A STUDY OF THE CHEMOTHERAPY OF LEUKAEMIA.

by

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INTRODUCTION.

Since the recognition of the disease one hundred years ago, the treatment of leukaemia has passed through two main phases. During the first fifty years treatment was by chemical agents. A large variety underwent clinical trial and some were found to temporarily influence the leukaemic process. Unfortunately many were highly toxic, and probably on account of this and our ignorance of their mode of action, they became known as cytotoxic agents. The discovery of x-rays in the early part of the present century led to a new approach in treatment, and radiotherapy thereafter was generally accepted, by most clinicians, as the treatment of choice for the chronic variety of leukaemia. During the past decade, however, interest has been renewed in chemotherapy, and it seems likely that we are now on the threshold of a second era of chemotherapy. Since the Second World War a considerable amount of research in this subject has been carried out in the United Kingdom and the United States of America and to a lesser extent in other parts of the world. As a result a number of chemical agents have been discovered which materially influence not only patients with chronic leukaemia but also those with the more rapidly fatal acute disease. Their introduction has come about in one of three ways: (a) the

observation that a compound being investigated for other reasons has a depressant effect on haemopoietic tissues - for example nitrogen mustard and urethane; (b) the synthesis of compounds related chemically to substances already known to be effective, such as in the case of triethylene melamine, triethylene phosphoramide, and 1:4-dimethanesulphonyloxybutane (Myeleran); and (c) the synthesis of analogues of nucleic acid precursors. Much attention is now being focussed on the last group of compounds. It is an entirely new field of research in chemotherapy and is considered by many investigators to be a more rational approach to the problem. Not only have some, e.g. the folic acid analogues and the purine analogue 6-mercaptopurine, resulted in dramatic remissions in patients with acute leukaemia, but with the aid of modern scientific techniques information has been obtained relating to their mode of action. In addition their importance as a stimulus to research into biochemical processes connected with nucleic acid metabolism in the normal, leukaemic and neoplastic cell as a whole, probably outweighs their present therapeutic value.

This thesis contains a study of the advances in chemotherapy which have taken place during the past ten years. For descriptive purposes it has been divided into four main sections. In the first

the history of chemotherapy has been described. This is followed by a section devoted to a comprehensive review of modern chemotherapeutic agents available for the treatment of both acute and chronic leukaemia. Allied neoplastic disorders shown to be responsive to these compounds have also been included in the text. A third section describes metabolic studies in normal and leukaemic patients and animals. The metabolism of the folic acid analogue, amethopterin, with particular reference to the problem of drug resistance is principally dealt with. Studies in the metabolism of folic acid and the citrovorum factor are also included. The final section deals with the clinical evaluation, in fifty patients, of a new compound, 6-mercaptopurine.

This thesis would not have been possible without the stimulus and advice of others. I am greatly indebted to the British Empire Cancer Campaign and the American Cancer Society for providing me with a research fellowship to study the problem of chemotherapy at the Sloan-Kettering Institute, Memorial Centre, New York City, United States of America. During the tenure of this scholarship studies were carried out under the guidance of Dr. C. P. Rhoads and Dr. J. H. Burchenal. To them I am most grateful. I should also like to thank Dr. Burchenal for permission to include Figs. 1, 2, 3, 4, 5, and 6, Dr. Doris

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HISTORICAL REVIEW

One hundred and ten years have lapsed since Craigie (1845) published his report in the Edinburgh Medical and Surgical Journal describing a patient in which "large numbers of pus and lymph cells" were observed in the blood after death. This description together with those of Bennett (1845) and Virchow (1846) are the first accurate accounts of the disease which Virchow named "Weisses Blut (Leukamie)" and which we have since called leukaemia.

The malignant nature of the disease has led to numerous forms of therapy, none of which to the present day have resulted in more than temporary improvement in the clinical and haematological state. Arsenic has the reputation of being the oldest form of effective treatment for leukaemia and its use was first described by Lissauer in 1865. Following the administration of Fowler's solution (Solution of Potassium Arsenite) to a patient suffering from leukaemia he observed marked symptomatic improvement, although in the absence of a method for ennumerating blood cells detailed information relating to changes in the blood was not recorded. Billroth (1871), Winiwarter (1877) and Morrill (1877) also commented on the efficacy of arsenic as a therapeutic agent in leukaemia, but it remained for Cutler and Bradford (1878) to accurately describe the effect of the drug on the

blood cells. They showed that Fowler's solution in gradually increasing doses caused a progressive decrease in both white and red cells. By substituting iron for arsenic the red cells increased, but a rise in the white cells occurred also and had, a month later, returned almost to their original level. Numerous publications now followed, (Sticker 1886; Thatcher 1889; Drew 1892; Osler 1892; Anderson 1893; Taylor 1894; Campbell 1894; White 1895; Bramwell 1899; McCrae (1900) all agreeing that improvement followed the use of Fowler's solution or other preparations of arsenic in cases of chronic leukaemia. The reign of arsenic as first choice in treatment of leukaemia ended following the discovery by Pusey (1902) and Senn (1903) of the beneficial effects of X-rays, and during the next thirty years radiotherapy became established as the treatment of choice in chronic leukaemia. Forkner and Scott (1931) and Forkner (1932) restudied the effect of arsenic and demonstrated the effectiveness of Fowler's solution in chronic myeloid leukaemia in particular and to a lesser extent in chronic lymphatic leukaemia. Patients with acute leukaemia were not benefitted although in some instances the total white cell counts were diminished. The interest aroused by Forkner's observations was, however, only temporary, and, apart from a few

physicians of the old school, the use of arsenic as a chemotherapeutic agent is now rarely considered.

Arsenic is given orally in the form of Fowler's solution, 5 minims three times daily, increasing one minim per day until a maximum dose of 20 minims three times per day has been reached, or toxic symptoms have intervened. Thereafter the dose is gradually diminished until a maintenance dose of five to eight minims three times daily is reached.

Although arsenic is generally considered to be the oldest form of treatment in leukaemia it must be remembered that quinine was reported by Hewson in 1852 as resulting in a return of an enlarged liver and spleen to normal size in the first case of leukaemia described in America. Wood (1852) observed a similar effect in his case. The diagnosis in these early cases was, however, uncertain. Blood studies were insufficient and the possibility that the clinical picture may have been confused with malaria cannot be excluded. However, later workers such as Bramwell (1899) described dramatic improvement following the administration of quinine, with disappearance of the enlarged spleen and a return of the leucocytes to normal levels within a few weeks. Muir (1909) also considered quinine of value but, generally, inferior to arsenic. Probably because of the advent

of x-rays, further studies of the effects of quinine in leukaemia have not been recorded.

It is generally recognised that iron has no specific effect in leukaemia but may be of value sometimes in assisting haemoglobin synthesis when iron deficiency anaemia develops. Roberts and Leonard (1869), however, reported 'a cure' following treatment with iron, but like all the reported 'cures' of leukaemia the lack of sufficient data makes it difficult to accept their diagnosis.

In 1875 Fox noted marked improvement following treatment of chronic leukaemia with phosphorus. In his account published in the Lancet and entitled "On the cure of leukaemia splenica by means of phosphorus" he reported that the white blood cells returned to normal, the spleen diminished in size and the anaemia disappeared. Broadbent (1875) and Gowers (1877) also described improvement following the administration of phosphorus in a patient with lymphatic leukaemia. Jenner (1876) and Moxon (1876) did not, however, observe the same beneficial effects. Little was heard of phosphorus as a chemotherapeutic agent in leukaemia thereafter and although the original observations of Fox, Broadbent and Gowers have never been substantiated by contemporary workers, the knowledge that arsenic and phosphorus have similar chemical properties

suggest it may, like arsenic, be able to temporarily bring about improvement in patients with chronic leukaemia. Another element closely related chemically to arsenic and phosphorus is antimony. Macfie (1920) describing a patient with both chronic myeloid leukaemia and malaria observed a marked, but temporary fall in the leucocyte count following the intravenous administration of tartar emetic. Lucia and Brown (1934a) and Lucia (1935) studied the effects of an antimonial compound in animals and human leukaemia respectively. A fall in the leucocyte count was observed in both, and in two patients with chronic myeloid leukaemia the splenomegaly diminished.

Iodine was administered to patients with leukaemia as early as 1877 by Morrill, and a year later by Cutler and Bradford without any beneficial effect being noted. Many years passed without any further observations being made. Then, as a result probably of the association of lymphoid hyperplasia with hyperthyroidism and the knowledge that the basal sedimentation rate was raised in leukaemia, interest was temporarily renewed. Friedgood (1932) reported the results of treatment of ten patients suffering from chronic lymphatic leukaemia, with Lugol's iodine solution. He was of the opinion that definite symptomatic, objective, and haematological improvement occurred. Damashek

et al. (1934) and Witts (1935) were unable to support these findings, but Israels (1935) noticed a reduction in the leucocyte count in two out of five patients with chronic lymphatic leukaemia. Damashek et al. (1934) performed a thyroidectomy in one of their patients after failure to respond to iodine with marked improvement in the clinical and blood picture. Witts (1935) was, however, unable to detect any improvement in a patient similarly treated and concluded that thyroidectomy did not seem justified as a form of treatment for leukaemia.

The only other reported chemical agent to be used in the nineteenth century was ergot, which both Da Costa (1875) and Walker (1896) considered to be beneficial and able to produce a diminution in size of the spleen. With the turn of the Century came the introduction of X-ray treatment and a general trend away from chemotherapy. Isolated reports appeared, however, concerning this form of treatment. Drysdale (1909) observed remarkable clinical and haematological improvement in a patient with chronic myeloid leukaemia following treatment with naphthalene tetrachloride. Colloidal gold, silver, and sulphur were given intramuscularly to two patients with chronic leukaemia by Cawadias and Monpherrate (1917) with no obvious improvement although the leucocyte count fell. The effect of

lead was studied by Hartmann (1931) and Krebs and Clemmesen (1934) in man and animals, but the results were unfavourable. Piney and Riach (1932) in a paper on the treatment of chronic myeloid leukaemia quoted Richter and Spiro (1910) as having observed a favourable haematological response to treatment with cinnamic acid. Only one new substance appeared to provoke much clinical interest in the period 1900-1939 and that was benzol, and it was largely from Germany and other continental countries that reports appeared of its use in chronic leukaemia. Following the observations of Selling (1910, 1911 and 1916) who described the toxic effects of benzol on the haemopoietic tissues, Korányi (1912) reported the first results of its use in the treatment of leukaemia. Chronic myeloid leukaemia appeared more responsive to treatment than chronic lymphatic leukaemia and a delay of approximately two weeks was observed before the drug became effective. Toxic manifestations were, however, common, especially gastro-intestinal upsets. Királyfi (1912) treated six cases of leukaemia and one with polycythaemia with definite benefit. Klein (1913) and Piney and Riach (1932) also found benzol an effective choice of treatment, while Kalapos (1935) in the most recent review of the subject concluded that from a study of over two hundred leukaemic

patients treated with a variety of agents over a twenty year period, benzol was quite as effective as radiotherapy. The same author believed that the dangers of benzol poisoning had been greatly overestimated because of the results of animal experiments where excessive doses were given. Injudicious treatment with benzol, he pointed out, was dangerous as it might lead to aplastic anaemia and damage to the liver and kidneys, but on the other hand, as he so correctly stated, X-rays might also lead to serious complications if not properly handled. Forkner (1938) was unable to find any evidence to incriminate benzol as a dangerous drug and although he considered Fowler's solution, at that time, probably the most reliable form of treatment, he felt that benzol was a useful chemotherapeutic agent.

Benzol is usually administered in doses of 4 ccs. daily, by mouth, in olive oil.

Apart from occasional publications such as those of Forkner and Scott (1931), Forkner (1932) and Kalapos (1935) which attempted to recreate interest in the values of arsenic and benzol, chemotherapy of leukaemia was not seriously considered again until after the Second World War. Renewed interest in this subject then occurred. The reason for this is not too certain. One must assume that it was related to the War at least in

some measure. Undoubtedly one group of drugs, the nitrogen mustards, appeared as a result of the interest surrounding mustard gas and its derivatives, as a direct consequence of war. Although Krumbhaar and Krumbhaar (1919) first described the effect of mustard gas derivatives on the blood and bone marrow it was not until the clinical trials carried out in the United Kingdom initially by Wilkinson and Fletcher (1947) and in the United States of America by Rhoads (1946) and Goodman et al. (1946), using methyl-bis (B-chlorethyl) amine hydrochloride and tris (B-chlorethyl) amine hydrochloride, that these derivatives were found to be effective, when given intravenously, in chronic leukaemia and in certain related diseases, particularly Hodgkin's disease. In an attempt to find a nitrogen mustard with a more selective effect on tumour tissue and less toxic and more convenient to administer, many new compounds were synthesised and tested initially against various animal tumours including leukaemia (Burchenal et al. 1948) and later, those considered suitable, against human leukaemia. The result was the introduction by Haddow, Kon and Ross (1948) of a nitrogen mustard derivative (B naphthyldi-2-chloroethylamine; R 48), which possessed nitrogen-mustard-like activity and was effective when given by mouth in inhibiting the growth of transplantable rat tumours. Mathews (1950)

and later other investigators, revealed that it produced temporary relief in chronic leukaemia and Hodgkin's disease. In the United States Karnofsky et al. (1951) reported on the effectiveness of triethylene melamine (TEM) as an oral nitrogen mustard. Other similar preparations have since been tested and in 1953 Haddow and Timmis described 1:4 Dimethanesulphonyloxybutane, or Myeleran, which Galton (1953, 1955) found to be effective in chronic myeloid leukaemia. A compound allied to TEM, triethylene thiophosphoramide (thio-TEPA) has also recently been described by Shay et al. (1953) and Zarafonetis et al. (1955) and found to be useful in the treatment of the chronic leukaemias.

About the time the clinical effectiveness of nitrogen mustard was being described, Haddow and Sexton reported in 1946 on the inhibiting effect of ethylcarbamate, ethyl phenylcarbamate, and isopropyl phenylcarbamate on tumours in mice. Subsequently Paterson and her colleagues (1946) reported the beneficial effect of ethyl carbamate (Urethane) in patients with chronic leukaemia. Since then many similar reports have appeared.

Colchicine derivatives have recently been the centre of much interest although it was as long ago as 1908 that Dixon and Malden described the influence of colchicine on leucocytes and the bone

marrow. Dustin (1934) showed colchicine to be an effective inhibitor of mitosis but it was not until 1953 that Santavy and Reichstein isolated a new alkaloid, deacetyl methylcolchicine (Colcemid; domecolcine), of low toxicity but effective in chronic leukaemia (Moeschlin et al. 1953; Wilkinson 1955).

The chemotherapeutic agents described above have been generally found effective only in chronic leukaemia. However, during recent years important advances have been made in the chemotherapy of acute leukaemia. In 1948 Farber published the remarkable observation that two folic acid conjugates, pteroyldiglutamic acid and pteroyltriglutamic acid, when given to children with acute leukaemia produced a rapid progression of the disease. Heinle and Welch (1948) supported this finding. This was the impetus to considerable research and one cannot help but feel that the introduction of the folic acid antagonist, 4-aminopteroylglutamic acid (aminopterin), and later 4-amino-N 10-methyl pteroylglutamic acid (amethopterin), together with Farber et al.'s (1948) account of the beneficial effects of aminopterin in acute leukaemia, was probably the stimulus for the immense interest in the subject of leukaemia chemotherapy which followed. Numerous analogues of nucleic acid precursors have been

synthesised and tested for anti-tumour activity at such centres as the Sloan-Kettering Institute, New York, U.S.A., where a large tumour screening programme has been in progress since 1942. 2, 6-diaminopurine (Burchenal et al. 1951), 2, 4-diamino-5 (3'4'dichlorphenyl) 6-methylpyrimidine (Murphy et al. 1954) and other compounds such as the dihydrotriazines (Modest et al. 1952; Farber et al. 1953) have all been shown to have anti-leukaemic properties, and able to produce occasional temporary remissions in acute leukaemia. One compound, 6-mercaptopurine (Burchenal 1953; Fountain 1954, 1954a, 1955) has been found to be effective in both acute leukaemia and chronic myeloid leukaemia. This form of chemotherapeutic research would appear to have a more rational basis, and may lead to an increase in our knowledge of the metabolism of mucleic acid and to the understanding of the metabolism of the neoplastic cell as a whole. In addition to the folic acid antagonists and 6-mercaptopurine, adrenocorticotrophic hormone and cortisone were described in 1949 by Pearson et al., and later by numerous other investigators as being able to bring about complete clinical and haematological remission in children, and occasionally in adults, with acute leukaemia. Unfortunately like all the other agents used in the treatment of leukaemia

in the past one hundred and ten years the response is only temporary and the ultimate prognosis remains the same.

REVIEW OF THE LITERATURE

A. ACUTE LEUKAEMIA.

Whereas certain chemical agents have been known for many years which favourably affect the course of chronic leukaemia, the treatment of acute leukaemia with such agents has come about within the past ten years. Although, as yet, of only limited therapeutic value the discovery of these compounds has stimulated much research in this field and has resulted in important advances both in therapeutics and in the understanding of the metabolism of the malignant cell.

The introduction of the folic acid antagonists is probably one of the most important fundamental advances in the treatment of neoplastic disease. The use of these antimetabolites as chemotherapeutic agents in acute leukaemia represents a biological approach to the treatment of what is generally considered to be a neoplastic disease, and is based on a knowledge of the metabolism of the leukaemic cell. Before the introduction of the folic acid antagonists, the natural course of acute leukaemia was one of continuous regression and death, usually within four or five months. A few patients had spontaneous remissions but such occurrences were unusual. To-day, with chemotherapeutic agents such as the folic acid antagonists, cortisone and ACTH, and more recently 6-mercaptopurine, remissions in children are to be expected in more than 50 per cent

of patients treated.

