given for seven days and then reduced to 3.3 mg./Kg./day and continued at this rate since no other therapy was available (fig. 2). The hemogram continued to show blast forms despite the subsequent leukopenia and the patient was hospitalized again on 4/18/57 in critical condition. Jaundice and ascites recurred two months after resuming 6-mercaptopurine and the patient expired on 6/8/57. Autopsy was not obtained.
TABLE 1.—Liver Function Studies in Patients with Toxic Hepatitis

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>van den Bergh</th>
<th>Cephalin Flocculation 48 Hours</th>
<th>Alkaline Phosphatase</th>
<th>Cholesterol Gm.</th>
<th>Icteric Index units</th>
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<tr>
<td></td>
<td></td>
<td>One minute mg.</td>
<td>Total mg.</td>
<td>Thymol units</td>
<td>48 Hours</td>
<td>A/G Units</td>
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<tr>
<td>1</td>
<td>T.R.</td>
<td>10/30/56</td>
<td>8.2</td>
<td>10.3</td>
<td>2.0</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>11/6/56</td>
<td>1.25</td>
<td>1.7</td>
<td>2.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12/6/56</td>
<td>.5</td>
<td>.7</td>
<td>2.1</td>
<td>Neg.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B.R.</td>
<td>12/20/56</td>
<td>Clinically</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expired</td>
<td>12/21/56</td>
<td>jaundiced with ascites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B.J.</td>
<td>6/1/57</td>
<td>5.7</td>
<td>8.5</td>
<td>0.9</td>
<td>3+</td>
</tr>
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<td></td>
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<tr>
<td>4</td>
<td>G.Z.</td>
<td>8/13/57</td>
<td>4.5</td>
<td>6.1</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
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<td>8/20/57</td>
<td>3.1</td>
<td>3.95</td>
<td>9.0</td>
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<tr>
<td></td>
<td>8/30/57</td>
<td>.0</td>
<td>1.3</td>
<td>2.0</td>
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</table>

**Case 2:** B. R., white female, aged 4, weight 18 Kg., was first seen on 1/5/56 at Sacred Heart Hospital because of progressive weakness, fever, pallor and splenomegaly. The leukocyte count was 1,300/cu. mm. and consisted of lymphocytes and prolymphocytes. Platelet count was 50,000/cu. mm. and hemoglobin 6 Gm. A diagnosis of acute lymphocytic leukemia was established.

The patient was started on 40 mg. of Meticorten* and 5 mg. of amethopterin/day and was in complete remission by 3/1/56. Prednisone was reduced to 5 mg./day and continued until November. The amethopterin was reduced to 1 1/4 mg./day on March 1 and continued until November 20. She was in complete remission during this 7 1/2 month period.

On October 24, pancytopenia reappeared with a platelet count of 90,000/cu. mm., a white cell count of 3,000/cu. mm., with an increase in lymphocytes. On October 26 she was placed on 2.5 mg./Kg./day of 6-mercaptopurine. Amethopterin was discontinued and prednisone was increased to 40 mg./day until 11/15/56. By November 20 the white cell count had dropped to 2,000, all lymphocytes, and the platelet count remained the same.

Bone marrow examination showed marked reinfiltration with lymphocytes typical of disseminated lymphosarcoma or acute lymphocytic leukemia of childhood. 6-Mercaptopurine was increased to 3.75 mg./Kg./day; her weight on that occasion was 20 Kg. A second bone marrow examination on December 12 again revealed a packed bone marrow with only 14% nonlymphocytic cells present. Splenomegaly had recurred. On December 13 prednisone was increased to 80 mg./day. 6-Mercaptopurine was maintained at 3.75 mg./Kg./day. Even so, she worsened and was admitted to Sacred Heart Hospital. The last three days of her illness were marked by jaundice, abdominal distention, acidosis, fever and a toxic state. 6-Mercaptopurine was discontinued. She was given one dose of Chlorambucil, 4 mg., the day before death. Her icteric and toxic state existed prior to the institution of this therapy. Two transfusions had been given, i. e., one on 1/20/56 and the other on 12/20/56 (fig. 2). Patient expired December 20, 1956. Autopsy was obtained (figs. 3A and B).

**Case 3:** B. J., white male, aged 7, weight 32 Kg., was first seen on 10/12/56. A diagnosis of acute lymphocytic leukemia was made elsewhere on 8/24/56 and he was given 30 mg./day of prednisone until 10/12/56. He had been in good health prior to the onset of the present illness.

*Prednisone by Schering Corp., Bloomfield, N.J.
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Fig. 3a. (top)—Case 2. Prominent bile thrombi and swollen or multinucleated cells are demonstrated in the liver.

