L-Asparaginase in the Treatment of Neoplastic Diseases in Children

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SUMMARY

Escherichia coli L-asparaginase was administered to 29 children with leukemia in dosage schedules of 200 i.u./kg/day for a minimum of 7 days or 5000 i.u./kg twice weekly for a minimum of 2 weeks. A complete remission was achieved in 7 patients (20%) by L-asparaginase alone and in 2 further patients by a combination of L-asparaginase and prednisone. Remission was maintained with p. o. methotrexate (30 mg/m² twice weekly) with a median duration of 90 days. Reinduction, generally with the original form of therapy, was possible in 2 out of 6 patients. Hypocellularity of the bone marrow was encountered in 2 patients. Twelve patients with a variety of solid tumors failed to show any response to L-asparaginase at different dosage schedules.

The most serious side effect was hypersensitivity affecting 13 patients; in 2, the blood pressure was unobtainable for several minutes. A serum sickness-like reaction occurred in 1 patient, pulmonary edema occurred in 2 patients, and diabetic ketoacidosis occurred in 1 patient. Other side effects were generally mild and included hepatic dysfunction, elevation in serum amylase and blood ammonia (27 out of 28 patients), and mild depression and lethargy (in 4 patients with elevated blood ammonia levels). Pancreatitis and renal failure were not observed. There were no fatalities.

INTRODUCTION

In 1953, Kidd (19) reported that the administration of guinea pig serum caused regression of certain mouse and rat lymphomas. Later, Broome (7, 8) demonstrated that this tumor inhibition was due to the L-asparaginase content of the guinea pig serum. The discovery by Mashburn and Wriston (22) that the L-asparaginase present in Escherichia coli had tumor-inhibiting properties made it possible to obtain a purified enzyme for evaluating antitumor activity in man. The initial reports by Hill et al. (17) and Oettgen et al. (26) suggested that it might be a promising agent for the treatment of acute leukemias and that its action was accompanied by a minimum of toxic side effects. The efficacy of L-asparaginase as an antileukemic agent was substantiated by other workers (10, 20, 28, 29, 32, 33). Initially, the side effects encountered were chills, fever, vomiting, allergic reactions, transient changes in liver function tests, and a decrease in plasma fibrinogen, serum albumin, and lipids. However, as clinical trials proceeded, it became clear that the drug was capable of producing severe toxicity including renal failure, pancreatitis, hyperglycemia, abnormalities of clotting factors, and central nervous system dysfunction (4, 14–16, 27, 34). This communication of our initial clinical experience in the treatment of childhood cancer with L-asparaginase substantiates the findings of others and describes additional clinical and laboratory features.

MATERIALS AND METHODS

E. coli L-asparaginase was obtained initially from the Squibb Institute for Medical Research, New Brunswick, N. J., and later from Merck, Sharp and Dohme Research Laboratories, West Point, Pa., through the National Cancer Institute. The range of specific activity of the Squibb preparation was 121 to 231 i.u./mg and that of the Merck enzyme was 300 to 350 i.u./mg, 1 i.u. being that quantity of enzyme which will release 1 μmole of ammonia per min from L-asparagine at pH 8.6 and 37°. Both preparations were supplied as a dry, water-soluble powder which was stable at 4° for several months; when reconstituted with 0.9% NaCl solution, it was stable for at least 7 days at 5°. In this study, L-asparaginase was reconstituted immediately before use with 0.9% NaCl solution.

Study Plan

Pretreatment evaluation included a physical and bone marrow examination, complete blood count, liver function studies, and estimations of the serum amylase, cholesterol, cholinesterase, and blood ammonia. Renal function studies performed in a number of patients included a urinalysis and estimations of the blood urea nitrogen and serum creatinine. Most of these investigations were repeated at weekly intervals and at the completion of treatment. In 10 patients, electroencephalographic recordings were obtained. Other investigations were performed as dictated by the clinical course.