The ability of these agents to produce remissions during which the patient is apparently normal, both clinically and haematologically, is probably of more fundamental importance, in our present state of knowledge, than the fact that the average survival time of children with acute leukaemia is now longer than in previously untreated patients. In addition their importance as a stimulus to research into biochemical processes connected with nucleic acid metabolism in the normal, leukaemic, and neoplastic cell as a whole, probably far outweighs their present therapeutic value.

In 1948 (Farber et al. 1948) during the course of clinical trials of a variety of incurable neoplastic conditions with two folic acid conjugates, pteroyl-diglutamic acid and pteroyl-triglutamic acid, gave them to eleven children with acute leukaemia. He observed in each case a rapid progression of the clinical course of the disease, what he termed an "acceleration phenomenon". A very rapid extension of the disease in the viscera and in the bone marrow occurred in comparison with untreated patients. Heinle and Welch (1948) also observed that a rapid clinical and haematological relapse occurred in three cases of chronic myeloid leukaemia given large doses of folic acid (pteroylglutamic

acid; PGA).

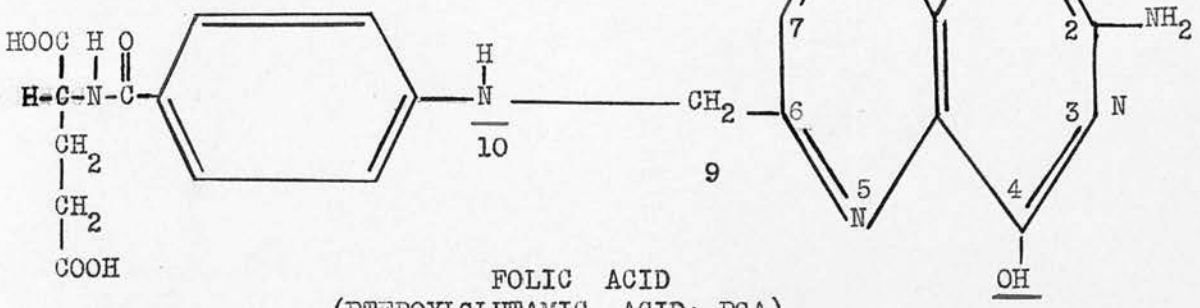
Such evidence that folic acid and its conjugates could induce apparent stimulation of abnormal leucocyte production led to the hypothesis that folic acid was an essential metabolite for leucopoiesis. It was this rationale that instituted the trials with the analogues of folic acid, commonly termed the folic acid antagonists, in patients with leukaemia.

Numerous analogues of folic acid have been prepared of which it is necessary to mention only a few. The first to show any effect on the leukaemic process was pteroylaspartic acid (Anfol A) prepared by substitution of aspartic acid for the glutamic acid residue of the folic acid molecule. N^{10} methyl-pteroic acid (Metfol B) also had some beneficial effect and led to the development of more potent antagonists of folic acid. The antagonist activity was found to be markedly increased by the replacement of the hydroxyl group by an amino group in position 4 of the pteridine ring, with the formation of 4-aminopteroylglutamic acid or as it is generally known, aminopterin (Seeger et al. 1947). Another analogue with similar activity was prepared by substituting a methyl group for a H atom in position 10 of the aminopterin molecule with the formation of 4-amino-N^{10}-methyl pteroylglutamic acid (Amethopterin)

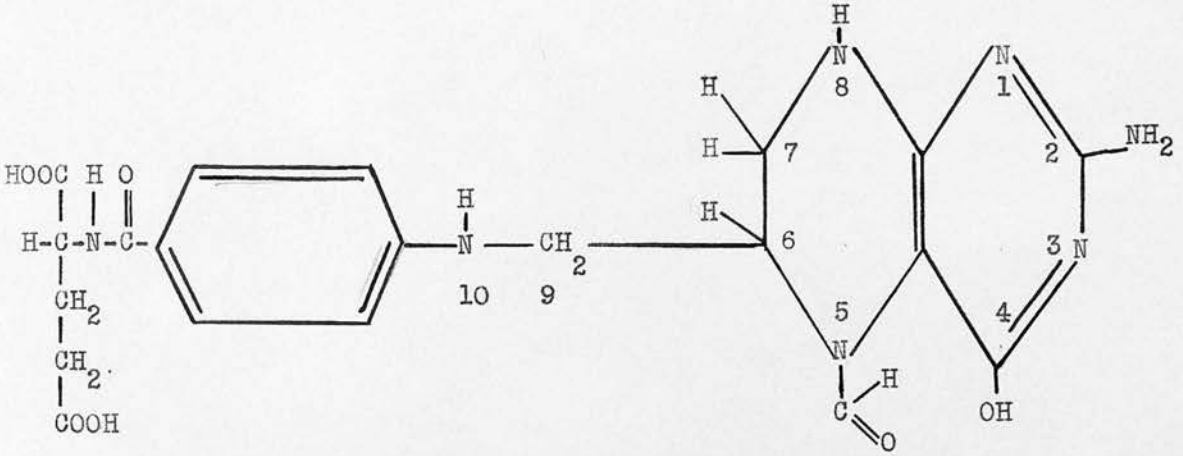
PABA

Pteridine

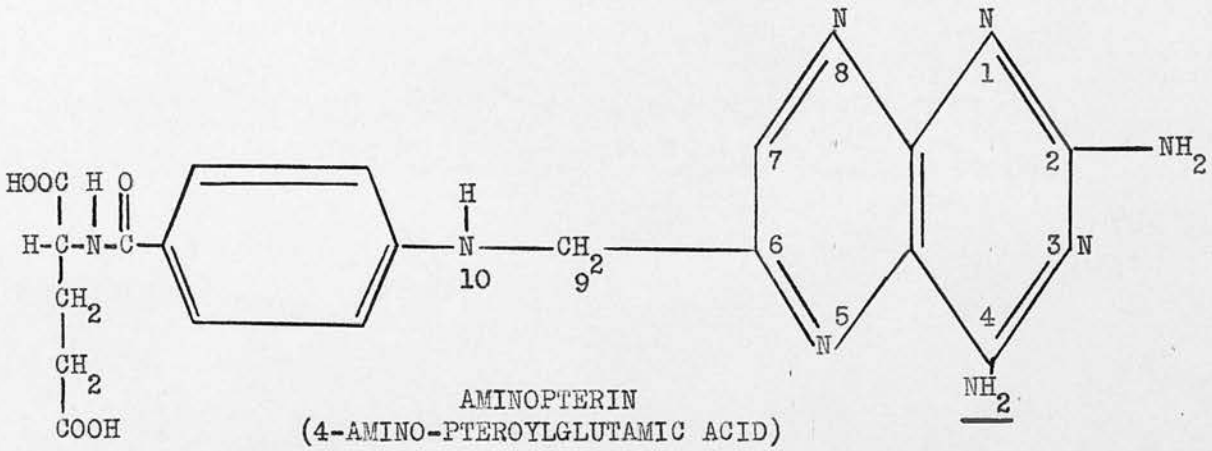
Glutamic Acid



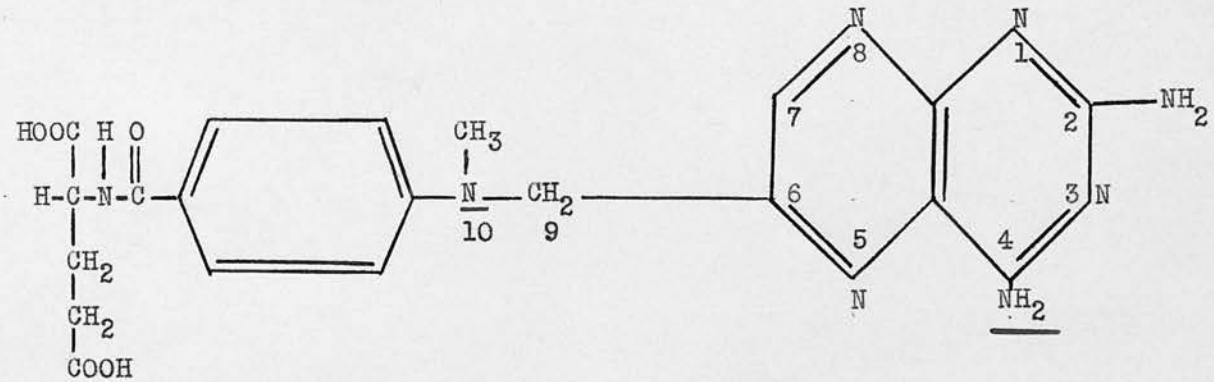
FOLIC ACID
(PTEROYLGLUTAMIC ACID; PGA)



5-FORMYL -5,6,7,8-TETRAHYDROPTEROYLGLUTAMIC ACID
CITROVORUM FACTOR (CF; LEUCOVORIN; FOLINIC ACID)



AMINOPTERIN
(4-AMINO-PTEROYLGLUTAMIC ACID)



AMETHOPTERIN
(4-AMINO-N^{10}METHYL-PTEROYLGLUTAMIC ACID)

(Franklin *et al.* 1949, Seeger *et al.* 1949, Jukes *et al.* 1950). The presence of a methyl group in the latter compound modified the toxicity in chicks but the anticipated reduction in toxicity in human subjects was not fulfilled.

Many more analogues of folic acid have been synthesised but aminopterin and amethopterin have proved to be the most effective for the treatment of acute leukaemia. Farber *et al.* (1948) in their initial report stated that of sixteen infants and children treated with aminopterin, ten showed clinical and haematological evidence of improvement. This observation was the stimulus which started large scale clinical trials of the folic acid antagonists throughout the United States of America and to a lesser extent in the United Kingdom and other parts of the world.

THE BIOLOGICAL ACTIVITY OF THE FOLIC ACID ANALOGUES

For the satisfactory understanding of the role of folic acid in neoplastic tissue it is necessary to consider the manifestations of folic acid deficiency in normal tissues.

It is well established that folic acid is an essential growth factor for a variety of lactobacilli (Jukes and Stokstad 1948), a ciliated protazoon Tetrahymina Geleii (Kidder 1946) and for the development of the pupae of the mosquito Aedes Egypti (Goldberg et al. 1944) and other insects (Fraenkel and Blewett 1947, Grob and Brunner 1946). In the absence of folic acid growth is prevented and hence the value of Streptococcus faecalis R. and Lactobacillus casei in mocrobiological assay procedures for folic acid.

Folic acid is also an essential metabolite for the growth of myeloid and erythroid tissues and in man it is now recognised that a deficiency is associated with the development of a megaloblastic anaemia. In monkeys, by feeding folic acid deficient diets, May and his associates have produced classical megaloblastic erythropoiesis associated with leucopenia, diarrhoea and lesions of the buccal mucosa (May et al. 1951, Sundberg et al. 1952). Similar observations described previously by Wills and Stewart (1935) as a

deficiency of Vitamin M are now generally recognised to have been due to folic acid deficiency. Folic acid deficient diets given to chicks in addition to inadequate growth and poor feathering led to anaemia, leucopenia and thrombocytopenia (Campbell et al. 1944, Hogan and Parrott 1940, Pfiffner et al. 1943). Rats receiving "purified" diets supplemented by sulphonamide drugs to suppress the biosynthesis of folic acid in the intestine, developed anaemia and granulocytopenia (Endicott et al. 1945, Spicer et al. 1942, Thiersch and Phillips 1949). In these few instances it is obvious that the cardinal feature of deficiency of folic acid is a derangement of haematopoiesis. In all cases when deficiency is produced by dietetic means a reversal to normal can be produced by administering folic acid or its conjugates, pteroyldiglutamic acid, pteroyltriglutamic acid or pteroylheptaglutamic acid (Endicott et al. 1945, Wright and Welch 1943, Daft et al. 1946, Hutchings et al. 1944, Suarez et al. 1946, Frost et al. 1946).

The use of folic acid analogues results in similar manifestations of folic acid deficiency. A crude methyl-substituted derivative of folic acid, referred to as X-methyl folic acid, was the first analogue found to antagonise the Vitamin in mammals. Characteristic signs of deficiency can be produced

in mice (Weir *et al.* 1948), rats (Franklin *et al.* 1947), chicks (Franklin *et al.* 1947a), and pigs (Cartwright *et al.* 1948) by feeding this compound. In pigs, a syndrome resembling the sprue syndrome resulted with the development of diarrhoea, loss in weight, a macrocytic anaemia and leucopenia. The manifestations of deficiency produced by this product are, similar to the dietary-induced deficiency, prevented or reversed by increasing the dietary level of folic acid. Aminopterin has been shown to produce a similar sprue-like syndrome in dogs (Thiersch and Phillips 1949a) with a characteristic megaloblastic bone marrow. By injecting guinea-pigs with 0.25 mgm. of aminopterin daily, Girdwood (1950) produced a moderately severe normoblastic anaemia. Evidence of folic acid deficiency induced by aminopterin has been recorded in other animal species by several investigators (Minnich and Moore 1948, Franklin *et al.* 1948, Swendseid *et al.* 1948, Oleson *et al.* 1948). One of the interesting features of the aminopterin- or amethopterin-induced deficiency is the fact that it is not readily prevented or reversed by folic acid, suggesting that these compounds are not true, competitive antagonists of folic acid.

THE MODE OF ACTION OF THE FOLIC ACID ANALOGUES

It may be said that an antimetabolite functions by replacing the metabolite in its particular enzyme system (Woolley 1952). Thus the folic acid analogues combine with folic acid enzyme systems, presumably because of their similar chemical structure. However, in view of the structure not being identical to that of the metabolite, they are unable to proceed through the remainder of the cycle of metabolic reactions. The end result is a blockage of the enzyme system and hence of the utilisation of folic acid. The degree of blockage of the enzyme system is dependent on the analogue in question and the presence of an amino group in position 4 of the folic acid molecule seems to be the most effective in preventing folic acid utilisation.

Various workers including Lampen *et al.* (1946) and Rogers and Shive (1948) have shown that the metabolic function of folic acid appears to be concerned with the synthesis of thymine and purine bases, which in turn enter into the metabolism of the nucleic acids, in particular the synthesis of desoxyribonucleic acid (Stokes 1944). Deficiency of folic acid results in an impaired formation of desoxyribonucleic acid (Prusoff *et al.* 1948) while excess of the metabolite enhances its formation

(Rege and Screenivasan 1950). Inhibition of nucleic acid formation by aminopterin and amethopterin would therefore appear to be due to blocking the synthesis of essential purine bases (Skipper *et al.* 1950, 1951, 1951a).

The action of folic acid antagonists cannot, however, be explained solely on the basis of an interference with the metabolism of folic acid. Studies by Franklin *et al.* (1948, 1949) indicate that folic acid can prevent the toxicity of aminopterin and amethopterin only when the analogues are administered near the minimal lethal dose. At higher doses of the analogues folic acid is ineffective. Since the demonstration of citrovorum factor (5 formyl, 5, 6, 7, 8-tetrahydropteroylglutamic acid; Leucovorin; Folinic Acid S.F.; C.F.), which has a close biological relationship to folic acid and is essential for the growth of the organism *Leuconostoc citrovorum* 8081, several workers have shown the more potent effects of this compound in reversing the toxic effects of the folic acid analogues. Thus Burchenal and Babcock (1951) have demonstrated the ability of C.F. to prevent the toxicity of massive doses of amethopterin in mice. Similarly C.F. has been found to be more effective than folic acid in preventing the growth inhibition of *S. faecalis R.*

(Broquist *et al.* 1950) and *L. citrovorum* (Sauberlich 1949) by aminopterin. It appears, therefore, that the 4 amino-analogues of folic acid act as competitive antagonists with respect to C.F. They also appear to act as non-competitive antagonists in relation to folic acid, such a mechanism being suggested by the work of Nichol and Welch (1950) who showed that aminopterin added to rat liver slices blocked the conversion of folic acid to C.F.

One of the interesting features of the actions of aminopterin and amethopterin is that their incorporation in folic acid enzyme systems appears to be almost irreversible. By injecting mice with amethopterin it can be readily shown with the aid of a plate assay technique (Burchenal *et al.* 1951a) that this compound becomes concentrated in tissues containing C.F. and folic acid, mainly the liver and kidney (Fountain *et al.* 1952).

Here it remains at almost the same concentration for a considerable period and is not readily replaced by either folic acid or C.F. at high doses (Fountain *et al.* 1953). Similarly human volunteers (Burchenal *et al.* 1951) showed that only 40-57 per cent of a 5 mg. oral dose of amethopterin was excreted in twenty-four hours, the majority within six hours. Using a modified technique a delay in

excretion of amethopterin was observed in twenty seven patients with acute leukaemia (see page 179) The serum level pattern and rate of excretion did not however show any obvious difference to those found in a limited series of control subjects. Swendseid et al. (1952) have also shown in patients with acute leukaemia that, following the administration of aminopterin, considerable although variable amounts are retained in the tissues. The same investigators have in addition demonstrated in human subjects suffering from leukaemia treated with aminopterin, that following a single oral dose of folic acid, larger quantities of the metabolite were excreted than would have occurred in patients not receiving aminopterin. The deduction is that the folic acid analogues are possibly retained in human tissues in a manner similar to that observed in experimental animals, and that A. the utilisation of folic acid is prevented by the blockage of the folic acid enzyme systems by the analogue, and B. the incorporation of the antimetabolite in these systems is almost wholly irreversible.

Other workers (Dougherty and Dougherty 1950, Higgins and Woods 1949) have suggested that a further action of aminopterin and amethopterin may be mediated by the adrenals, although in mice the

antileukaemic properties of these compounds do not appear to be prevented by adrenalectomy (Law et al. 1949). Hanlon et al. (1950) found no evidence of adrenocortical stimulation in leukaemic subjects treated with aminopterin.