Fig. 3b.—Case 2. This sternal marrow demonstrates large numbers of lymphocytes. Many of these are atypical lymphocytes as shown by Wright smears. Only scattered, normal marrow elements remain.
Physical examination revealed a mild Cushingoid state, but no lymphosplenomegaly. Partial atrophy of the calf muscles was present and dorsiflexion of the feet poorly performed.

The initial leukocyte count on 8/24 '56 was 45,300 cu. mm. On 9/5/56 it was 119,000 with 91% lymphocytes and 9% prolymphocytes. Platelets were 19,300/cu. mm. and hemoglobin 13.0 Gm. On 10/12/56, the hemogram was normal.

Prednisone was reduced to 5 mg./day and amethopterin begun at 2.5 mg./day for 24 days, reducing it thereafter to 1.25 mg. day (fig. 1). He remained in full remission until February 1, 1957 when headaches, diplopia and staggering gait with widened base indicated either brain tumor or leukemic central nervous system infiltration. Because of the normal hemogram and normal-sized lymph nodes and spleen, a neurosurgical consultation was sought.* Ventriculograms and pia-arachnoid biopsy were made and leukemic infiltration was proven. Therapy partially corrected his blindness and CNS symptoms until 5/3/57 when symptoms returned. Vigorous therapy was instituted, using 80 mg. of prednisone and 3.75 mg. of amethopterin/day. Partial remission occurred on March 1, 1957 and amethopterin was reduced to 2.5 mg./day and prednisone to 10 mg./day. Relapse occurred on 4/1/57. Amethopterin was discontinued and 6-mercaptopurine was begun at 3.2 mg./Kg./day (figs. 1 and 2). On 5/29/57 convulsions forced hospitalization at Sacred Heart Hospital, Spokane, Washington. Physical examination revealed a markedly Cushingoid youngster with dilated pupils, bilateral optic atrophy and diffuse paronychia. The sclerotics were icteric and the skin yellow. Stools were clay-colored. The liver was tender and palpable down 2½ fingerbreadths and abdominal distention with shifting dullness noted. The hemogram on admission was: leukocytes 1,200/cu. mm. with 94% lymphocytes, platelets 26,000/cu. mm. and hemoglobin 14.4 Gm. Urine was bile positive. A septic state with deepening jaundice persisted. Liver function studies were abnormal (table 1). The patient expired June 9, 1957, in coma. Autopsy was obtained (figs. 4 and 5).

The patient did not receive any blood or plasma transfusions.

Case 4: C. Z., white male, aged 7, weight 25 Kg., was well until approximately September 1, 1956, when frequent bruising and generalized lymphadenopathy appeared. He was hospitalized at Sacred Heart Hospital on September 27, 1956 and received .5 mg. of aminopterin from September 29 through October 12. Because of progressive fatigue, vomiting, fever and sore throat he was referred to our care on October 15, 1956.

Physical examination revealed a pale, critically ill youngster with nasal bleeding, generalized purpura and firm, grade II cervical and inguinal lymphadenopathy. The tip of the spleen was palpable. The initial hemogram was 4,400 leukocytes/cu. mm., which consisted of virtually 100% prolymphocytes, 4.5 Gm. of hemoglobin and 20,000 platelets per cu. mm. The youngster was treated with 60 mg. of prednisone and 2.5 mg. of amethopterin/day. On October 16, 1956 he was given 1 unit of fresh blood taken from his mother (fig. 2). By November 1 he was in beginning remission and by December 20, 1956 he was in complete hematologic and clinical remission. On January 15, 1957 he was successfully operated upon for an acute gangrenous appendix. He was continued on 1.25 mg. of amethopterin/day until March 18, at which time the hemogram consisted of 68,000 leukocytes which were nearly all prolymphocytes and lymphoblasts, 14.1 Gm. of hemoglobin and platelets 34,000/cu. mm. The spleen was enlarged. Purpura had recurred. Prednisone was resumed, 40 mg./day, amethopterin discontinued and the patient placed on 6-mercaptopurine, 3.0 mg. Kg. /day. By April 10 the platelet count had increased to 100,000. The white count was reduced to 4,000/cu. mm. with a nearly normal differential. Prednisone was gradually reduced to 5 mg./day by May 1. 6-Mercaptopurine was reduced to 2.0 mg./Kg./day on April 10, and on May 15 further reduced to 1.0 mg./Kg./day. The patient continued in complete remission until June 25, when a second relapse, characteristic of acute lymphocytic leukemia, occurred. 6-Mercaptopurine was increased to 3.0 mg./Kg./day and prednisone to 60 mg./day. During the period of July 1 through July 6, 4 mg./Kg./day of 6-mercaptopurine were given and then reduced to a maintenance

* Alfred R. Kessler, M.D., Spokane, Wash.
Fig. 4a. (top)—Case 3. Low power view of the liver demonstrates lobular architecture altered only in that the hepatic cords are no longer distinct. There are very few inflammatory cells. There are no neoplastic cells.