Biochemical Investigations

The blood ammonia was determined from venous blood, drawn into heparinized tubes and chilled instantly, by a modification of the method described by Conway (12). The serum amylase was estimated by the method of Caraway (11) and the serum cholinesterase by the method of Michel (23).
Total and direct bilirubin was measured by the method of Malloy and Evelyn (21), serum cholesterol was measured by the method of Zurkowski (36), serum alkaline phosphatase was measured by the method of Bowers and McComb (5) and expressed in Bodansky units, and serum albumin was measured by means of paper electrophoresis by the Durrum technique (3). Serum aspartic transaminase was estimated by the method of Reitman and Frankel (31). The normal values for these constituents in our laboratory are depicted in Table 4.

Patient Selection

Acute Leukemia (Table 1). Thirty-two children, 30 with acute lymphoblastic leukemia and 2 with acute myelogenous leukemia, refractory to or in relapse following conventional agents, were treated with L-asparaginase. The median time from diagnosis to therapy with L-asparaginase was 17 months. Their ages ranged from 2 to 13 years. Previous chemotherapy included at least 4 of the following agents: methotrexate, 6-mercaptopurine, vincristine, arabinosyl cytosine, and cyclophosphamide. All patients but 1 (Case 5) had previously received corticosteroids in combination with 1 or several of these agents. During L-asparaginase therapy, blood and platelet transfusions were administered as necessary, and vigorous treatment of infection formed part of the supportive care. Allopurinol and adequate hydration were prescribed for the control of hyperuricemia.

Corticosteroids were administered if the initial platelet count was below 50,000/ml (prednisone, 1 mg/kg/day) or to prevent a possible hypersensitivity reaction during desensitization (hydrocortisone, 100 mg/day i.v., or prednisone). Those patients who received corticosteroids for 7 consecutive days in association with L-asparaginase were considered to have received a course of corticosteroids.

The therapeutic response in these patients was evaluated in terms of the establishment of a bone marrow remission with an adequately cellular marrow, the blast count being less than 7% of the total nucleated cells counted. A partial remission was defined as a bone marrow blast count between 7 and 14%. The duration of remission was the duration of complete and partial bone marrow remission.

Other Tumors (Table 2). Twelve patients with a variety of solid tumors, all with palpable or radiological evidence of tumor, were treated with L-asparaginase. Their ages ranged from 1 year, 2 months to 18 years, 6 months. All had received previous chemotherapy, including actinomycin D, vincristine, cyclophosphamide, vinblastine, procarbazine, nitrogen mustard, and corticosteroids, and all but Patient 4 had received radiation therapy. In these patients, a complete response was defined as the disappearance of objective signs of cancer and a partial response as an incomplete decrease in tumor size of at least 25%.

Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Dosage (i.u./kg/day)</th>
<th>Duration of Treatment (days)</th>
<th>Dosage to Induce Remission(s)</th>
<th>Duration of Remission(s)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>21</td>
<td>63,000</td>
<td>90</td>
<td>Concomitant prednisone administration. Hypersensitivity reaction with 2nd course.</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>F b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>19</td>
<td>66,360</td>
<td>90</td>
<td>All remissions associated with prednisone; 3rd remission preceded by hypocellular marrow. Severe allergic reaction while on maintenance. Total dose received, 177,800 i.u.</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>F b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>13</td>
<td>50,700</td>
<td>120</td>
<td>Partial remission with 2nd course; complete remission not induced despite escalation in dosage. Received 1,257,500 i.u. over 7.5 mo. without reaction.</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td>61,600</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td>65,600</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>11</td>
<td>60,000</td>
<td>175</td>
<td>Pulmonary edema developed after 10th dose. Serum cholinesterase, 0.3.</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>12</td>
<td>126,000</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>14</td>
<td>70,000</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>14</td>
<td>70,000</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>15</td>
<td>30,000</td>
<td>30</td>
<td>Severe anaphylactic reaction with pulmonary edema during desensitization.</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>68,000</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>14</td>
<td>68,000</td>
<td>35</td>
<td>Remission preceded by hypocellular marrow.</td>
</tr>
<tr>
<td>8</td>
<td>5000 d</td>
<td>14</td>
<td>735,000</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5000 d</td>
<td>11</td>
<td>480,000</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>200–10,000</td>
<td>10</td>
<td>F b</td>
<td></td>
<td>Initial decrease in rising WBC with subsequent deterioration.</td>
</tr>
<tr>
<td>10</td>
<td>200–10,000</td>
<td>10</td>
<td>F b</td>
<td></td>
<td>Initial decrease in rising WBC with subsequent deterioration.</td>
</tr>
<tr>
<td>11</td>
<td>1000</td>
<td>8</td>
<td>F b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1000</td>
<td>18</td>
<td>F b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Maintained with methotrexate (30 mg/sq m).
b Failed induction or reinduction.
c Maintained with L-asparaginase (1000 i.u./kg twice weekly).
d Twice weekly schedule.
Table 2