That the folic acid antagonists do not wholly exert their influence through interference with folic acid enzyme systems in the viscera has been shown by experiments in which changes in the bone marrow of the rat have been observed in the absence of the abdominal viscera including the liver, kidneys and adrenal gland (Karnofsky et al.). This suggested a direct action on the haemopoietic tissues, and the possibility that the folic acid antagonists might in addition be cytotoxic agents. The experimental data which conclusively demonstrates their antifolic activity, the ability of C.F. to reverse the antileukaemic effect of amethopterin in leukaemic mice (Burchenal et al. 1950, Burchenal et al. 1949), the prevention of toxicity in human subjects with C.F. (Burchenal 1952) and the appearance occasionally in patients undergoing treatment, of evidence of folic acid deficiency in the form of megaloblastic erythropoiesis, indicates, however, their main action to be antagonists of folic acid.

Recently Jacobson (1954, 1954a, and 1954b) has

shown that one of the principal modes of action of folic acid antagonists is to interfere with the function of C.F. in the growth of both normal and pathological cells. C.F. was demonstrated to play an essential part during mitotic division and without this factor during treatment with aminopterin, bone marrow cells from acute human and mouse leukaemia, which had entered mitosis, were unable to advance beyond the metaphase stage. Similar observations were made on the normal intestinal epithelium of the mouse. The same author also made the interesting observation that aminopterin was inactivated when cultured with various tissues including mouse and human leukaemic cells, but the mechanism of inactivation was obscure.

In patients with acute leukaemia rapidly proliferating leucocytes have been shown to have a content of C.F. approximately five times that of the normal leucocyte (Swenseid et al. 1951). The same investigators (Swenseid et al. 1952a) also reported that leukaemic patients excreted less of a test dose of folic acid as folic acid than did normal individuals. The latter observation has been supported by Girdwood (1953). These findings suggest that the primitive leucocytes concentrate C.F. because of their relatively high metabolic

requirements and it has been postulated that on this basis they would be more susceptible to the action of the folic acid antagonists and so provide a rational basis for the use of these compounds in the treatment of acute leukaemia. Similar studies, however, carried out by the author in collaboration with Drs. Burchenal, Murphy, Ellison and Hutchison failed to reveal any difference in the demands for exogenous C.F. between the leukaemic and non-leukaemic subject.

THE CLINICAL APPLICATION OF THE FOLIC ACID ANTAGONISTS IN THE TREATMENT OF ACUTE LEUKAEMIA.

Initially the 4-amino derivates of pteroylglutamic acid were given intramuscularly, but it has now been shown that both aminopterin and amethopterin are equally effective by mouth. Absorption of amethopterin has been demonstrated to be equally rapid by the oral or intramuscular route (Burchenal *et al.* 1951a). If a 5 mgm. dose of amethopterin is administered to a normal individual on a fasting stomach, appreciable levels of the drug can be detected in the serum fifteen to thirty minutes later with a rapid disappearance from the blood in three to twenty-four hours. Only where there is impaired real function, as may occur in the later stages of acute leukaemia, are detectable amounts of amethopterin found in the

serum after twenty-four to forty-eight hours. It is when renal excretion is impaired that great care must be exercised, as serious toxic manifestations are likely to occur.

The initial dose of amethopterin for a child is 2.5 or 5.0 mgm. (half to one tablet) depending on the age. The equivalent dosage of aminopterin is 0.5 or 1 mgm. (half to one tablt). In adults up to 10 mgm. of amethopterin and 3 mgm. of aminopterin may be administered daily.

Both compounds appear to be equally effective in producing remissions, the only advantage of amethopterin being that the dose is higher and therefore more easily regulated.

Whether or not patients are treated in hospital or as out-patients is dependent upon their general condition, the presence or absence of high fever, severe haemorrhage or infection, etc. In the author's experience at the Memorial Centre, New York City, all patients whose immediate prognosis was good were treated as out-patients. They were seen twice weekly when full clinical and haematological examinations were carried out, excepting bone marrow examination, which was usually performed at fortnightly intervals. During periods of remission patients were seen at less frequent intervals. Patients requiring blood were

transfused in the out-patient clinic and in the absence of haemorrhage were usually allowed to return home. Fever necessitated the use of antibiotics (penicillin, aureomycin, terramycin, etc.), which were prescribed for out-patients by the family doctor.

The usual plan of therapy in children is to prescribe 2.5 mgm. of amethopterin daily (or an equivalent dose of aminopterin) at the outset. This is continued until a remission occurs as evidenced by a return of the bone marrow to normal function. Regulation of the dosage is dependent on the state of the patient's bone marrow, the peripheral white count and whether or not toxic manifestations occur. A failure to respond, as shown by a deterioration in the patient's general condition, a stationary or rising leucocyte count, a failure of the number of leukaemic cells in the bone marrow to fall, and an enlarging spleen or increasing lymphadenopathy, is an indication to increase the dose of amethopterin. A low platelet count when secondary to the disease, with or without associated haemorrhage, is not a contra-indication to treatment, for it is only by suppressing the leukaemic process that haemorrhage can be controlled for any length of time.

The appearance of a remission as evidenced by a return of the patient to apparently normal health

with a normal or greatly improved haematological status, may occur at varying intervals after the onset of therapy. It may be necessary to continue treatment for only two or three weeks, while in other instances evidence of a remission may not occur for as long as three months.

When a remission results therapy is sometimes withheld until signs of relapse occur. Such intermittent therapy has been advocated in the hope that premature drug resistance might be prevented. **Farber** (1951), however, maintains that treatment should be continued during a remission in an attempt to delay a relapse. It is doubtful whether either regime holds any significant advantage over the other.

Contra-indications to increasing the dosage of the antagonist are the presence of renal dysfunction and the onset of signs of toxicity. As there is marked individual variation in the tolerance to these compounds it is essential that signs of developing toxicity should be watched for in all patients. With the onset of toxic symptoms treatment is suspended temporarily until they subside, after which treatment is restarted, usually at a lower dosage. In many instances the folic acid antagonists must be given until toxic manifestations develop, as not infrequently the onset of toxicity precedes a remission.

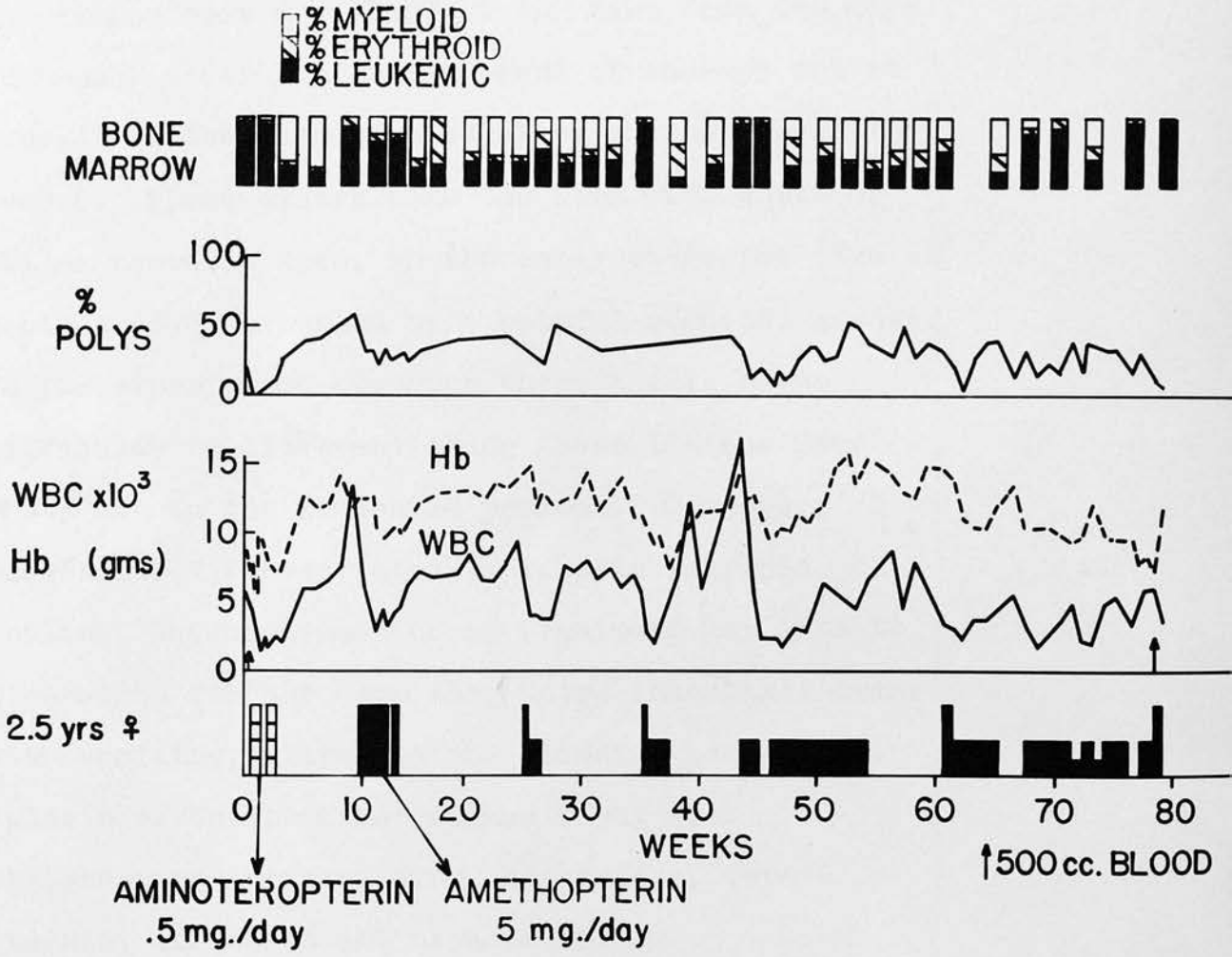


FIG. 1. Prolonged improvement, in a child with acute leukaemia, following treatment with folic acid antagonists.

TOXIC MANIFESTATIONS

The earliest sign of toxicity of amethopterin and aminopterin may occur at any time from two days to months after the commencement of therapy and is usually in the form of ulceration of the buccal mucosa. These ulcers take the form of a white or yellow necrotic area, in the early stage the size of a pin head, surrounded by a painful reddened mucosa. To the experienced observer there should be no difficulty in differentiating these lesions from those due to the leukaemic process. Temporary cessation of therapy usually results in rapid healing, but continuation of treatment may lead to ulceration further down the gastro-intestinal tract with vomiting, diarrhoea and bleeding per rectum. Aplasia of the bone marrow may occur with pancytopenia and resulting haemorrhage, severe anaemia, infection and high fever.

Alopecia is a later sign and may not be recognised in the early stages. Skin rashes may develop, but these are usually evidence of sensitivity to the drug and not infrequently respond to treatment with antihistamine drugs.

Megaloblastic erythropoiesis indicative of folic acid deficiency is not uncommonly found, and although not necessarily associated with toxic manifestations elsewhere is an indication to discontinue or reduce the dosage of the antagonist.

By withholding treatment at the onset of toxicity, together with the institution, if necessary, of blood transfusions and antibiotics, recovery is the rule in the majority of patients.

There seems to be no advantage in giving folic acid or C.F. once the toxic manifestations have developed. Experiments in animals have shown that C.F. will prevent the toxicity of amethopterin if given immediately prior to the antimetabolite (Burchenal and Babcock, 1951). No significant protection is afforded when a similar dosage of C.F. is given four or more hours after the administration of amethopterin. That C.F. is capable of preventing toxicity in human subjects has been shown by Burchenal (1952). In a patient extremely sensitive to amethopterin who developed signs of toxicity after amethopterin 2.5 mgm. daily for eight days, 3 mgm. of C.F. were given at the same time as the antagonist and the patient was then able to tolerate up to 60 mgm. of amethopterin daily for twenty-one days. Schoenbach et al. (1950) recorded a somewhat similar observation on two patients.

It was hoped that with the introduction of C.F. toxicity might have been prevented without interfering with the chemotherapeutic action of the folic acid antagonists. It has been shown in mice that the antileukaemic effect of amethopterin is prevented

by C.F. (Burchenal et al. 1950, Burchenal et al. 1949) and although a similar effect seems likely in human subjects it has not yet been conclusively demonstrated. Several observers believe C.F. to hasten the leukaemic process in man, and the consensus of opinion is that it holds no place in the treatment of acute leukaemia with the folic acid antagonists.

EVALUATION OF THE RESULTS OF TREATMENT OF ACUTE LEUKAEMIA WITH THE FOLIC ACID ANTAGONISTS.

Many clinicians consider that every patient with neoplastic disease should be given the opportunity of any new therapy, and for this reason presumably a therapeutically controlled series is rarely available. Untreated acute leukaemia with its unpredictable course, renders the evaluation of results of therapy extremely difficult, a problem which is made more complex by the absence of a satisfactory series of controls. Before evaluating the results of antifolic treatment one must take into account other factors which may influence the course of the disease, and the incidence of spontaneous remissions.

Spontaneous Remissions in Acute Leukaemia

It is well recognised that temporary spontaneous remissions may occur during the course of acute leukaemia. Evidence of such remissions extends back as far as 1878 when Eisenlohr reported regression of organ enlargement together with a fall

in the leucocyte count in a male adult. Heuck (1879) reported a similar remission and in more recent years the subject of spontaneous remissions has been reviewed by Forkner (1938), Dreyfus (1948), Birge et al. (1949), Jimenez de Asua (1951), Southam et al. (1951) and Tivey (1954).

An analysis of the reported cases of spontaneous remissions in many instances is made difficult by the lack of necessary haematological data, in particular studies of bone marrow cytology. As Southam et al. (1951) stress in the discussion of remissions in acute leukaemia, a precise definition of terms is essential. The term remission is sometimes loosely applied to any improvement in the general clinical status such as might frequently follow blood transfusion. This seems undesirable as in the vast majority of instances no change in the basic pathological state occurs. It is now generally recognised, since the introduction of chemotherapeutic agents able to favourably alter the haematological status in acute leukaemia, that a remission infers a reversal of the peripheral and bone marrow blood picture towards normal - complete or partial depending on the degree of improvement - in addition to clinical improvement. With such criteria Southam et al. (1951) in an excellent review of patients treated at the Memorial Centre, New York, from 1926-48, were able to detect only two instances

of complete spontaneous remissions in their series of one hundred and fifty cases, both being in children, although from the data available a total of 8.7 per cent spontaneous remissions of varying degree was assumed, 4.0 per cent of which appeared to be complete.

From a study of the literature spontaneous remissions appear to be rare, and the variation in their reported incidence seems probably due to some fundamental differences in definition of the term remission. Diamond and Lubby (1951) found that 8.6 per cent. of three hundred children observed prior to 1947 exhibited evidence of a partial or complete remission. Dante et al. (1951) noted one in thirty-seven children, Jersild and Mehlsen (1951) two in sixty children, Zuelzer (1949) eighteen in seventy-five children, Smith and Bell (1950) three in seventy-four children, Rodgers et al. (1951) none in one hundred and forty children. Including the figures reported by Southam et al. (1951) the above publications comprise nine-hundred and twenty-one cases of acute leukaemia in children of which sixty-two (6.7 per cent.) showed evidence of spontaneous remissions of varying degree. (Table 1) Reports of the incidence of spontaneous remissions in adults are few. Rosenthal (1950) noted six in two thousand patients, while Damashek et al. (1950) quoted figures of 1-2 per cent. Ross (1950) stated

Investigators	No. of Patients	Spontaneous Remissions	
		No.	Per Cent.
Southam et al. (1951)	83	12	14.4
Diamond and Luhby (1951)	300	26	8.6
Darte et al. (1951)	37	1	2.7
Jersild and Mehlsen (1951)	60	2	3.3
Zuelzer (1949)	75	18	24.0
Smith and Bell (1950)	74	3	4
Rodgers et al. (1951)	152	Nil	-
Brandberg (1943)	140	Nil	-
Total	921	62	6.7

TABLE 1. Incidence of Spontaneous Remissions reported in Children with Acute Leukaemia.

that they never occurred. Southam *et al.* (1951) detected one in sixty-seven cases but the same author in a review of fifty-one cases of spontaneous remissions, thirty-eight of which had been previously reported in the literature, twenty-one were over the age of nineteen years. Tivey (1954) analysed one hundred and two cases of spontaneous remissions from the literature, fifty-one of which were those previously examined by Southam *et al.* (1951). He noted the age and sex incidence to be approximately equal, suggesting that such remissions when they occur are as likely to be seen in adults as in children. Another finding of interest was that remissions occurred in all morphological varieties of leukaemia. Of seventy-nine cases where the type of leukaemia was recorded, forty occurred in the lymphoblastic, twenty-five in the myeloblastic and fourteen in the monocytic variety Thirty-four of the remissions in those with lymphoblastic leukaemia occurred in children while twelve of the fourteen patients with monocytic leukaemia developing spontaneous remissions were adults. It seems likely that such a distribution is to a large extent due to the fact that lymphoblastic leukaemia is more common in children, while monocytic leukaemia is usually seen in adults.