Fig. 4b.—Case 3. High power liver demonstrates bile pigment in Kupffer cells and several hepatic cells. Note multinucleated cells.
Fig. 5a. (top)—Case 3. Cerebrum. Leukemic cuffing around blood vessels in the cerebrum.

Fig. 5b.—Case 3. Area of necrosis of the bone marrow indicating intense therapy insufficient to destroy leukemic cells noted in figure 5a.
level of 3 mg./Kg./day. Prednisone was gradually reduced. Prompt remission recurred. By mid-July, 84% of a 4,500 white blood count was polymorphonuclear and platelets had returned to normal. The patient remained well until July 31 when an acute, painful, marked cervical lymphadenopathy and leukopenia developed.

Because of the acute aleukemic relapse the dose of 6-mercaptopurine was increased to 4 mg./Kg./day, beginning August 1. Amethopterin was added at 2.5 mg./day. Prednisone was continued at 15 mg./day. By August 6 the spleen, though palpable, had reduced in size, as had the cervical nodes. By August 12 the white count had increased to 4,000 with an increasing percentage of polymorphonuclears, hemoglobin 12.35 Gm. and platelets 185,000/cu. mm. He had, however, lost his appetite and was complaining of abdominal distention; the liver was now palpable down 2 fingers and tender. Shifting abdominal dullness was present. The youngster was obviously jaundiced (table 1); accordingly, 6-mercaptopurine and amethopterin were discontinued. He was placed on 60 mg/day of prednisone and a high carbohydrate diet. By August 20 there was clearing of the jaundice, improvement in appetite and less abdominal distention. The liver tenderness had disappeared and the ascites had cleared.

However, because of progression of lymphosplenomegaly he was given .75 mc. P² intravenously on September 4, 1957. No effect was noted and on September 13, 1957 he was admitted to Sacred Heart Hospital, moribund, with acute tracheobronchitis. The second and third transfusions were given on September 6 and 10, 1957 (fig. 2). The hemogram was 42,200 leukocytes/cu. mm., consisting of prolymphocytes and lymphoblasts, platelets 63,000/cu. mm. and hemoglobin 10.6 Gm. The patient expired on September 14, 1957. Autopsy was obtained (fig. 6).

RESULTS AND DISCUSSION

Toxicology studies of 6-mercaptopurine have demonstrated liver damage in rats and dogs¹,²; however, its occurrence at usual treatment dosage in man has not been recorded. Wintrobe³ has observed several cases of jaundice in which 6-mercaptopurine was suspected as the toxic agent.

The usual dosage is 2.5 mg./Kg., continued for at least one month before assuming failure; at that time dosage may be increased up to 5 mg./Kg. Leukopenia per se is definitely not the sole indication for stopping the drug, since the disease process may present with leukopenia while at the same time the bone marrow is virtually all replaced by leukemic lymphocytic infiltration. Treatment is more apt to be pushed to higher dosage for longer periods if 6-mercaptopurine is the last drug used than if it is used initially. If 6-mercaptopurine is used initially, recognition of hepatitis becomes of more importance, since cessation of the drug may allow for use of an anti-folic agent which may result in a period of six to eight months of complete remission.

6-Mercaptopurine therapy of two to five months preceded the appearance of jaundice at all dosages. The variation in dosage used is shown in figure 2. The 5.0 mg./Kg. accepted maximum dose was exceeded for one week only in the treatment of Case 1 (fig. 2) and did not immediately precede the development of jaundice. All patients, however, were receiving dosages greater than the usually recommended level of 2.5 mg./Kg. at the time hepatitis developed. The range of dosage at that time was from 3.3 to 4.0 mg./Kg./day. Figure 1 summarizes the various factors in the leukemic life of the four cases.

The differential diagnosis of the hepatitis in the first case weighed heavily
Fig. 6a. (top)—Case 4. Liver, low power. Leukemic infiltration demonstrated on the low power. Normal liver architecture two weeks after laboratory liver function had returned to normal.