<table>
<thead>
<tr>
<th>Case</th>
<th>Type</th>
<th>Age (yr)</th>
<th>Duration of disease (yr)</th>
<th>Dose (i. u./kg)</th>
<th>Days</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neuroblastoma</td>
<td>1-2/12</td>
<td>5/12</td>
<td>2000</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Neuroblastoma</td>
<td>4-1/2</td>
<td>1-1/2</td>
<td>200</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rhabdomyosarcoma</td>
<td>5-9/12</td>
<td>1-1/2</td>
<td>200</td>
<td>9</td>
<td>L-Asparaginase in ascitic fluid.</td>
</tr>
<tr>
<td>4</td>
<td>Malignant melanoma</td>
<td>15</td>
<td>1</td>
<td>200</td>
<td>14</td>
<td>Thrombocytopenia.</td>
</tr>
<tr>
<td>5</td>
<td>Hodgkin’s disease</td>
<td>13</td>
<td>4-1/2</td>
<td>200</td>
<td>11</td>
<td>Thrombocytopenia.</td>
</tr>
<tr>
<td>6</td>
<td>Hodgkin’s disease</td>
<td>8-1/2</td>
<td>1/2</td>
<td>200</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Osteogenic sarcoma</td>
<td>18-1/2</td>
<td>2-1/2</td>
<td>200</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Osteogenic sarcoma</td>
<td>15-1/2</td>
<td>1</td>
<td>200</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Atypical Ewing’s sarcoma</td>
<td>16-1/2</td>
<td>4</td>
<td>200–1000</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Burkitt’s lymphoma</td>
<td>6-1/2</td>
<td>1-1/2</td>
<td>200</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Burkitt’s lymphoma</td>
<td>16</td>
<td>1-1/12</td>
<td>5000a</td>
<td>4 doses</td>
<td>Depression, lethargy.</td>
</tr>
<tr>
<td>12</td>
<td>Wilms’ tumor</td>
<td>6</td>
<td>1/2</td>
<td>1000–10,000</td>
<td>12</td>
<td>Progressive jaundice.</td>
</tr>
</tbody>
</table>

a Twice weekly.

Table 3

Complications during therapy with L-asparaginase

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. of patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypersensitivity reactions</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Urticaria</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Angioneurotic edema</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Bronchospasm</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cyanosis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Arthropathy</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Erythematous plaques at venipuncture sites</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hypotension (shock)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Diabetic ketoacidosis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hematological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC, &lt;2000 (median nadir, 10th day)</td>
<td>8/36 courses</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia (median nadir, 12th day)</td>
<td>20/36 courses</td>
<td></td>
</tr>
</tbody>
</table>

Treatment Schedule

The first few patients were hospitalized to receive the medication, and as experience accumulated it was administered on an outpatient basis. The possible development of hypersensitivity or other toxic reactions while the drug was under investigation precluded its administration under anesthesia or by the family pediatrician.

Therapy commenced with a continuous i.v. infusion of 5% dextrose-water followed by an intradermal test dose of 0.05 i.u. L-asparaginase. The pulse, blood pressure, and respiration were monitored at 10-min intervals for 30 min. A positive test was defined as the development of anaphylaxis or induration or erythema in excess of 5 mm in diameter at the intradermal site. In the absence of a reaction, chlorpheniramine maleate (2 to 4 mg) was administered i.v., and 0.5 hr later the full dose of L-asparaginase was injected by slow i.v. push over a period of 2 min. The dextrose infusion was retained for a further 0.5 hr as a vehicle for urgent medication in the event of anaphylaxis.

Fig. 1. Arthropathy of the fingers and hands. Erythematous plaques at previous venipuncture sites where L-asparaginase had been administered.