In several of the reported cases, remissions

were preceded by one or more factors, which have led many to suspect that such remissions may not in fact be truly spontaneous. It has for long been a common supposition that remissions generally are causally related to an infectious process. Diamond and Luhby (1951) reported that 75 per cent. of children with remissions had severe acute infections immediately preceding the onset. Tivey (1954) considered that pyogenic infection was an associated factor in about one third of his series, and in 50 per cent. remission was preceded by a febrile illness. Southam et al. (1951) could find no relationship of remissions to preceding infection. Eisenlohr (1878) and Heuck (1879) in their early reports observed a relationship of infection to spontaneous remissions. The former author observed a case in which typhoid fever occurred. During the acute illness the clinical and haematological evidence of leukaemia regressed only to re-appear three weeks after recovery from the fever. In Heuck's cases the occurrence of empyema was associated with a decrease in the leucocyte count and diminution in size of the spleen. Marischler (1896), Neutra (1903) and Dock (1904) reviewed the early cases and suggested that a variety of infections might be the forerunners of a temporary improvement in the leukaemic state. Recently, Tivey (1954) has, in addition,

associated virus diseases, including chicken pox, measles and infectious mononucleosis with "spontaneous" remissions. Wintrobe (1951) has occasionally observed temporary improvement following virus and coccal infections but he considers it a rare and unusual occurrence. Taylor (1953) reported remissions in cases of acute monocytic leukaemia who developed glandular fever. Jaffe (1932) is of the opinion that the response to infection depends on the persistence of sufficient functioning myeloid tissue to produce mature polymorphonuclear cells.

Considerable differences of opinion exist regarding the relationship of infection to remissions. Southam (1951) stated that the evidence seemed to be only circumstantial and found it difficult to accept a causal relationship between infection and remission in a disease characterised by marked susceptibility yet rarely showing evidence of remissions. Such is the opinion of many and although the personal observations of others must be considered, more conclusive evidence seems desirable before infection per se can be accepted as the factor responsible for spontaneous remissions.

Other factors have been described as being associated with remissions. These include non-specific trauma, various extracts including those

of urine and faeces (myelokentric and lymphokentric acids) (Miller *et al.* 1947) and blood transfusions. In the evaluation of treatment of acute leukaemia with chemotherapeutic agents only the latter has to be seriously considered.

It seems probable that the more liberal use of supportive measures such as blood transfusions, antibiotics, the maintenance of a normal acid base equilibrium, etc., as well as the increased skill in caring for these patients may have also affected the natural course of the disease. Southam *et al.* (1951) were of the opinion that repeated blood transfusions and the use of antibiotics in the treatment of patients at the Memorial Centre, New York, during the years 1946 and 1947 had no effect in inducing remissions, although there was some evidence of a slight but significant increase in survival time. Bierman (1950) also found that blood transfusion and antibiotics increased the survival time in children from 5.6 to 8.9 months, in an untreated control series, to 8.9 months. Tivey (1954) found in his analysis of one hundred and two cases of spontaneous remissions that blood transfusion may have been an associated factor in thirteen instances. Hayhoe and Whitby (1955) are of the opinion that simple transfusion alone is likely to lead to a remission of greater or lesser degree in approximately one third of patients,

the outcome of treatment being usually manifest within a week. They also believe that second remissions may be induced by these means and only recommend hormonal or chemotherapeutic methods when it is clear that blood transfusion is not being effective. In their series, fourteen of forty-one patients were considered to have developed remissions following blood transfusion alone, and it was observed that the lymphoblastic type of leukaemia respond more frequently than the other varieties. Evidence of definite haematological improvement was, however, not recorded conclusively and in only one instance was definite improvement in bone marrow cytology observed. It is also of interest that the majority had received considerable quantities of transfused blood. The opinion of Whitby and Hayhoe is not that of others, and in the author's present series of twenty-eight patients with acute leukaemia there was no indication that haematological remissions were in any way related to routine blood transfusions, although symptomatic improvement was frequently observed.

French workers have stressed the role of blood transfusions in the causation of remissions and have used exsanguino-transfusion as a form of therapy (Bernard and Bessis 1948, Bessis and Bernard 1947 and 1948). In a clinical study of sixty patients receiving this treatment Bessis and

Dausset (1950) noted clinical improvement with regression of enlarged organs in approximately 50 per cent., while a significant number (20 per cent.) showed haematological improvement of some degree. Improvement was observed to occur largely in children, adults not being affected to the same extent. Dreyfus (1948) in reviewing the literature found that in twenty-two examples of spontaneous remissions blood transfusions were the only constant form of therapy and had been given in nineteen. The findings of the French investigators led to the suggestion that normal blood or plasma contained a blood-cell maturation factor absent or deficient in patients with leukaemia (Bernard and Bessis 1948; Dreyfus 1948) which has gained support from Schwind (1947) who noted partial maturation of leukaemic myeloblasts following fresh plasma transfusions. Further studies relating to the fundamental mechanism of the remissions in these patients seems, however, desirable.

In view of the variety of factors which have been considered to predispose to remissions one is led to consider the possibility of a common basic mechanism. As remissions in a percentage of patients follow the administration of adrenocorticotrophic hormone (ACTH) or cortisone the possibility that the rare spontaneous remission may be due to adrenal stress might be postulated. Tivey

(1954) also supported such a mechanism and considered that exchange transfusions might act in this way or even result in a direct transfer of ACTH and/or cortical steroids.

The duration of spontaneous remissions is variable. Diamond and Luhby (1951) found them to be less than ten weeks while Tivey (1954) in his series which included those of Southam et al. (1951) found the median duration in fifty-four children to be 5.2 weeks and in forty-eight adults 9.4 weeks. Individual remissions lasting as long as four years have, however, been described (Fauvert et al. 1948, Schiro and Weiss 1946). Rarely has more than one spontaneous remission been observed but Birge et al. (1949), Rappoport and Kugel (1947) and Whitby and Christie (1935) have reported such an occurrence while Miller et al. (1947) described partial remissions on two occasions in each of three patients treated with crude myelokentric acid. Bassen and Kohn (1952) reported four separate spontaneous remissions in the same individual.

The average survival time of patients developing spontaneous remissions seems to be appreciably longer than those who do not (Southam et al., 1951).

In summary it may be said that whereas so-called spontaneous remissions occasionally occur they would appear to be extremely rare. The true incidence is

doubtful but from the reports examined comprising almost one thousand children with acute leukaemia, not treated with modern chemotherapeutic agents such as folic acid antagonists, some evidence of clinical and haematological improvement occurred in 6.7 per cent. of cases. Blood transfusions when massive may predispose to remissions but Hayhoe and Whitby's (1955) suggestion that one third of adult cases of acute leukaemia have true remissions following transfusion is not accepted by the author. The variety of infections described as leading to remissions suggests that the organism is not directly responsible for the remission. Many factors have been described as associated with such remissions which throws some doubt as to their true spontaneity and leads to the suggestion that all these factors may lead to a common basic mechanism and that remissions in patients not treated with chemotherapeutic agents may be the result of adrenal stress in the susceptible individual. Remissions may occur irrespective of age, sex and morphological type of leukaemia but in view of the incidence of the particular types varying with age, remissions are more likely to be observed in acute lymphoblastic leukaemia in children, while remissions in monocytic leukaemia and to a lesser extent myeloblastic leukaemia are generally to be seen in adults.

Spontaneous remissions resemble those following

treatment with the folic acid antagonists in certain respects, including the variable but generally relatively short duration and the associated significant increase in survival time. Bassen and Kohn (1952) observed an initial period of leucopenia before the onset of spontaneous remission in their patient and suggested the similarity between spontaneous and drug induced remission. Although accurate statistics of the incidence of spontaneous remissions in adults is lacking, the reported remissions appear to equal those in children. Remissions in adults following treatment with aminopterin or amethopterin are on the other hand extremely uncommon. Similarly antifolic treatment seems to affect mainly lymphoblastic leukaemia whereas spontaneous remissions may develop in any variety of leukaemia. There would therefore appear to be certain differences, as well as similarities, between the spontaneous and drug induced remissions.

In conclusion it may be stated that supportive treatment may account for some increase in survival time, and that occasional spontaneous remissions may occur. Neither of these factors are however likely to interfere with the evaluation of present day chemotherapeutic agents to any extent. Comparison of a series of patients treated with the folic acid antagonists at the Memorial Centre, New

York, between 1948-51 with Southam et al's. (1951) series of untreated cases is interesting (Burchenal 1952a). Of one hundred and thirty-six cases treated with the folic acid antagonists thirty-eight survived more than one year and there was an overall remission rate of over 50 per cent. In one hundred and fifty untreated cases only two survived more than a year and the overall remission rate was 8.7 per cent. Although the folic acid antagonists appear to have played the major therapeutic role in the induction of remissions, with regard to the survival time account must be taken of the supportive treatment as well as the effect of the chemotherapeutic agents. Thus Farber (1951) now speaks of "Total Care", to infer a combination of all the available means to increase the comfort, well-being and survival time of the patient.

RESULTS OF TREATMENT OF ACUTE LEUKAEMIA WITH THE FOLIC ACID ANTAGONISTS.

At the Second Conference on the Folic Acid Antagonists in the treatment of Leukaemia, held in February, 1951, at Boston (Proceedings of the Second Conference on Folic Acid Antagonists in the Treatment of Leukaemia, 1952), it was reported that 68 per cent. of four hundred and twenty-five children with acute leukaemia had been improved by therapy with the folic acid antagonists. At the Memorial Centre, New York, forty-four out of one hundred and nineteen children developed complete

remissions but only one out of thirty-six adults (Burchenal 1952a). The poor response in adult patients is now generally recognised and the value of aminopterin and amethopterin is almost confined to the treatment of acute leukaemia in children.

The evaluation of therapy by various investigators in the United States of America is shown in Table 2. The detailed criteria of complete and partial remissions tend to vary. Farber (Proceedings of the Second Conference on the Folic Acid Antagonists, 1952) considered a complete remission to be present when the patient returned to temporary normal health, with the presence of less than 7 per cent. blast cells in the bone marrow. A partial remission was indicated by a reduction of blast cells in the bone marrow to less than 30 per cent. Burchenal, at the same Conference, stated that a remission was complete when the bone marrow contained 20 per cent. or fewer stem cells or lymphocytes, as frequently there was difficulty in differentiating between stem cells and young lymphocytes. In a more recent publication (Burchenal et al. 1953) this figure was changed to 30 per cent. In some instances clinical improvement occurs without any noticeable change in the haematological status. Whether this is a consequence of treatment with the folic acid antagonists alone is questionable. The variation in the remission rate from one

(All patients received therapy for over seven days and survived three weeks or longer after beginning of therapy)

Investigator	Total No. of Patients	Complete Remissions		Partial Remissions and/or Clinical Improvement	No Response	Overall Improvement
		Per cent.				Per cent.
Burchenal	76	14	18.4	29	33	56.5
Farber	190	54	28.4	76	60	68.0
Stickney	24	8	33.3	5	11	54.0
Quilligan	34	22	64.7	5	7	80.0
Pierce	27	15	55.6	8	4	85.2
Sacks	14	6	42.8	2	6	57.1
Davis	31	19	61.3	9	3	90.0
Sylvester	30	20	66.6	3	7	76.6
Heinle	43	-	-	-	7	63.0

TABLE 2. Incidence of Remissions in Children with Acute Leukaemia treated with the Folic Acid Antagonists

(Data compiled from The Proceedings of the Second Conference on the Folic Acid Antagonists in the Treatment of Leukaemia, Boston, U.S.A., 11th March 1951, and published in Blood, Vol. 7, Supplement, January 1952).

institution to another is probably accounted for in part by the different interpretation as to what constitutes a complete and partial remission, although there is no doubt that higher remission rates are obtained by those most experienced in the use of these compounds.

Many other reports have been published concerning the ability of the folic acid antagonist to bring about clinical and haematological remissions in acute leukaemia. In the United Kingdom Dacie et al. (1950) noted nine remissions in thirteen patients treated with aminopterin, of which seven occurred in children. Wilkinson (1948 and 1953) and Wilkinson and Gardikas (1951) reported remissions of varying degree in ten out of thirty-eight patients of various ages. Other investigators included Pierce and Alt (1948), Jacobson et al. (1948), Stickney et al. (1949), Weber et al. (1950), Damashek et al. (1950), Meyer et al. (1950), Bruton and Price (1952), Poncher (1952) and Dresner and White (1952). All agree that remissions are more likely to develop in children and in the lymphoblastic type of leukaemia.

The duration of individual remissions is extremely variable. They may last only a few weeks or many months. Damashek (1950) has noted individual remissions lasting thirteen months while Burchenal et al. (1951b) reports a range of four to

ninety-six weeks, the majority lasting five to eight weeks. Second remissions are generally accepted as being less common, although Burchenal et al. (1951 b) found that only two of twenty patients, in whom treatment had been discontinued during the period of complete remission, failed when relapse occurred to respond to a second course of treatment. Eventually, however, the disease becomes refractory to the folic acid antagonists, although individual cases of multiple remissions are known.

RESISTANCE TO THE FOLIC ACID ANTAGONISTS IN ACUTE LEUKAEMIA.

It has been shown that a substantial percentage of children with acute leukaemia develop evidence of a partial or complete remission following treatment with aminopterin or amethopterin. The varying response may in part be dependent on the cell type, as most observers agree that the lymphoblastic variety of acute leukaemia responds best, fewer remissions occurring in the myeloblastic type, while acute monocytic leukaemia appears to be unaffected by these compounds.

In many instances it seems likely that the leukaemic cells rapidly acquire resistance to the folic antagonists. Experience has shown that all patients ultimately become resistant and it is expected that a child who has responded once or even two or three times will eventually fail to benefit

from further therapy.

An attempt at explaining the development of resistance to the 4-amino analogues of folic acid, a circumstance of major importance, has been undertaken by several investigators. By passing leukaemic cells, generation after generation, through mice treated with amethopterin, a strain of leukaemia resistant to amethopterin has been produced (Burchenal et al. 1950a). Passage of resistant cells through untreated mice failed to show any reduction in the degree of resistance, suggesting that it was a true mutation. If such animal studies could be applied to human leukaemia, which is open to doubt, it would suggest that resistance does not appear to be due to tolerance on the part of the patient as a whole, but to the acquisition of tolerance specifically by the abnormal leucocyte. Animal studies by Burchenal et al. (1951c) have shown that although the leukaemic cells were originally exposed only to amethopterin a cross resistance to all the 4-amino-analogues of folic acid was observed. However, the same investigators (Burchenal et al. 1951d) have demonstrated that a strain of mouse leukaemia highly resistant to the 4-amino analogues of folic acid is suppressed by 9-methyl-folic acid suggesting that this particular leukaemic cell does not develop an alternative metabolic pathway allowing

it to survive without the need for folic acid. Lack of cross resistance to cortisone (Burchenal et al. 1951e) and a purine antagonist, 2, 6-diaminopurine (Burchenal et al. 1951c), in the amethopterin resistant strain of mouse leukaemia has also been observed. Law and Boyle (1950) and Law (1951) have also developed a transplantable lymphoid leukaemia in mice resistant to the folic acid antagonists and showing an ability of the leukaemic cells to utilise the antagonist. Law (1954) has reviewed the phenomena of resistance and dependence. The results of these two groups of workers suggested the possibility that the leukaemic cell rapidly acquires or develops an ability to metabolise the 4-amino-analogues of PGA. Support for this concept was obtained by Burchenal and his colleagues who after developing a strain of streptococcus faecalis highly resistant to amethopterin, designated S. faecalis/A, (Burchenal et al. 1951f) showed that S. Faecalis/A was capable of maximum growth on aminopterin and amethopterin in the absence of a folic acid supplement (Hutchison and Burchenal, 1952). Further study, however, indicated that these folic acid analogues contained varying amounts of folic acid as impurity (Hutchison and Burchenal, 1953) so the initial suggestion that the resistant organism might be metabolising the antagonists was probably incorrect. A similar

explanation may account for the suggestion by Kidder et al. (1951) that aminopterin can substitute for folic acid in promoting the growth of Tetrahymenageleii and so disprove the thesis that conversion of the analogues to form folic acid occurs (Hutchison and Burchenal (1952)). Experiments in mice previously given amethopterin have shown that no appreciable diminution in concentration of the antimetabolite follows implantation of resistant (Ak4R) leukaemic cells (page 166), again suggesting that the resistant cell fails to metabolise amethopterin at least in assayable quantities.

In addition to the theory that the resistant leukaemic cell may convert aminopterin to utilisable folic acid, resistance to amethopterin has also been suggested to be due to deamimation to form a weaker antagonist, N^{10}-methylpteroylglutamic acid (methopterin). It has been demonstrated that amethopterin is not deaminated in normal mouse tissues (Fountain et al. 1953) and the indication is that the animal with resistant leukaemia also fails to do so (Page 202)

Resistance has been considered by some as possibly due to an increased synthesis of folic acid and C.F. by the resistant cell. Studies by the author (Page 224), suggest no appreciable difference in total C.F. and folic acid concentration in the livers of mice with AK4 (amethopterin)

sensitive) and AK4R (amethopterin resistant) leukaemia. C.F. has been found to be ineffective in influencing the growth of sensitive and resistant cells and indeed Law and Boyle (1951) have indicated that C.F. may interfere with the utilisation of amethopterin. Nichol (1954) found that suspensions of lymph nodes and spleens from leukaemic mice formed C.F. from synthetic folic acid when incubated in a medium containing glucose, ascorbate and serine or formete. The enzymatic formation of C.F. in this system was inhibited by amethopterin. Amethopterin resistant (Line 1/A) leukaemic tissue, however, did not utilise folic acid more efficiently for the formation of C.F. than the corresponding sensitive lines. In this respect resistant leukaemic cells differ from the amethopterin resistant strain of S. faecalis which has the ability to form more than one hundred times as much C.F. from folic acid as the parent antagonist - sensitive strain (Broquist et al. 1953, Hutchison and Burchenal 1952a, Nichol et al. 1953). Hutchison and Burchenal (1954) are also of the opinion that resistant leukaemic cells are unable to form more C.F. from exogenous folic acid than the sensitive cells. However, their experiments suggested a higher concentration of both total and free C.F. in the spleens of mice with resistant (Line 1/A) leukaemia as compared with those

with the amethopterin sensitive (Line/1) leukaemia. In addition their results suggested that the resistant cell liberates the conjugated C.F. more readily.