Fig. 6b.—Case 4. High power demonstrating the edge of an area of a leukemic infiltration. Mild lipid metamorphosis in otherwise essentially normal liver cells, representing post-treatment phase.
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in favor of serum hepatitis, since blood transfusions had been given 4½ months previously. At that time the high direct-acting bilirubin (table 1), without significant and sustained changes in the cephalin flocculation and thymol turbidity tests, cast doubt on the diagnosis. Further, prompt clearing of the jaundice during a critical phase of leukemia likewise seemed unlikely had serum hepatitis been present. Finally, relapse of the jaundice state after an interval of seven months did not seem likely. The subsequent three patients were clearly not affected by blood transfusions as the etiologic agent as shown in figure 2. Case 3 received no transfusions.

The possibility of an association with steroid therapy was considered but dismissed in view of the wide experience with these drugs and lack of evidence for hepatogenic implication. Whether the combination of steroids with 6-mercaptopurine is significant cannot be answered from the data. The jaundice, in any event, cleared promptly in Cases 1, 3 and 4, while continuing steroids and discontinuing 6-mercaptopurine.

The incidence of hepatitis as a complication of 6-mercaptopurine has been 14 per cent, based upon the experience of one of us (SKM) in the treatment of 29 patients with acute leukemia with 6-mercaptopurine.

Clinical laboratory evidence of the hepatitis was shown by amber urine positive for bile, elevation of direct-acting bilirubin, positive cephalin flocculation and thymol turbidity (table 1). Two cases, 1 and 4, recovered promptly upon withdrawal of 6-mercaptopurine. Liver function improved in two to three weeks. This was complete as evidenced by the subsequent examination of the liver in Case 4 (fig. 6). Progression of the leukemic process, including liver infiltration (fig. 6) did not affect the clearing of the hepatitis.

Histologic examination of the liver demonstrates an essentially similar picture in Cases 2 and 3. In both cases there is a pronounced stasis of bile as indicated by numerous bile thrombi. A prominent disruption of the normal hepatic architecture in the form of a complete loss of the hepatic cords is seen. Many individual hepatic cells are swollen by a metachromatic granular cytoplasm. Many of these cells are multinucleated. There is considerable variation in the size and chromatin pattern of the nuclei. Bile pigment is prominent in the Kupffer cells of Case 3 and in many of the hepatic cells.

There is no evidence of the focal hepatic necrosis which was described in normal animals kept on much higher doses of 6-mercaptopurine.1,2

The histologic picture seen in our cases is most similar to that reported in chlorpromazine jaundice,4 except that the cytologic changes in the individual hepatic cells were much more striking than those resulting from chlorpromazine.

The biliary stasis and cytologic abnormalities certainly are more typical of chlorpromazine jaundice as described by Kelsey4 than of either the viral or toxic types of hepatitis described by Popper,5 although the cytologic changes in the individual hepatic cells are similar to those described by Wood6 in an early case of serum hepatitis.

It is suggested that future cases of injury to the liver may be recognized at onset by careful examination to detect liver enlargement and tenderness.
occurring with nausea. Confirmation by laboratory studies, especially serum bilirubin, cephalin flocculation and thymol turbidity, will aid in establishing the diagnosis. We have had two patients develop nausea and right upper quadrant tenderness subsequent to our observations above, and the symptoms and findings promptly cleared when 6-mercaptopurine was stopped.

**SUMMARY AND CONCLUSIONS**

Four patients are presented who developed toxic hepatitis while being treated for leukemia with 6-mercaptopurine and steroids. The onset was rapid and suggested an acute hepatitis with intrahepatic blockage. Pathologic examination of the liver in two patients who expired with hepatitis is presented and the pathology described. In the third autopsied case the hepatitis had resolved while receiving massive steroid therapy, even though the leukemia progressed rapidly when 6-mercaptopurine was withdrawn. Early recognition of the toxic hepatitis becomes especially important where 6-mercaptopurine is the first drug to be used in the management of the patient with acute leukemia.

**SUMMARIO IN INTERLINGUA**

Es presentate le casos de quatro patientes qui disveloppava hepatitis toxic durante que illes esseva tractate, pro leucemia, con 6-mercaptopurina e steroides. Le declaration esseva rapide e suggereva hepatitis acute con blocage intrahepatic. Es describite le pathologia del hepate in duo patientes qui moriva con hepatitis. In le tertie caso con datos necroptic, le hepatitis se habeva resolvite in le curso del therapia a doses massive de steroide, sed le leucemia progredeva rapidemente quando le administration de 6-mercaptopurina esseva interrumpite. Le prompte recognition de hepatitis toxic es specialmente importante in casos in que 6-mercaptopurina es le prime droga usate in le tractamento de patientes con leucemia acute.

**REFERENCES**

3. Personal communication.
Hepatitis in Association with Prolonged 6-Mercaptopurine Therapy

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