Most allergic reactions occurred within this period. Despite these measures, sensitization could not be predicted or averted. The intradermal test dose was therefore abandoned, and, as an alternative method, a slow i.v. infusion of L-asparaginase in 250 ml of dextrose-water over a 45-min period was introduced. This did not prevent anaphylaxis.

Dosage for Acute Leukemia

Daily Schedule. Two hundred i.u./kg/day were given for 10 to 21 days. In some patients in whom remission was not achieved but improvement occurred, the duration of treatment was extended to 28 days. In 2 patients whose white blood cell count rose several days after an initial decrease in the circulating peripheral blasts, the dosage was increased from...
200 to 1000 i.u./kg/day over several days. One patient received 1000 i.u. daily for 18 days in an attempt to lower an elevated WBC.

**Twice Weekly Schedule.** Five thousand i.u. twice weekly for 2 weeks were prescribed to children who could not attend the clinic daily. Seven daily injections or 2 injections on the twice weekly schedule were referred to as a "course" and were a minimum requirement for evaluation.

**Desensitization.** An attempt was made to desensitize those patients who developed a hypersensitivity reaction to L-asparaginase. This procedure required hospitalization under constant surveillance and the immediate availability of facilities to meet any emergency. Initially, with a continuous i.v. infusion, the skin test dose of 0.05 i.u. was repeated. In the absence of a reaction, 0.5 hr later, i.v. hydrocortisone and chlorpheniramine maleate were administered. This was followed by 1 i.u. of L-asparaginase injected i.v. The dose was doubled at 15-min intervals unless a reaction occurred. Desensitization was considered successful when the cumulative amount reached the desired level without reaction. Desensitization procedures were attempted in 5 patients, and in 2 patients they were successful. One of these 2 again developed anaphylaxis several days later, and further desensitization in this patient was not attempted. Desensitization was abandoned after the 4th day if the requisite dosage had not been achieved. Each desensitization procedure commenced with 1 i.u. irrespective of the level previously attained. In no instance did successful desensitization result in remission.

**RESULTS**

**Acute Leukemia (Table 1).** Of the 32 patients studied, 3 patients (1 with acute lymphoblastic leukemia and 2 with acute myelogenous leukemia) did not complete a course of therapy and were not evaluated. Twenty-one of the 29 patients received 200 i.u./kg/day for 7 to 28 days with a median duration of 19 days. There were 7 complete remissions (Cases 1 to 7) of which 2 (Cases 1 and 3) were associated with concomitant prednisone administration. The median duration of therapy until onset of remission was 14 days (range, 11 to 21 days), and the total dosage required to produce these remissions varied from 30,000 to 70,000 i.u. (median, 61,000 i.u.). The 3 patients in whom the daily dose was increased above 200 i.u./kg did not achieve a remission despite initial responses. Of 5 patients completing a course of 5000 i.u./kg twice weekly for 2 weeks, 2 developed a complete remission (Cases 8 and 9). At the completion of therapy of Case 8, the bone marrow was hypocellular and repeat examination 1 week later demonstrated true remission. Of the 16 courses of L-asparaginase which were associated with corticosteroid therapy, there were 4 remissions (3 of these in Case 3).

The 1st L-asparaginase remission was maintained with p.o. methotrexate (30 mg/sq m twice weekly). The duration of this remission varied from 30 to 175 days (median, 90 days).

In 6 patients, reinduction was attempted with L-asparaginase, generally with the original dosage. In 2 patients, further remissions were obtained (Case 3, 2 further complete remissions; Case 4, a partial remission). The duration of the 2nd and 3rd remissions in Case 3 was 55 and 25 days, respectively; in Case 4, the partial remission lasted 35 days. An attempt to prolong the duration of the 2nd remission in Case 4 with L-asparaginase as maintenance therapy was unsuccessful, and increase of the dose failed to induce another remission. Immediately following the 3rd course of L-asparaginase in Case 3, the bone marrow was hypocellular with a moderate number of histiocytes; true remission was not documented until 1 week later. Maintenance with L-asparaginase to prolong the duration of this remission was also attempted; but 3 weeks later a severe hypersensitivity reaction developed, and the drug was discontinued. Relapse occurred 5 days later.