Other theories such as methylation, acetylation or esterification of the 4-amino group or alteration in the permeability of the cell membrane have been suggested (Burchenal et al. 1951g). Nichol (1954a) working with the resistant strain of S. faecalis has also some evidence that the cell walls may be less permeable to amethopterin.

In summary, several possible explanations have been put forward regarding the mechanism of resistance to the folic acid antagonists. Alternate metabolic pathways, conversion of the antifolics to less potent antagonists or to utilisable folic acid or an increased synthesis of citrovorum factor have been suggested. In addition the possibility that the resistant leukaemic cell is unable to readily absorb the antagonist has to be considered in addition to the more clinical approach that nests of leukaemic cells may be resistant from the outset and gradually replace the sensitive ones destroyed by anti-folic treatment. The posibility that the terminal failure of the leukaemic patient to respond to the folic acid antagonists may not be due to drug

resistance in the accepted meaning of the term cannot at this stage be altogether excluded. Many patients failing to benefit from antifolic therapy show a decline in the leucocyte count but without improvement in the differential cell count, haemoglobin or platelet level, suggesting treatment is eliminating leukaemic cells but normal haemopoietic development is suppressed. A possible explanation of the development of a remission and eventual resistance can be summarised as follows:- (a) Remission in acute leukaemia results from suppression of the leukaemic process followed by regeneration of normal blood cells from functioning haemopoietic tissue; (b) "Resistance" results when the bone marrow fails to regenerate after the inhibition of growth of leukaemic cells. "Resistance" would therefore be a part of the natural history of leukaemia and due to a fundamental defect in normal haematopoiesis in turn brought about by the leukaemic process. Although this may be an explanation of "resistance" in human leukaemia, in animals and bacteria cells resistant to amethopterin have been developed. It is therefore essential that work in this field be continued in the hope that it will help to elucidate this problem.

Adrenocorticotrophic Hormone (ACTH) and Cortisone in the Treatment of Acute Leukaemia.

Since 1950 several reports have appeared

relating to the effectiveness of ACTH and Cortisone in producing temporary remissions in acute leukaemia. As early as 1942, Heilman and Kendall had observed temporary regression of lymphoid tumours in mice administered cortisone (compound E) although this work was not published until 1944. Murphy and Sturm (1944) also demonstrated the effectiveness of ACTH and adreno-cortical hormones in protecting against transplanted leukaemia in rats. In the same year, Dougherty and White (1944) showed the effect of these hormones on lymphoid tissues and the depressive effect on circulating lymphocytes. Valentine, Craddock and Lawrence (1948) reviewed the subject of the relationship of the hypophyseo-adrenal cortical system to lymphoid tissue structure but themselves at that time were unable to demonstrate a clear cut association. In animals and in human subjects (Hills et al. 1948) the administration of ACTH is followed by an increase in circulating neutrophils and a diminution in lymphocytes and eosinophils. Later workers (Burchenal et al. 1950b) Sugiura et al. 1950) showed some degree of regression of malignant processes in animals. The vast amount of experimental work showing the effect of the adreno-cortical hormones on haemopoietic, and in particular lymphoid tissue, naturally led to its trial in the treatment of leukaemia.

ACTH and cortisone appear to be equally

effective in their action on the leukaemic process, although ACTH stimulates the patient's adrenal glands to secrete several steroids whereas cortisone merely supplies an excess of a single adrenal hormone. The dosage of the hormone varies to a certain extent with the age of the patient but it should be stressed that if remissions are to develop adequate dosage is essential, in children up to 200 mgm. daily of cortisone by mouth and twice this dosage if necessary in adults. ACTH is given intramuscularly, usually four times daily, for a total dose of 100 mgm. in children and 200 mgm. in adults.

The intravenous administration of ACTH is highly satisfactory and in children 25-40 mgm. may be given in an intravenous infusion over a period of eight to twelve hours. This method of administration is occasionally dramatic. Within a day or two the bleeding completely ceases, normoblasts and granular cells replace the leukaemic cells in the bone marrow, which usually becomes hypocellular, associated with a reticulocytosis and rise in haemoglobin. Platelet production increases and normal white cell maturation appears. The lymph glands and spleen decrease in size and the patient, within seven to fourteen days, regains apparent normal health. Cortisone and intramuscular ACTH may produce the

same effect but usually not so rapidly. Treatment with oral cortisone may have to be continued for several weeks in order to produce a remission, but if undesirable side effects should occur, such as severe hypertension and metabolic upsets, therapy is usually suspended. Cushing's facies, hirsutism and acne usually clear rapidly when treatment is stopped and are therefore not dangerous. It is the author's impression that if a child with acute leukaemia is going to respond to hormonal therapy, a response will usually occur soon after therapy is started and before the onset of unpleasant side effects.

Results of Treatment with ACTH and Cortisone

Pearson et al. (1949) in a preliminary report showed that in six patients with neoplastic disease of lymphatic tissue, including chronic lymphatic leukaemia, a marked decrease in size of lymph glands, spleen and other evidence of disease followed treatment with ACTH and cortisone. The same group of workers in 1950 demonstrated the effectiveness of these adrenal-cortical hormones in inducing remissions in patients with acute leukaemia. Numerous publications supporting these observations followed. Farber et al. (1950) noted ten remissions, of which five were described as complete, out of a total of seventeen children, the duration of the remissions being less than ten weeks

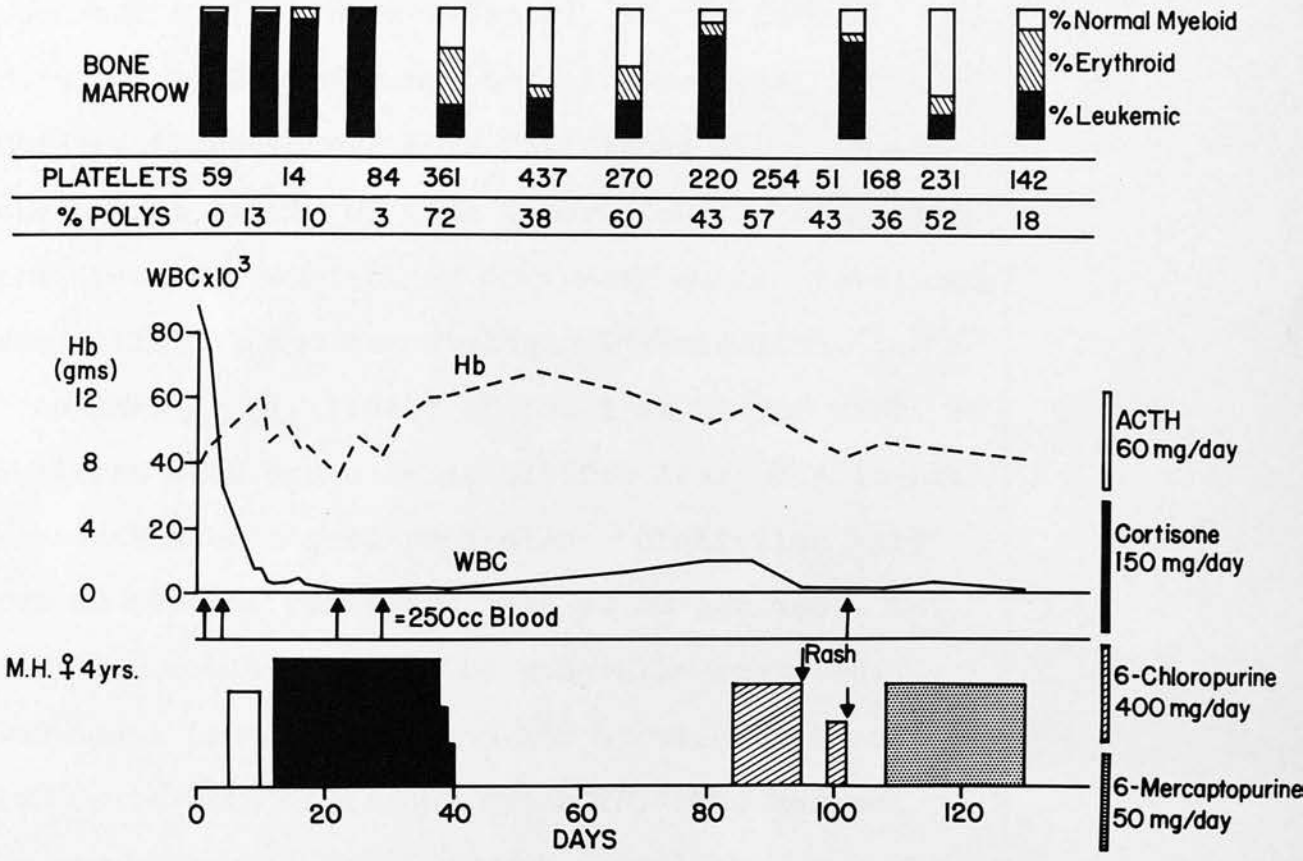


FIG. 2. Complete remission in a child with acute leukaemia following treatment with ACTH and cortisone. A second remission was induced later by 6-chloropurine and 6-mercaptopurine.

in all cases. Wintrobe *et al.* (1950), Stickney (1950), Spies *et al.* (1950), Snelling *et al.* (1951), Schulman *et al.* (1951) and Rosenthal *et al.* (1951) reported similar observations. At the Second Clinical ACTH Conference held in December, 1950, at Chicago (Proceedings ACTH Conference 1951) it was shown that eighty of one hundred and seventy-five children and fourteen of forty-six adults developed good clinical and haematological remissions. Rosenthal *et al.* (1951) stated that 69 per cent. of children with acute lymphoblastic leukaemia in his series showed a good remission. Statistics vary but an average remission rate of 50 per cent. in children would probably be generally accepted. Burchenal (1952a) reviewed the treatment of acute leukaemia with cortisone and ACTH. The Medical Research Council Panel report (1952), however, only mentioned improvement in six out of twenty children with acute leukaemia, and three out of sixteen adults. A second report (1953) showed improvement to have occurred in three of a further sixteen patients. Damashek (1952) criticised the dosage used in this group of patients and stated that good remissions were generally observed in 50-60 per cent. of cases of lymphoblastic leukaemia in children and when ACTH or cortisone was given simultaneously with aminopterin of which he had observed fifteen cases a remission may be induced in almost every case.

Hayhoe and Whitby (1955) observed five remissions in their series of ten adult patients, three of the remissions being complete. Wilkinson (1955a) reports a remission rate of 31.5 per cent. in his series of seventy-six patients of various ages.

Remissions are generally accepted as being less common in adults than in children, although more likely to occur than if treated with the folic acid antagonists. Acute lymphoblastic leukaemia is more responsive than other types to hormone therapy as is expected in view of the known effect on lymphoid tissue (Dougherty and White 1944). Acute monocytic leukaemia invariably fails to show any form of response to ACTH or cortisone (Pearson et al. 1950, Burchenal 1952a, Hayhoe and Whitby 1955). ACTH and cortisone induced remissions generally last from a week to three months, the majority of investigators agreeing that the average duration of a remission is rather less than that obtained with the folic acid antagonists. Second remissions may occur in children but adults usually fail to respond a second time. Resistance to therapy occurs sooner or later in all cases and the eventual outcome is the same as that which follows treatment with the folic acid antagonists. An interesting and important feature in this respect is the apparent absence of cross resistance between the steroids and the folic acid antagonists (Kingsley

Pillers et al. 1952) suggesting that ACTH And cortisone have a different primary mode of action on the leukaemic process from that of the folic acid antagonists. These workers were of the opinion that the two series of compounds were effective in producing further remissions in approximately 50 per cent. of patients showing resistance to one type of therapy. The suggestion by Damashek (1952) that remissions in acute leukaemia in children may be expected in almost every case when a combination of aminopterin and hormones was used tends to support this finding. Farber (1950), Marie and his co-workers (1951) and Bernard and Mathé (1952) have also recommended "combination therapy" and an apparent synergistic effect together with a lower degree of toxicity has been suggested by the French workers. Kelty and Beard (1953) and Maguin (1953) also considered combined therapy to be advantageous. Others agree that a higher initial percentage of remissions are likely to occur in children but prefer to use sequential therapy as they believe that resistance to both compounds is likely to occur earlier when used in combination. Nevertheless it is accepted that combination therapy in adults is no improvement on ACTH or cortisone alone. Since the folic antagonists alone are rarely of value in adults it would be remarkable if the combination

produced more favourable results, and indeed would indicate a true synergistic relationship.

The mode of action of cortisone and ACTH in leukaemia is unknown. As has been suggested, the absence of cross resistance (Kingsley-Pillers et al. 1952) suggests it is different to that of the folic acid antagonists, although as mentioned previously there is some evidence that the antagonists may in part act via the adrenals. Chemical studies by Pearson et al. (1949 and 1950) and Snelling et al. (1951) showed a relative increase in excretion of uric acid and creatine which appeared to be proportional to the disappearance of leukaemic cells. Whatever the mechanism may be this suggests a dissolution of leukaemic tissue. The clinical findings of diminished cellularity of the bone marrow during treatment coincident with a decrease in the proportion of blast cells and a subsequent re-appearance of normal marrow constituents points to this being the case, as does the absence of any evidence of maturation of the while cell precursors.

Survival Time of Treated and Untreated Acute Leukaemia

One of the most objective single measurements of the effectiveness of chemotherapy in acute leukaemia is probably the duration of survival, although the number and duration of remissions and the symptomatic benefit afforded by treatment must

also be considered when assessing the value of a chemotherapeutic agent.

Survival time in a disease such as acute leukaemia may be measured from the time of diagnosis or from the onset of the first symptoms. Both methods are open to obvious criticism and are likely to be only a rough approximation. The duration from the time of diagnosis when examining a series of cases from a single clinic seems to the author to be more accurate, at least for comparative purposes, than relying upon the patient's judgement as to the onset of definite symptoms of leukaemia.

Warren (1929) found that only two patients in his series, out of a total of one hundred and thirteen, survived longer than six months, and that 74 per cent. were dead within two months. More recent figures relating to survival time in patients not receivinv chemotherapy have been reported by Dale (1949). Of a total of thirty-six only one survived longer than one year and 75 per cent. were dead within six months. Southam et al. (1951) in a comprehensive study of one hundred and fifty patients found an average duration from the onset of the first symptoms of just over twenty weeks. They reported 50 per cent. as being dead by the seventeenth week and 75 per cent. by the twenty-eighth week. Only two survived longer than

one year. The same workers noted a gradual increase in the survival time over the years between 1926 and 1948. This they considered due to supportive measures such as blood transfusions, sulphonamides and later penicillin and other antibiotics. Deep x-rays, radioactive phosphorus, urethane and nitrogen mustard had no influence on survival time. Bierman (1950) also found an increase in survival time, from 5.6 to 8.9 months, in patients receiving supportive treatment. A recent statistical analysis by Tivey (1952) of four hundred and twenty-eight cases recorded in the literature showed that 50 per cent. died within four months of the first symptoms and only 5 per cent. survived as long as one year.

Little information of statistical significance has been published concerning the effect of chemotherapy on survival time. Poncher *et al.* (1952) reported an increase in the mean from twenty-three to forty weeks as a consequence of treatment with folic acid analogues. Colebatch and Williams (1950) also noted an increase in life expectancy, and in his small series found the survival time almost doubled as a result of chemotherapy. On the other hand, Haut *et al.* (1955) who analysed statistically the results recorded in the literature and included one hundred and three of their own patients were of the opinion that

treatment with the folic acid antagonists, ACTH and cortisone and 6-mercaptopurine had not significantly increased the total survival time. They did, however, find that in those patients who achieved at least one remission, the median survival was enhanced from four and a half to eight months.

There seems little doubt that in many instances life has been prolonged as a result of what might be termed (a) supportive measures and (b) specific chemotherapeutic agents, but it seems doubtful whether the survival time in the group which fail to show haematological improvement as a result of treatment, is materially affected. A comparison of Southam et al's (1951) figures prior to 1948 and those obtained from the Memorial Centre, New York, between 1948-51 after the institution of specific chemotherapy is of interest. Of one hundred and thirty-six patients treated with the folic acid antagonists, thirty-eight (28 per cent.) survived more than a year. In the group of one hundred and fifty not receiving treatment only two (1.3 per cent.) survived more than a year. Similarly in the untreated group of leukaemic children reported by Tivey (1952) only 5 per cent. survived as long as one year. Since the introduction of 6-mercaptopurine Burchenal et al. (1954) have observed that by the sequential use of agents known to favourably influence the leukaemic

process, the number of patients surviving longer than a year has risen to 52 per cent.

The increase in survival time as a consequence of therapy, is a positive indication that agents such as the folic acid antagonists, ACTH and cortisone and 6-mercaptopurine can temporarily arrest the leukaemic process. When viewed as a whole, the increase is depressingly small, but the knowledge that in a percentage of patients prolonged remissions with complete freedom from symptoms may occur, gives added impetus to further research.

Recent Trends in Chemotherapeutic Research

Although the need for fundamental biochemical information is of prime importance, the lack of such knowledge led to a new approach in cancer therapy. At several Centres in the United States of America large tumour screening programmes are in progress (Stock (1950). Considerable numbers of chemical compounds are tested annually for growth inhibitory properties in animal tumours, including mouse leukaemia. Those found effective, and of low toxicity are tested for therapeutic properties in patients with various neoplstic diseases including leukaemia. This type of research in many instances is largely empirical, but many compounds are chosen on a more rational basis. With the knowledge that folic acid was essential for the synthesis of

nucleic acid and that the folic acid antagonists were effective in the treatment of acute leukaemia, analogues of other essential metabolites have been prepared and tested. Several analogues of adenine and guanine have been synthesised of which one, 2, 6-diaminopurine has resulted in remissions in five patients out of a series of twenty-five treated with this compound (Burchenal et al. 1951b) 8-Azaguanine has not been found to be of any material benefit in the human subject although in animal leukaemia it has been found to strikingly influence the disease when given in conjunction with the folic acid antagonists (Goldin et al. 1952; Law 1952). The most effective purine antagonist to be prepared and tested up to the present is 6-mercaptopurine. This compound has been shown to bring about remissions in a high percentage of children with acute leukaemia and also occasionally in adults with this disease (Burchenal et al. 1953; Fountain 1954, 1955). 6-Mercaptopurine is considered in detail later (page

6-Mercapto-2-aminopurine (thioguanine), synthesised by Elion et al. 1952, and 6-chloropurine, synthesised by Bendich et al. have both brought about remissions in chronic myeloid leukaemia without any evidence of toxicity (Burchenal, 1954) and recently have been found to produce remissions

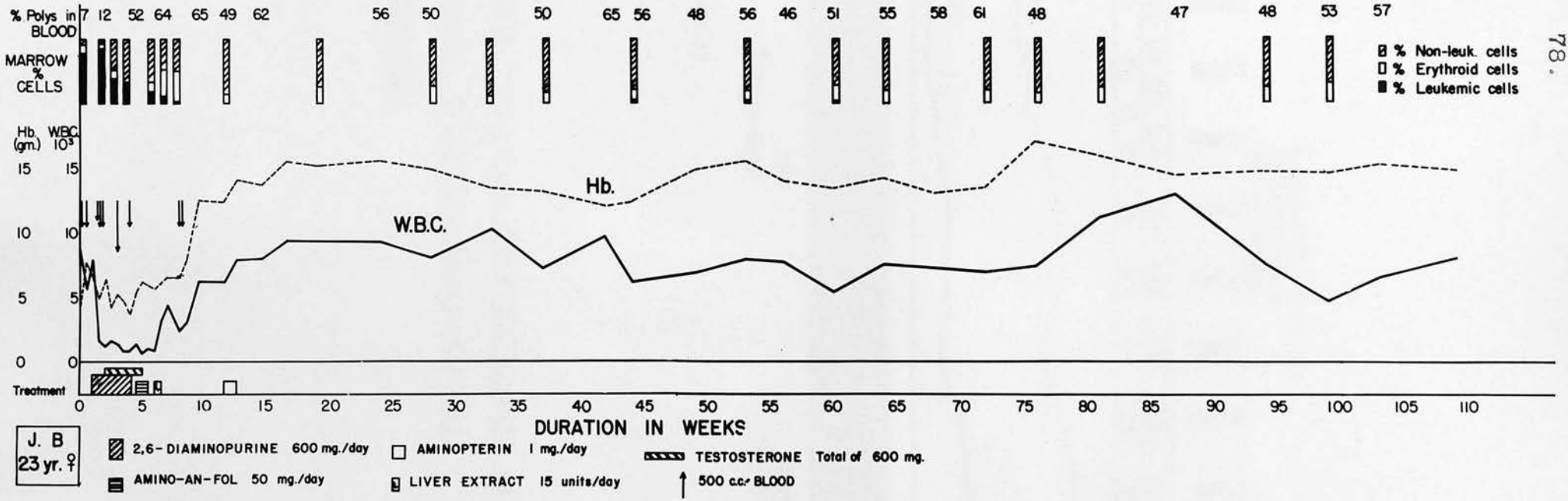


FIG. 3. Prolonged remission in a young adult female with acute leukaemia following treatment with 2,6-diaminopurine.

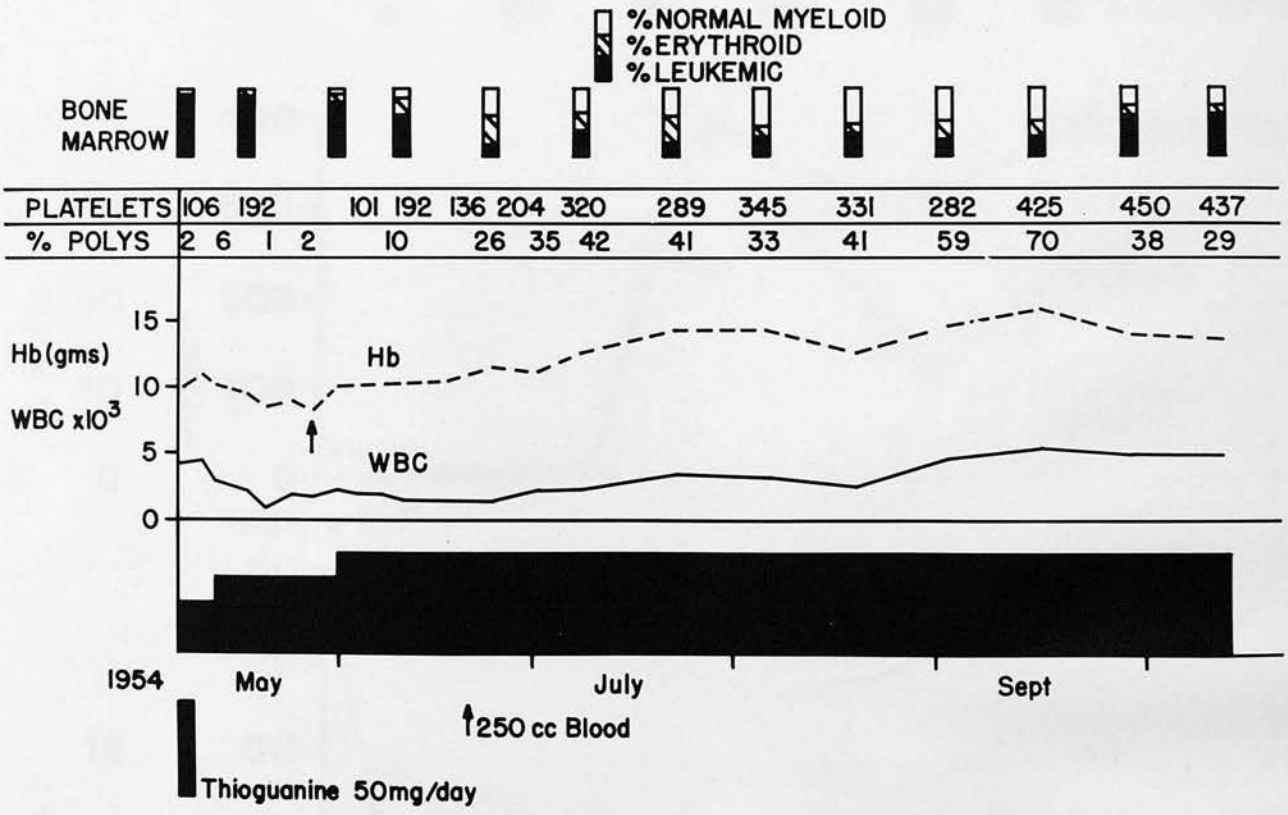


FIG. 4. Complete remission, in a child with acute leukaemia, following treatment with thioguanine.

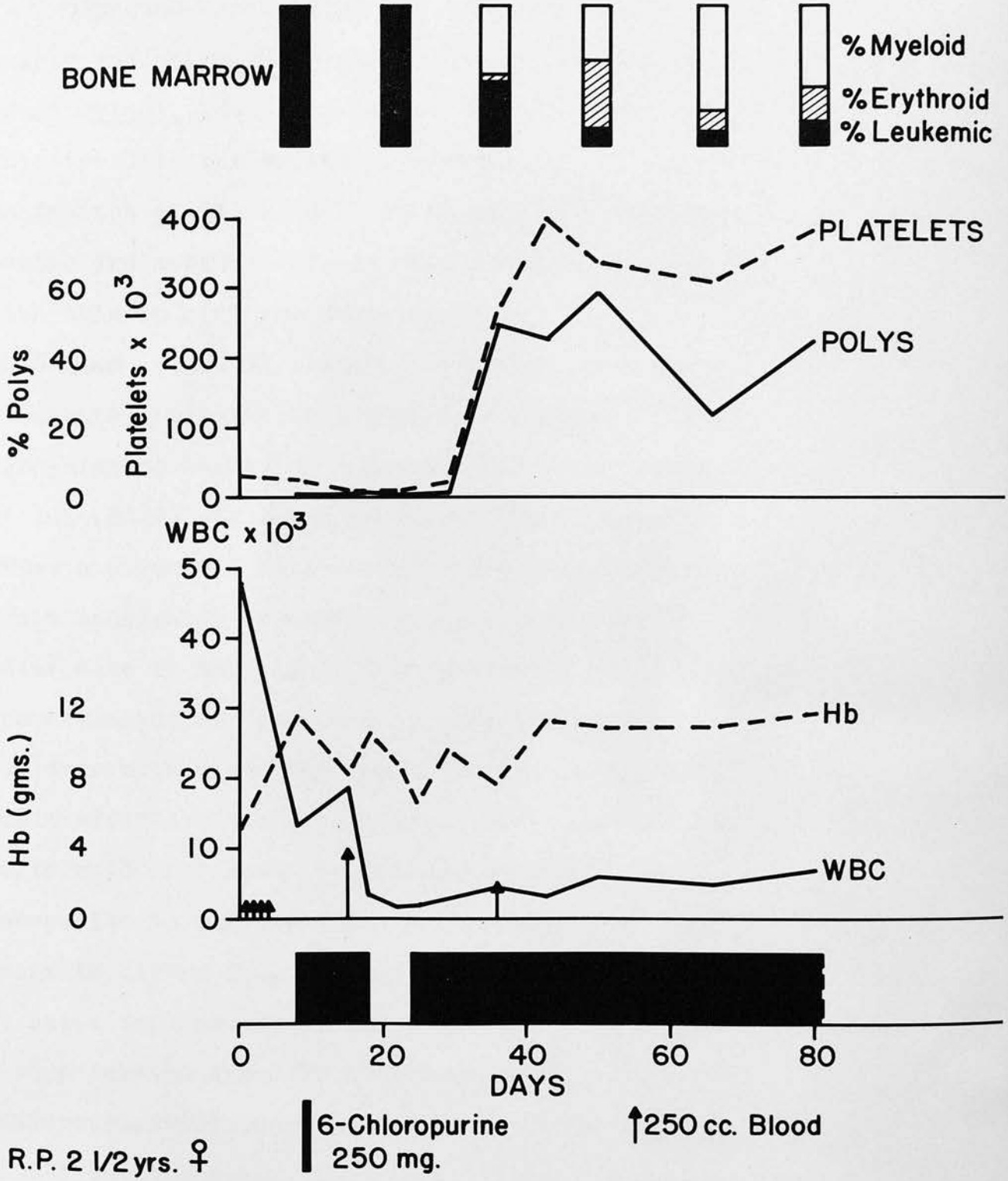


FIG. 5. Complete remission, in a child with acute leukaemia, following treatment with 6-Chloropurine.

occasionally in acute leukaemia (Figs. 4 and 5).

Compounds containing the 2:4-diaminopyrimidine moiety including those with antimalarial activity (Falco et al. 1949) are competitive antagonists of folic acid in the growth of lactobacillus casei (Hitchings et al. 1952). In animals they produce states indicative of folic acid deficiency and with this in mind the possibility was suggested that such compounds might be effective in acute leukaemia. Several have been synthesised and one, 2, 4-diamino-5-(3'4'-dichlorophenyl)-6-methylpyrimidine (DDMP) was found to exhibit inhibitory effects on animal tumours (Clarke et al. 1952) and mouse leukaemia (Burchenal et al. 1952). At a later date it was reported as producing haematological improvement in three of twelve children with acute leukaemia (Murphy et al. 1954). Toxic effects similar to those associated with the folic acid analogues was noted, including the conversion to megaloblastic erythropoiesis. DDMP seems to differ from amethopterin, however, in that it has a more prolonged toxic effect.

A related group of compounds, the dihydrotriazines have been shown by Farber et al. (1953) to have a temporary antileukaemic activity in children. Undesirable side-effects preventing them from being of practical value at the present time have, however, been observed.

Another antimetabolite, O-diazo-acetyl-L-Serine (azaserine) (Bartz *et al.* 1954; Ehrlich *et al.* 1954; Stock *et al.* 1954) has been found to occasionally produce temporary haematological improvement in children, but relapse tends to follow after a short interval (Ellison *et al.* 1954).

Pyridoxine deficiency in mice, produced by administering the analogue desoxypyridoxine, has been shown to result in lymphopenia and granulocytosis (Weir *et al.* 1949; Weir and Heinle 1950) and its action has been compared with that of cortisone. Desoxypyridoxine has been tested in human leukaemia but no evidence that it produces any material benefit has been recorded.

Apart from possible antimetabolites, and more specifically substances which may interfere with nucleoprotein metabolism, compounds related in chemical structure to the compounds already known to affect the leukaemic process e.g. the nitrogen mustards, form a basis for testing for antileukaemic activity.

Burchenal (1954) has suggested that until more than quantitative differences are discovered between the normal and leukaemic cells, it is doubtful whether any single agent will have sufficient specificity to control the disease. Until more fundamental knowledge be known concerning the metabolic derangement in leukaemia, a combination

of several compounds able to produce inhibition at different levels of a single metabolic pathway necessary for the leukaemic cell, may yield more satisfactory therapeutic results. Such "combined therapy" has, of course, been used with success in the treatment of infectious diseases, particularly tuberculosis.

Various investigators have reported on the additive effects of a combination of drugs in the leukaemic animal. Goldin, Greenspan and Schoenbach (1952), using a combination of aminopterin and 8-azaguanine observed an increased survival time in leukaemic mice. Amethopterin and 8-azaguanine were also found by Law (1952) to have striking potentiating effects in mouse leukaemic while Nadel and Greenberg (1953) observed a synergistic inhibitory action of amethopterin and the diaminopyrimidine, 2, 4-diamino-5-(3'4'-dichlorophenyl) 6-Methylpyrimidine (DDMP), upon leukaemia L 1210 in mice, with an increase in survival time of 92 per cent. Various combinations of amethopterin, nitrogen mustard, a nitrogen mustard derivative, triethylenemelamine, and erythromycin were found by Barvick and Goodson (1954) to reduce the tumour growth and increase the survival time of mice with sarcoma 180, and as early as 1949 Skipper presented data suggesting a possible antileukaemic synergism between urethane and the nitrogen mustard, methyl-

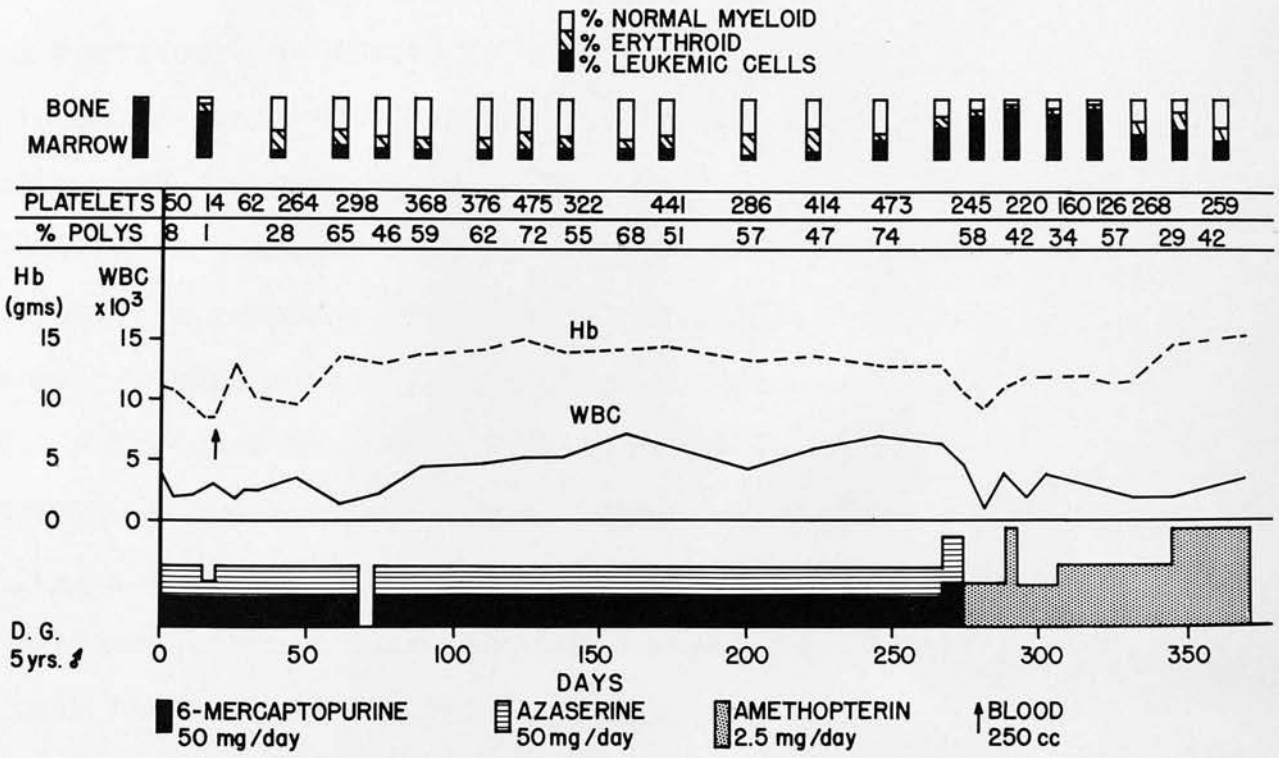


FIG. 6. Complete remission, in a child with acute leukaemia, following treatment with 6-mercaptopurine and azaserine. The possibility that the remission was due to 6-mercaptopurine alone cannot be excluded.

bis (B-chloroethyl) amine.