**Solid Tumors.** A detailed analysis of the various solid tumors treated with L-asparaginase is summarized in Table 2. The dosage varied from 200 i.u. to 10,000 i.u./kg daily in all but Patient 11, who received 5,000 i.u./kg twice weekly. There were no responses, and progressive deterioration was noted throughout the period of treatments. Thrombocytopenia occurred in 2 patients and was probably related to tumor of the bone marrow. In all other patients with solid tumors, treatment with L-asparaginase was not attended by clinically important leukopenia, anemia, or thrombocytopenia.

**Toxicity (Table 3).** Hypersensitivity reactions affected 13 patients who had initially tolerated the drug without difficulty. They usually occurred about the 8th day of the daily schedule or with the 3rd dose of the twice weekly schedule. The complete spectrum, which was seldom seen in any one patient, comprised cough, vomiting, abdominal pain, maculopapular eruptions, urticaria, angioneurotic edema,
cyanosis, bronchospasm, and cardiovascular collapse. A serum sickness-like reaction occurred in 1 patient (Fig. 1). The appearance of maculopapular eruptions or urticaria was an indication for interruption of therapy and the administration of i.v. hydrocortisone. Additional resuscitative measures were instituted when indicated. In 2 patients with anaphylactic reactions (Cases 3 and 6), the blood pressure was unobtainable for several minutes. The reactions were generally accompanied by tachycardia, but not infrequently bradycardia was noted after the acute phase had subsided. There were no fatalities, and most reactions were controlled with antihistamines and hydrocortisone.

Pulmonary edema, confirmed by radiological examination of the chest, was detected in 2 patients. One incident (Case 7) occurred as part of an anaphylactic reaction during the process of desensitization. The 2nd occurred in Case 6 several hours after the 10th daily dose of L-asparaginase and was associated with dyspnea, cough, cyanosis, bilateral rales, and mental confusion. An immediate response to oxygen and morphine therapy was obtained. L-Asparaginase therapy was not interrupted; 5 days later, a remission was demonstrated together with radiological resolution of the pulmonary congestion.

Fever attributable to L-asparaginase was considered to be present in 3 patients. It disappeared after discontinuation of therapy. Although no significant fall of hemoglobin occurred during the 11 courses of therapy in the solid tumor patients, it was not possible to assess the effect of L-asparaginase on red cell production in the leukemic group as supportive blood transfusions obscured the picture. An increase in the reticulocyte count was noted both in patients in whom remission developed and in whom relapse persisted. It is possible that normal bone marrow regeneration was compromised by prior myelosuppressive therapy in some patients.

Leukopenia (WBC, <2000/cu mm) was noted in 8 of the 36 courses of treatment in patients with acute leukemia; the nadir occurred on the 10th day of therapy. In patients with solid tumors, leukopenia was not a problem in the 11 courses of L-asparaginase therapy given. Thrombocytopenia (platelets, <50,000/cu mm) occurred in 20 of the 36 courses in the leukemic patients and in 2 of the 11 courses in the solid tumor patients, although it is probable that in the latter this was due to tumor invasion of the bone marrow.

Abnormalities in liver function were common but mild (Table 4) and usually became evident midway during the course of therapy. The more prominent derangements were elevation of the serum levels of aspartic transaminase, alkaline phosphatase, and bilirubin, both conjugated and unconjugated. In some cases, there was a reduction of the serum albumin, cholesterol, and cholinesterase concentrations, but, although these falls were statistically significant, they were insufficient to warrant modification of therapy.

Renal function was not disturbed in any of the patients studied; the blood urea nitrogen and serum creatinine concentrations and urinalysis remained within normal limits. Pancreatic function was, however, abnormal in a few patients. A moderate rise in the serum amylase was noted in 3 patients, although there were no episodes of acute pancreatitis. Transient diabetic ketoacidosis occurred in 1 patient several days after commencement of L-asparaginase therapy. The blood sugar rose to 475 mg/100 ml and remained at similar levels for 3 consecutive days in association with ketonuria. In this case, therapy with L-asparaginase was not interrupted; the ketonuria and hyperglycemia responded to insulin therapy, and the course of L-asparaginase was completed without further untoward effect.