Up to the present time few reports have been published concerning the effectiveness of combined therapy in human leukaemia. Burchenal (1954a) has found no evidence of synergism between amethopterin and cortisone, or amethopterin and 6-mercaptopurine. Although certain investigators (Damashek 1952; Farber 1950; Marie et al. 1951; Bernard and Mathe 1952; Kelty and Beard 1953; Maguin 1953) consider the initial response of children with acute leukaemia is better when a combination of cortisone and a folic acid antagonist is given, this does not necessarily indicate a synergistic effect. Kingsley-Pillers et al. (1952) have shown an absence of cross resistance between these two compounds and it seems likely that sequential therapy would produce just as good if not better results than combined treatment. The French workers, however, are of the opinion that a synergistic effect does exist. Azaserine and 6-mercaptopurine although resulting in striking additive effects in animals (Clarke et al. 1954) have shown little effect when given to children with leukaemia. Innes and Rider (1955) have recently reported evidence suggesting a synergistic relationship between urethane and a nitrogen mustard derivative, B-napthyldi-2-chloropropylamine, designated R151, in the treatment of multiple myeloma, which provides a further stimulus in the

search for more effective combinations for the management of patients with leukaemia.

In the search for new chemotherapeutic agents for acute leukaemia it seems probable at the present day that the main attention will be focussed on nucleic acid synthesis. It is, however, also essential that studies relating to the mode of action of cortisone and ACTH and the mechanism of resistance of the leukaemic cell to these hormones and other chemotherapeutic agents, be pursued. Although disappointment followed the encouraging early results with the folic acid antagonists, the important developments in the chemotherapy of acute leukaemia in the space of only a few years leads one to believe that further advances may be looked forward to with optimism.

B. CHRONIC LEUKAEMIA.

NITROGEN MUSTARDS AND RELATED COMPOUNDS

The effects of mustard gas derivatives on haemopoietic tissue was first reported after the first world war. Krumbhaar and Krumbhaar (1919) described the action of these compounds on blood and bone marrow and their cytotoxic action was reported by Pappenheimer and Vance (1920). About the same time Lynch et al. (1918) and Warthin and Weller (1919) published accounts of the medical aspects of mustard gas poisoning and its general systemic effects, while Flury and Wieland (1921) studied the effects of these compounds on the gastro-intestinal tract and on the normal electrolyte and fluid balance.

The possible value of mustard gas in the local treatment of cancer was reported by Beremblum in 1929. He observed that the induction of warts in mice by the repeated application of tar could be almost completely prevented by the addition to the tar of 0.1 per cent. mustard gas. Adair and Bagg (1931) showed that tar cancers in mice could be controlled by the surface application of mustard gas in absolute alcohol and in human subjects good therapeutic results were obtained in cases of skin cancer. It was not until 1935 that this particular therapeutic application of the mustards was pursued further. Berenblum in that year found that several compounds closely related

to mustard gas were able to produce pronounced inhibition of tumour induction in mice. Three years later Maier (1938) described the depressant effects of mustard gas on the leucopoietic tissues which were followed in 1939 by a similar report by Drews.

With the advent of the second world war, intensive interest in the subject of nitrogen mustard derivatives was developed in both the United Kingdom and the United States of America. Wilkinson, as early as 1942, observed that certain B-halogenated alkylamines had marked depressant effects on haemotopoiesis. Leucopoiesis appeared to be affected earlier than erythropoiesis and a rapid improvement was seen to follow the withdrawal of these toxic agents (Wilkinson and Fletcher 1947). Such an observation led to the consideration of the possible therapeutic effects of these substances in both chronic lymphatic and chronic myeloid leukaemia and later to acute leukaemia, Hodgkin's disease and polycythaemia. Wilkinson and Fletcher (1947) reported the effects of two derivatives, methyl-bis (B-chlorethyl) amine hydrochloride and tris (B-chlorethyl) amine hydrochloride in these conditions. When given intravenously they found these nitrogen mustards to be most beneficial in chronic myeloid leukaemia, in one patient a remission of eight months following treatment.

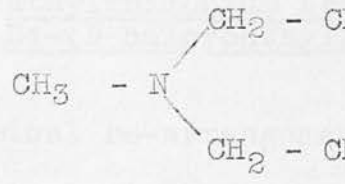
Dramatic improvement was also seen to occur in Hodgkin's disease but less satisfactory results were observed in chronic lymphatic leukaemia.

The nitrogen mustards were introduced into medical practice in the United States of America in 1946 for the palliative treatment of leukaemia and other malignant diseases of the reticulo-endothelial system. Rhoads (1946) investigated the therapeutic properties of the same two compounds as used by Wilkinson, in a series of one hundred and sixty patients suffering from neoplastic disease, chiefly leukaemia, Hodgkin's disease and related disorders. Good results were obtained in patients with Hodgkin's disease but X-ray therapy was still considered advisable in the early, slowly progressive case with localised disease. Nitrogen mustards were concluded to be of most value in producing symptomatic relief when the disease became widespread and systemic effects, such as fever, were present. Some patients with lympho-sarcoma and chronic lymphatic leukaemia were improved as were those with chronic myeloid leukaemia. The effects of the nitrogen mustards in other forms of cancer were not encouraging. Goodman et al. (1946) published detailed clinical reports of the therapeutic results obtained in this series of patients. Following these initial observations numerous reports have appeared in the

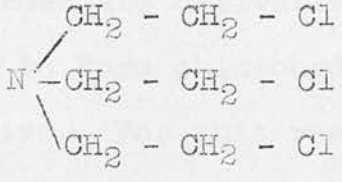
literature substantiating the findings of those early workers and indicating that the nitrogen mustards have a place in the management of malignant disease of the reticulo-endothelial system.

The chemistry, mechanism of action and pharmacology of the nitrogen mustards has been the subject of intensive research and has been reviewed at length by Gilman and Philips (1946) and Philips (1950). The two mustard gas derivatives which have been found most satisfactory for clinical purposes are those described by Wilkinson and Fletcher (1947) and Rhoads (1946) and are:-

(a) Di-(B-chloroethyl) methylamine hydrochloride, the 'bis' compound



and (b) Tri-(B-chloroethyl) amine hydrochloride, the 'tris' compound



The 'bis' compound has been most widely used as most observers have claimed it to be less toxic. Wilkinson (1953), however, considers the 'tris' compound to be better and less likely to give rise

to phlebosclerosis. It seems probable that both compounds have identical therapeutic properties.

The two nitrogen mustards are very soluble in water in which they undergo a distinctive intramolecular transformation (Fig. 7), with release of halide ion to form cyclic ethylenimonium derivatives which are the intermediates responsible for chemical reactivity of nitrogen mustards (Philips 1950) and which produce powerful nucleotoxic and cytotoxic effects on enzyme systems and mitosis in rapidly proliferating cells.

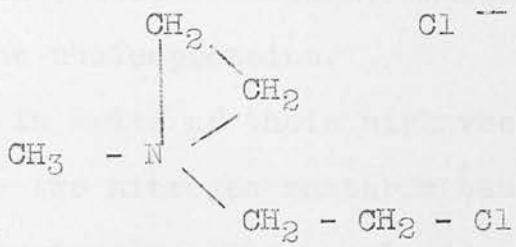


Fig. 7.

Ethylenimonium transformation of Di-(B-chloroethyl) methylamine.

This chemical re-arrangement of the nitrogen mustard molecule in aqueous solution is to some extent reversible, but more important is the hydrolysis which these intermediate derivatives undergo in aqueous solution to form chlorohydrims which are relatively inactive. For this reason solutions of the nitrogen mustards should be prepared immediately before administration otherwise much of their potency will be lost through hydrolysis.

The exact mode of action of the nitrogen mustards in destroying growing cells is unknown.

Philips (1950) comments on the similarity in actions of these compounds and those of ionizing radiation, a view put forward by others including Boyland (1948) who has used the term 'radiomimetic' to describe the biological properties of these chemical agents. The susceptibility of cells seems dependent on their proliferative activity and the site of action is the nucleus. Skipper et al. (1951) have observed inhibition of nucleic acid synthesis following administration of nitrogen mustards, while Dustin (1948) noted that mitotic poisons, like radiation, caused depolymerisation of the nucleoproteins.

In spite of their high vesicant properties these two nitrogen mustards can be given safely intravenously. The usual mode of administration is to inject the solution of nitrogen mustard into the rubber drip tube of a saline infusion. By such a method extravasation into the subcutaneous tissues with local inflammation, pain and necrosis may be prevented and venous thrombosis possibly lessened.

The dosage schemes of nitrogen mustards vary widely. The initial recommendation was 0.1 to 0.2 mg. per kg. of body weight administered on consecutive or alternate days until between three and six doses had been given. The maximal single dose advised was 8 mg. In selected cases larger doses of 0.2 to 0.3 mg. per kg. have been given in two injections, twenty-four to forty-eight hours

hours apart (ApThomas and Cullumbine 1947). Wilkinson (1953) advised the use of a standard dose of 6 mg. in adults which was repeated two or three days later or at longer intervals depending on the leucocyte count and response of the patient. Sensitivity to the nitrogen mustards varies considerably in different subjects which must always be a consideration when estimating the dose of the drug to be employed. In the author's experience the scheme suggested by Wilkinson (1953) is highly convenient and is a satisfactory standard method of prescribing these compounds. In view of the effects of the nitrogen mustards on the bone marrow it does, however, seem advisable to refrain from repeating a course of treatment until six to eight weeks has passed since the last course.

One of the adverse criticisms of nitrogen mustard therapy is the toxic side-effects which are liable to occur.

When injected into animals in toxic doses these compounds have been shown to provoke an early transient rise in the haemoglobin level and erythrocyte count followed by a rapid development of anaemia and leucopenia (Cameron 1942). Loss of weight and diarrhoea occurred and at post-mortem scattered haemorrhages in the gut were seen. Examination of the bone marrow revealed that the white cells were more depleted than the red.

Cameron and Rydon (1942) showed that the leucotoxic action was mainly on the polymorphonuclear cells and their precursors, while the lymphocyte series was affected to a lesser extent. In higher doses, Philips (1950) observed marrow aplasia together with a rapid shrinkage of lymphoid tissue which became devoid of lymphocytes.

It is usual for human subjects to experience nausea or vomiting within the first four to eight hours of injection. Attempts to counteract the severity of this effect have been made. Shullenberger et al. (1949) suggested the simultaneous administration of 150 to 200 mg. of pyridoxine hydrochloride (vitamin B_6). Craver (1948), however, considered that pyridoxine should be administered intramuscularly ten to thirty minutes after the nitrogen mustard to avoid chemical interaction between the two substances. Wilkinson (1953) found that anti-histamine drugs given at the time of injection and repeated four hours later controlled or reduced the severity of these symptoms. Other workers (Wintrobe and Huguley 1948; Kennedy and Aub 1949) recommended the use of sedatives, e.g. opiates or barbiturates to minimise the gastro-intestinal reactions.

The effect on the haemopoietic system may be marked but at the dosage level used therapeutically Gellhorn and Jones (1949) found that depression of

haematopoiesis was usually reversible, and thrombocytopenia of a degree to produce serious haemorrhagic manifestations has been rare (Wintrobe and Huguley 1948).

Depression of all elements of the blood forming tissuesfollows the administration of nitrogen mustard, but the effect on the leucocytes is the most pronounced. The fall in granular cells is more marked than that of the lymphocytes and usually reaches its maximum in two to three weeks. The level of lymphocytes in the peripheral blood falls during the first week reaching its maximum in about six to eight days, while thrombocytopenia may not reach its maximum until the third week after treatment. Within six weeks of treatment the white cells and platelets usually return to normal, the presence of a few myelocytes in the peripheral blood and monocytosis heralding the rise in the polymorph count, as frequently seen in patients recovering from acute agranulocytosis. A mild reduction in the volume of packed erythrocytes may occur reaching its peak in about three weeks but occasionally anaemia may be improved by the effect of nitrogen mustard on the neoplastic process. Depression of both mature and primitive cells in the bone marrow occurs but marrow regeneration has been stated as usually following within a period of six weeks. (Spurr *et al.* 1947)

Haemorrhagic manifestation associated with a prolonged clotting time and the appearance of heparin-like anticoagulant in the blood have been described (Smith et al. 1948). A similar coagulation defect has been induced in experimental animals by toxic doses of nitrogen mustard or x-rays (Jacobson et al. 1948; Allen et al. 1948).

Although a return of the bone marrow, leucocyte and platelet counts to normal is generally considered usual following a course of nitrogen mustard, the author has found that marrow aplasia with cytopenia and bleeding is more prominent in those having received nitrogen mustard therapy. An analysis by the author of the terminal blood pictures at the Memorial Hospital, New York, of eighty patients having died of Hodgkin's disease, many of which had received several courses of nitrogen mustard, or a nitrogen mustard derivative, triethylene melamine, indicated the hazard of intoxication following their administration. Table 3 indicates the last recorded haemoglobin levels, leucocyte and platelet counts before death. The interval between blood examination and death was less than two weeks in over 75 per cent. of patients, the vast majority actually being within a few days of death.

	No.	Hb. over 80%	60 - 79%	40 - 59%	Below 40%
HAEMOGLOBIN	76	10 (13%)	22 (29%)	36 (48%)	8 (10%)

	No.	Over 15,000	10 - 15,000	4 - 10,000	1 - 4,000	Under 1,000
LEUCOCYTES (per c.mm.)	77	9 (11.5%)	8 (10.5%)	12 (15.5%)	32 (41.5%)	16 (21%)

	No.	Over 150,000	100 - 150,000	50 - 100,000	Below 50,000
PLATELETS (per c.mm.)	60	13 (21.7%)	5 (8.3%)	12 (20%)	20 (50%)

TABLE 3. Haemoglobin, Leucocyte and Platelet Levels in Patients in the Terminal Stage of Hodgkin's Disease

Most patients received blood transfusions late in the disease and the haemoglobin levels as a result did not reflect the true degree of anaemia. Severe anaemia was, however, observed in approximately 60 per cent. of patients irrespective of blood transfusions. More revealing, however, was the profound leucopenia, in 21 per cent. the leucocyte count being under 1,000 per c.mm., while 62.5 per cent. had counts below 4,000 per c.mm. Platelets were also greatly reduced in 50 per cent. of patients in which counts were carried out in the later stages of the disease, the count being below 50,000 per c.mm. Thrombocytopenia was associated with haemorrhage in twenty-seven patients (i.e. 33.7 per cent.), bleeding being confined, as would be expected, mainly to the group of patients whose platelet counts were below 50,000 per c.mm. (Table 4.).

In a condition such as Hodgkin's disease where the accepted forms of treatment, irradiation and chemotherapy, have a toxic, inhibitory effect on blood formation, the therapeutic agent is usually incriminated as the cause of the low leucocyte or platelet count. It should be remembered, however, that the disease itself may in part predispose to depression of haematopoiesis. In the series under discussion only five patients received no treatment. Two of these patients had blood pictures characteristic

No.	No. with platelets <50,000	No. with haemorrhage	*Platelets in patients with haemorrhage		
			Normal	50-100,000	<50,000
60	30	27	1	2	22

TABLE 4. Incidence of Thrombocytopenia and Haemorrhage in Patients with Terminal Hodgkin's Disease.

*Platelet count not performed in two patients.

No.	Normal marrow	Normal cellularity plus disease	Hypocellular marrow	Acellular marrow
71	10	12	37	12

TABLE 5. Cellularity of Bone Marrow at Autopsy in Hodgkin's Disease.

No.	Normal marrow	H.D. infiltration of marrow	"Complete" replacement of marrow by disease	Hypocellular No disease
71	10	44	5	12

TABLE 6. Incidence of Bone Marrow Invasion by Hodgkin's Disease. Autopsy Figures.

A. Leucopenia

Patients treated with x-rays alone	No. with leucopenia W.B.C.'s < 4,000 per c.mm.	Patients treated with x-rays and nitrogen mustard	No. with leucopenia W.B.C.'s < 4,000 per c.mm.
25	8 (32%)	50	37 (74%)

B. Thrombocytopenia

Patients treated with x-rays alone	No. with platelets < 150,000 per c.mm.	Patients treated with x-rays and nitrogen mustard	No. with platelets < 150,000 per c.mm.
10	7 (70%)	48	40 (83%)

TABLE 7. Incidence of (A) Leucopenia and (B) Thrombocytopenia following X-rays and Nitrogen Mustard in Patients with Hodgkin's Disease (where Blood Counts were available for analysis).

A. Leucopenia

Patients treated with x-rays	Leucopenia after x-rays and before nitrogen mustard therapy	Patients treated with x-rays and nitrogen mustard	Leucopenia after x-rays and nitrogen mustard
63*	15 (24.5%)	50	37 (74%)

B. Thrombocytopenia

Patients treated with x-rays	Thrombocytopenia after x-rays and before nitrogen mustard therapy	Patients treated with x-rays and nitrogen mustard	Thrombocytopenia after x-rays and nitrogen mustard
45*	15 (30%)	48	40 (83%)

TABLE 8. Incidence of (A) Leucopenia and (B) Thrombocytopenia following X-rays and Nitrogen Mustard in Patients with Hodgkin's Disease (where Blood Counts available for analysis)

*12 patients who had combined x-ray and nitrogen mustard therapy are not included.

of aplastic anaemia and the bone marrow at autopsy was found infiltrated by disease. It was interesting to observe that in 70 per cent. of cases the bone marrow taken at autopsy from four sites (sternum, rib, vertebra and ilium), showed infiltration by the disease (Table 6). In addition the bone marrow was found to have a reduced cellularity in 70 per cent. of cases (Table 5).

How much x-rays play a part in producing depression of bone marrow activity is difficult to say but there is no doubt that excessive doses of nitrogen mustard may lead to a profound leucopenia and thrombocytopenia. Comparison of the blood pictures in patients receiving x-rays alone and those receiving x-rays and nitrogen mustard (Tables 7 and 8) showed clearly that the main cause of the high incidence of hypoplasia or aplasia of the bone marrow was due to nitrogen mustard, but probably in 25 to 30 per cent. of cases, x-rays and bone marrow replacement by disease may have been the predisposing factors.