Blood ammonia concentrations, measured in 28 patients during the course of asparaginase therapy, were raised in all but 1 case, the concentrations varying from 31 to 714 μg/100 ml. The levels were normal before the commencement of therapy. Other evidence of hepatic impairment (moderately raised levels of serum aspartic transaminase or bilirubin) was present in only 4 of the patients. Abnormalities in affect, behavior, consciousness, or coordination suggestive of hepatic precama were not prominent features. Four of the 27 patients with raised blood ammonia concentrations suffered from moderate depression and lethargy, but only 1 of these patients had any evidence of electroencephalographic abnormality (moderate slowing of the rhythm but no abnormal wave forms). The remainder of the 10 electroencephalograms studied, in patients in whom the blood ammonia concentration exceeded 200 μg/100 ml, were within normal limits.

DISCUSSION

Although the mechanism of the antineoplastic action of L-asparaginase is not fully understood, it appears that, unlike the majority of normal cells, certain neoplastic cells have an absolute requirement for exogenous sources of L-asparagine, probably to meet their requirements for protein synthesis. Other important pathways of asparagine utilization also have not been excluded (6, 30). Studies in rodent tumors have demonstrated that the antitumor effect of L-asparaginase is related to a specific depletion of L-asparagine which results in the death of those cells that are deficient in the enzyme asparagine synthetase (18). This enzyme provides an endogenous pathway for the synthesis of L-asparagine from L-aspartic acid in the presence of a source of ammonia, for example, L-glutamine, and is related to the development of resistance to L-asparaginase treatment in man (1, 15). The process by which L-asparaginase appears to affect only malignant cells by selective starvation has been referred to as amino acid depletion therapy (17).

Unfortunately, few human tumors are sensitive to L-asparaginase therapy. In this study, clinically important responses were observed in acute lymphoblastic leukemia, and no responses to therapy were detected in any of the 12 patients with solid tumors who were treated. This is in agreement with the results of other studies. Responses in lymphosarcoma, reticulum cell sarcoma, and malignant melanoma have been reported but the most consistently responsive human cancer has been acute lymphoblastic leukemia (15, 20, 32, 33). The response rate in our series of patients with acute lymphoblastic leukemia (29%) corresponds with the rate (26%) found in the study by Haskell et al. (15), in contrast to the response rate of 62% reported by Tallal et al.
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These authors also described responses in patients with acute myelogenous leukemia.

The duration of the first response to L-asparaginase therapy was disappointingly short, 30 to 175 days with a median of 90 days, when maintained with twice weekly p.o. methotrexate. This compares favorably with the median duration of complete remissions of 9 weeks, despite attempted maintenance in most cases with either 6-mercaptopurine or methotrexate reported by Haskell et al. (15) and a median duration of 60 days reported by Tallal et al. (32). Some of the latter patients received L-asparaginase as maintenance therapy. In the series reported by Leventhal et al. (20), the median duration of remissions was 58 and 64 days with various forms of maintenance therapy. The present study also indicates that reinduction of remission in acute lymphoblastic leukemia was possible with L-asparaginase but the duration of the 2nd and subsequent remissions was very brief (3 to 8 weeks). Resistance to L-asparaginase was easily acquired and could not be overcome by increasing the dose. This observation and the danger of hypersensitivity discourages its use in a maintenance program.

Previous reports have indicated that the production of normal bone marrow is relatively unimpaired during L-asparaginase therapy (15, 17, 27). Myelosuppression in the present study could have been related to disease activity or to the effect of previous chemotherapy. However, that marrow regeneration was also compromised by L-asparaginase was demonstrated by the hypocellularity that preceded remission in 2 patients. It is possible that, as experience accumulates, further evidence of this phenomenon will be seen.

Potentially the most serious of the untoward reactions encountered were the hypersensitivity reactions. Skin testing and the use of antihistaminics and corticosteroids were not found to be of value in averting these reactions. The hypersensitivity reactions resembled those which may be medicated by reaginic (Type 1) or precipitating (Type 3) antibodies (13). Unfortunately, facilities for the measurement of these antibodies were not available. The acute reactions responded satisfactorily to the administration of i.v. hydrocortisone and s.c. Adrenalin; the serum sickness-like reaction was treated symptomatically.

The E. coli preparation of L-asparaginase may be contaminated with trace amounts of endotoxin. These can mimic many of the side effects that occurred, particularly in laboratory animals (37). However, the role of endotoxin in the pathogenesis of the “allergic” reactions discussed in this study is uncertain. The L-asparaginase preparations used were not pyrogenic in rabbits. There was no dose-reaction relationship; patients receiving 5000 i.u./kg twice weekly were no more likely to have reactions than those receiving the lower daily dose.

The derangements in liver function detected in our patients were mild, the test abnormalities developing midway during the course of treatment. Hypoalbuminemia, hypofibrinogemia, and hypocholesterolemia associated with L-asparaginase therapy have been reported previously (4, 9, 14, 27, 29). The reduction in the serum cholinesterase values observed is probably another index of hepatic dysfunction. Although the i.v. injection of L-asparaginase in rabbits was associated with features reminiscent of cholinesterase deficiency [death, preceded by convulsions; capillary constriction; profuse salivation; and respiratory paralysis (1)], clinical features suggestive of this syndrome were not encountered in our patients.

The pathogenesis of the toxic effects on the liver is presently unknown; biopsies have shown a toxic hepatitis with fatty changes (29, 33, 35). The hepatotoxic effects of endotoxin in laboratory animals are well recognized (37) as is also the ability of L-asparaginase to prevent the fatty liver caused by ethionine or carbon tetrachloride (2). It is therefore uncertain whether asparagine deficiency or the presence of contaminant endotoxin underlies the hepatic dysfunction induced by L-asparaginase therapy. The fact that the nonspecificity of the E. coli preparation in clinical use is an amidohydrolase with weak glutaminase activity is a further complicating factor. Following treatment with L-asparaginase, circulating glutamine has been converted to glutamic acid (24), and this also could contribute to the toxic effects observed with the administration of this drug.

Hyperglycemia and glycosuria during therapy with L-asparaginase has been reported by others (29, 34); in one patient, it was associated with ketosis (25). Whitecar showed that the hyperglycemia that results from L-asparaginase therapy may be due to a decrease in insulin synthesis (34). The pathogenesis of the diabetic ketoacidosis in our patient is uncertain; there were no clinical episodes of acute pancreatitis, and insulin assays were not undertaken. Of interest is the report that endotoxin in laboratory animals may produce hyperglycemia and depletion of liver glycogen in addition to the derangements of liver function (37).

The neurological abnormalities induced by L-asparaginase in this study were minimal. Oetgen et al. (27) observed lethargy, somnolence, or confusion in 33% of their trials in children although distinct clinical neurological lesions were not observed. An abnormal electroencephalogram was seen in a number of patients, but ammonia levels were not reported. Haskell et al. (16) reported that neurotoxicity was a major problem affecting one-half of the adults treated, but none of the children. They considered that depletion of L-asparagine or L-glutamine might interfere with central nervous system protein synthesis or that increased levels of L-aspartic and L-glutamic acid resulting from therapy might affect brain metabolism. Two forms of diffuse brain dysfunctional syndrome were observed by Ohnuma et al. (29); the liberation of large amounts of ammonia by enzyme was estimated from the increases in L-aspartic acid values for “blank” ammonia values in asparaginase determination. In our series, raised blood ammonia levels following therapy were clearly not directly related to cerebral dysfunction since they were detected in 27 of the 28 patients studied. The marked increase in ammonia levels that may occur during therapy with L-asparaginase (17) may possibly be toxic if associated with liver function abnormalities (15). However, the parameters of liver function in the present study were not grossly abnormal and possibly account for the absence of neurological features.

The pathogenesis of the pulmonary edema in Case 6 is not entirely resolved. Initially cholinesterase deficiency was postulated, but other manifestations of the syndrome were
absent; there was no evidence of anaphylaxis and the spontaneous resolution within 5 days militates against infection.

It is obvious that L-asparaginase obtained from *E. coli* has a wide variety of side effects. Perhaps these are not unexpected since the enzyme has a profound effect on protein synthesis and is itself a protein. Also, absolute purification does not appear to have been obtained, and the role played by endotoxin awaits clarification. The most serious side effect, carrying the greatest morbidity, was anaphylactic shock. This was frequently severe enough to warrant discontinuation of the drug. The toxic effects were not limited to any particular dosage or system and were unpredictable. With increased use, further complications should be anticipated. In this context, it is strongly recommended that L-asparaginase be used under expert supervision and with facilities to meet any emergency immediately available.

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