Clinical Application of the Nitrogen Mustards.

Since the original reports of Rhoads (1946) Goodman et al. (1946) and Wilkinson and Fletcher (1947) a voluminous literature has collected on the subject. Most of the reports of their therapeutic value concerns the treatment of Hodgkin's disease and related lymphomas, but a number of investigators

have published accounts on their experiences with the nitrogen mustards in the treatment of the leukaemias. In the United Kingdom Wilkinson (1953) has presented his results of treating one hundred and twenty-six cases of chronic leukaemia with tri-(B-chlorethyl)-amine hydrochloride. In 74 per cent. of the patients, clinical and haematological improvement followed treatment. In 54 per cent. the duration of the remission was, however, less than three months, 36 per cent. had improvement lasting from four to twelve months while in 10 per cent. the remission exceeded one year's duration. Comparing the survival time of patients with chronic leukaemia treated with x-ray irradiation and those receiving nitrogen mustard therapy Wilkinson observed that the latter group compared extremely well and were by no means inferior. In general, he considered the indications for treatment of chronic leukaemia with nitrogen mustard were deteriorating health, anaemia, a rising leucocyte count, loss of weight, sweating, increasing splenomegaly and glandular enlargement. No beneficial effects were observed in patients with acute leukaemia except in a few where the leucocyte count was high. In these instances a fall in the leucocyte count rapidly followed treatment but no improvement in the distribution of white cells or in platelet or red cell

production was noted. This is in agreement with the author's experience that nitrogen mustards have no specific effect on the acute leukaemic process and are on the whole not to be recommended. Goodman et al. (1946) found that nitrogen mustard therapy was of no value in the terminal stages of chronic leukaemia but in others the results of treatment were comparable with those obtained with x-ray therapy. Spurr et al. (1948) found that remissions in patients with chronic myeloid leukaemia were rarely longer than three months and concluded that they were no longer than those induced by x-ray therapy. Burchenal et al. (1949a) analysed the results of treatment of thirty-eight patients with leukaemia. In those with acute leukaemia no consistent clinical remissions were seen, but occasional temporary symptomatic relief occurred. They concluded that the results obtained in chronic myeloid leukaemia were similar to those with irradiation and that in chronic lymphatic leukaemia only those patients in good condition before treatment did well, those with far advanced disease generally responding poorly. Craver (1948) considered that as a rule longer remissions and one accompanied by greater reduction in the splenomegaly can be produced at least initially by x-ray treatment. In his experience nitrogen mustard induced remissions were short lived, and lasted only a month or so. Goldman et al.

(1948) noticed temporary benefit from treatment in both patients with chronic myeloid and chronic lymphatic leukaemia. Bauer and Erf (1950) considered nitrogen mustards of no real asset in the treatment of leukaemia and was probably contra-indicated. Other reports have appeared concerning the treatment of leukaemia with these compounds including those by Karnofsky et al. (1947), Wintrobe And Huguley (1948) and Damashek (1949). Wintrobe and Huguley (1948) found that seven out of eleven patients with chronic myeloid leukaemia showed a good response to nitrogen mustard, but when the disease became refractory to x-ray therapy, nitrogen mustard also usually failed. Karnofsky and his colleagues concluded that nitrogen mustard did not produce any therapeutic effects which could not be accomplished with x-rays.

Most observers agree that remissions follow treatment in the early cases. The remissions, however, are generally of short duration and are no longer than those following x-ray treatment, although nitrogen mustard, as Wilkinson (1953) has pointed out, may result in the survival time being equal to that of the patient receiving radiotherapy it has no real advantage over x-rays, and because of this, their mode of administration and of their less readily controlled toxicity, few physicians are now using this method of treatment of chronic

leukaemia.

Treatment of diseases other than Leukaemia with Nitrogen Mustard

Hodgkin's disease

Goodman et al. (1946) studied the results of nitrogen mustard therapy in twenty-seven patients with Hodgkin's disease, most of which were advanced or had become resistant to x-rays. Nitrogen mustard caused a partial or complete disappearance of the tumour masses, a fall in temperature, and an increase in both appetite and weight; this was followed by symptom-free remissions of from two weeks to seven months. Since response to subsequent courses of nitrogen mustard was unsatisfactory, it was considered that the main indication for its use was in the later stages of the disease, particularly when resistance to therapeutic doses of x-rays developed. Wintrobe et al. (1947) and Karnofsky et al. (1947) agreed that nitrogen mustard was particularly likely to be of benefit in the x-ray resistant case of Hodgkin's disease. ApThomas and Cullimbine (1947) working in Manchester, England, reported the first comprehensive account in the United Kingdom of the effect of this form of treatment in patients with Hodgkin's disease. They concluded that it was a useful agent in the palliative treatment of extensive disease and pointed out that symptoms could still be alleviated by nitrogen mustard in cases where

radiotherapy had been abandoned. Bone marrow depression was considered a limiting factor as far as dosage was concerned and leucopenia and thrombocytopenia appeared to be more severe after the 'tris' compound. Both the 'bis' and 'tris' forms of the drug seemed to have identical clinical effects on the disease. Radiotherapy was considered the choice of treatment initially because (1) the mean period of remission after x-rays was longer; (2) vomiting was less troublesome; (3) leucopenia was certain and sometimes severe after nitrogen mustard therapy. Wintrobe and Huguley (1948) reported that in seventeen out of thirty-two patients with generalised Hodgkin's disease the results were good, and that thirteen had survived for an average of over fifteen months. Craver (1948), based on an experience of treating one hundred and two patients, concluded that in the presence of generalised disease with associated symptoms, viz. fever, night sweats, pruritus, etc., nitrogen mustard was the treatment of choice. Nitrogen mustard did not, however, replace x-rays in those patients with localised disease. Goldman et al. (1948) came to similar conclusions and reviewing the literature found that over 90 per cent. of the reported cases of Hodgkin's disease who had received mustard therapy obtained benefit.

Nabarro (1949) was also of the opinion that in

generalised Hodgkin's disease nitrogen mustard was more effective than x-rays. In his series of twenty-one patients only five, however, responded satisfactorily to treatment. Erf and Bauer (1949) demonstrated the brevity of the remissions which may follow treatment. Of their forty-three patients only seventeen remained in remission six weeks after treatment. In a later publication these workers (Bauer and Erf, 1950) reported good immediate results in all but two of fifty-nine cases.

Hodgkin's disease involving bone has been reported by Damoshek et al. (1949) to be unaffected by nitrogen mustard. Kamofsky et al. (1947) have also commented on the lack of radiological improvement in osseous lesions but found that pain may be relieved. Wintrobe and Huguley (1948) however observed healing in some instances of spinal involvement, and relief of backache in other cases without radiological evidence of vertebral lesions.

Many other reports have been published relating to the value of nitrogen mustard in Hodgkin's disease including those by Reinhard et al. (1950), Alpert (1950), Spurr et al. (1950) and Bethell et al. (1950). The consensus of opinion suggests that it is a valuable additional therapeutic agent but radiotherapy is still the

treatment of choice in the early stages. Nitrogen mustard therapy appears to be most suitable indicated in the following clinical types of Hodgkin's disease:

1) Advanced cases which fail to respond to therapeutic doses of x-rays, i.e. "radio-resistant" disease.

2) Where the disease is widespread and associated with marked constitutional symptoms (fever, sweating, pruritus, weight loss, etc.)

3) Those with fever but without demonstrable enlarged lymph nodes,

and 4) Patients with visceral involvement, pleural effusion and ascites.

Irrespective of the dramatic improvement, which may occasionally be observed in patients with advanced Hodgkin's disease following nitrogen mustard therapy, there seems to be little effect on the survival time of patients as a whole. Boland (1951) compared the survival time of (A) one hundred and five patients treated with irradiation alone seen between 1940-1944, with (B) a group of eighty-seven patients first seen during 1946-47. Twenty-seven of the latter group had nitrogen mustard at sometime during their illness. The three year survival time rate in (A) was 33 per cent. and in (B) 40 per cent. The average duration of life was three months longer in Group (B) and the author

suggested it may be related to the short nitrogen mustard induced remissions occurring late in the disease.

The possibility of other modes of administration of nitrogen mustard has been investigated. Bierman et al. (1951) suggested that the intra-arterial route may be of benefit in selected patients while several investigators have suggested intra-pleural injection in patients with pleural effusion associated with Hodgkin's disease. The personal experiences of the author are that intra-arterial administration has shown no better results than the usual intravenous route and is a difficult and impractical procedure. Temporary symptomatic relief may, however, be obtained for patients with pleural effusion by intra-pleural injection of nitrogen mustard after first removing as much fluid as possible by paracentesis. Re-accumulation of fluid in these cases seems to be delayed by such treatment.

Lymphosarcoma.

Nitrogen mustard has also been shown to be occasionally of value in the palliative treatment of terminal cases of lymphosarcoma. Sometimes, as in Hodgkin's disease, the results are dramatic, but successive relapses prove less amenable to treatment. Jacobson et al. (1946) obtained significant remissions, varying in duration from

three to eighteen months in four out of six patients with lymphosarcoma. Rhoads (1946) was of the opinion that the highly aggressive and rapidly growing type of lymphosarcoma did not seem to be significantly affected even by maximal doses. Wintrobe and Huguley (1948) found that only three of their series of eleven patients with lymphosarcoma responded well. Justin-Besancon et al. (1948) concluded that only a certain percentage of cases were sensitive to this treatment and the results on the whole were less encouraging than those of Hodgkin's disease. Ben-Asher (1949) and Reinhard et al. (1950) came to similar conclusions. Herve (1948) and Spurr et al. (1950) were, however, more favourably impressed.

The indications for nitrogen mustard therapy in lymphosarcoma would appear to be similar to those in Hodgkin's disease. Whereas many clinicians consider lymphosarcoma and especially the more rapidly growing form to respond less readily than Hodgkin's disease to nitrogen mustard, symptomatic relief may follow in the later stages of the disease and it is therefore worthy of trial.

Polycythaemia Vera.

Treatment with nitrogen mustard may result in a remission of symptoms and a return of the blood picture to normal. Jacobson (1946), in an early report, stated that in all five patients he treated

symptomatic and haematological improvement occurred, and in some cases improvement was maintained for over a year. Di Guglielmo (1949) found that a four-day course of nitrogen mustard usually resulted in the erythrocyte count returning to a normal level. A delay of up to two months was observed before the maximum effect of therapy occurred. Craver (1948); Goldman et al. (1948); Faloon and Gorham (1948) also reported beneficial effects of nitrogen mustard therapy, but concluded that the results were no more favourable than could be expected from x-ray therapy. Spurr et al. (1950) treated a group of ten patients with nitrogen mustards. A total of fifteen courses was given and the range of remissions varied from two to thirty months. In eight cases, remissions of five months or more were produced and in two the remission lasted three months or less. Bauer and Erf (1950), however, considered it dangerous to use nitrogen mustard for the treatment of polycythaemia in case vomiting caused cerebral haemorrhage.

Although only small groups of patients with polycythaemia have been treated and, therefore, a complete evaluation of nitrogen mustard therapy has not been obtained, most observers agree that its effect is no better than that of other forms of treatment - especially x-rays. The toxic side effects encountered with nitrogen mustard therapy

are also against this form of therapy, while the ease of administering treatment with x-rays, and in the author's opinion, radioactive phosphorus (P_{32}) in particular, must also be considered when considering the assets of the various therapeutic agents. In a series of thirty patients treated by the author at the Leeds General Infirmary during the past four years, twenty-four have developed remissions following P_{32} of over nine months duration, and only four responded unsatisfactorily. The good therapeutic results and the absence of side-effects suggest that P_{32} is still more satisfactory than present day chemotherapy.

Mycosis Fungoides

Craver (1948) treated four patients with this disease and suggested that the chronic type of case without bulky tumours of the skin may do fairly well but that in the advanced or aggressive cases, while striking rapid, partial remissions may occur, they will probably be followed by rapid relapses. There is, however, little evidence that nitrogen mustard is more effective than radiotherapy in the treatment of this disease. Block and Murphy (1948) noted improvement in one patient with mycosis fungoides and showed that the lesions, although becoming acellular, still retained the characteristics of the disease following treatment. Philpott et al. (1947) also obtained good results with nitrogen

mustard in one patient and Schafer and Lehner (1949) published an account of a case which responded well to nitrogen mustard when other forms of therapy had failed. Henstall et al. (1947) noted dramatic improvement in one patient following nitrogen mustard therapy, and Herve (1948a) observed a complete regression of all tumours within thirty days of treatment with marked symptomatic improvement. Local relapse occurred three months after the injection of nitrogen mustard.

In certain instances nitrogen mustard would appear to be beneficial in patients with mycosis fungoides and must be considered as an available therapeutic agent in the management of this disease. With the knowledge that the skin lesions are in the early stages are highly radio-sensitive, x-rays should be considered the treatment of choice at that stage, for it has not been demonstrated that remissions following nitrogen mustard are ever an improvement on those obtained by x-rays.

Miscellaneous Diseases.

Various diseases apart from the reticulosis have been treated with nitrogen mustard. Diaz et al. (1951) reported favourable results in rheumatoid arthritis, but this was not substantiated by Cohen et al. (1953). Snider (1948) reported improvement in four cases of sarcoidosis and Goodman et al. (1946) considered that some benefit

followed the administration of nitrogen mustard in cases of melanosarcoma. As a result of the beneficial effects in leukaemia and Hodgkin's disease, nitrogen mustard has been given to a wide variety of neoplastic diseases in the hope that it may produce at least symptomatic relief. The only form of cancer in which definite improvement has consistently been reported is bronchogenic carcinoma. Boyland and Warwick (1947) in the Twenty-Fourth Annual Report of the British Empire Cancer Campaign reported good responses with nitrogen mustard in several cases of bronchial cancer as evidenced by alleviation of pain and cough, and absorption of pleural exudates. Similar results have been reported by other workers including Rhoads (1946) and Karnofsky et al. (1948). Karnofsky and his colleagues reported symptomatic improvement lasting from two weeks to two months in 74 per cent. of thirty-five patients with inoperable cancer of the lung. Objective evidence of disease such as those associated with superior mediastinal obstruction, metastatic lesions and pleural effusion diminished following nitrogen mustard. In the rapidly growing, anaplastic lung cancer improvement was noted to frequently follow treatment and these authors considered that nitrogen mustard may be more effective and possibly less hazardous than x-ray treatment. The combination of x-rays and nitrogen

mustard did not produce more satisfactory results. Skinner and his fellow-workers (1948), however, considered that inoperable lung cancer was best treated by nitrogen mustard combined with x-rays. Craver (1948) treated thirty-three cases of carcinoma of the lung and observed occasional dramatic improvement, while Lynch (1950) analysing his series of sixty patients noted that in 69 per cent. subjective improvement occurred while in over 50 per cent. there was definite objective evidence of improvement.

Although the results are only of a temporary nature, and the response to a second course of treatment poor (Karnofsky et al. 1948), nitrogen mustard would seem to hold a place in the palliative treatment of lung cancer. If x-rays are contra-indicated, and if pain, dyspnoea, pleural effusion or other distressing symptoms are present a trial should be given to nitrogen mustard.

ORAL NITROGEN MUSTARD DERIVATIVES.

Following the successful clinical trials of the intravenous nitrogen mustards, research was directed towards finding a nitrogen mustard with a greater selective effect on tumour as compared with the normal tissue, and therefore with less toxic side effects. In addition new derivatives have been sought for which are active when taken orally. The former efforts have, thus far, not been successful

but progress has been made towards a more convenient method of nitrogen mustard therapy through the discovery of the tumour-inhibiting effects of

A. B-naphthyldi-2-chloroethylamine (R.48), followed later by a similar preparation, B-naphthyldi-2-chloropropylamine (R.151).

B. 1:4-Dimethanesulphonyloxybutane (Myleran: GT 41).

C. Triethylene melamine (TEM)

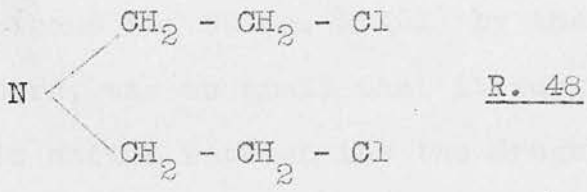
D. Triethylene phosphoramide (TEPA) and Triethylene thiophosphoramide (thio TEPA)

A. B-naphthyldi-2-chloroethylamine (R. 48)

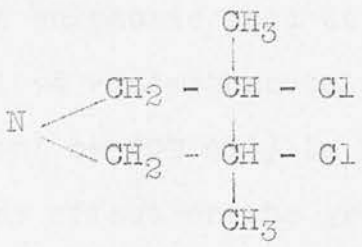
Haddow et al. (1948) studying the inhibitory effects on tumours of a variety of aromatic nitrogen mustards, selected two for clinical trial. Preliminary investigations (Haddow1948), however, revealed that one of these compounds, bis (B-chloroethyl) aniline (R 74) had a marked depressant effect on the bone marrow. More encouraging results were obtained with B-naphthyldi-2-chloroethylamine (R 48) which led to clinical trials by several groups of workers through the Medical Research Council. Matthews (1950) was the first to publish his results. Five patients with Hodgkin's disease all showed some improvement but this was of short duration (four to eight weeks) and further treatment was less

effective. Three cases of chronic myeloid leukaemia were treated and remissions lasting approximately three months occurred in two. A further two remissions of similar duration occurred in one following repeated courses of treatment. Two out of four cases of chronic lymphatic leukaemia were benefited and a prolonged remission was also observed in a patient with polycythaemia. Patients with acute leukaemia and reticulosarcoma were not effected. Toxic symptoms were mild and generally consisted of gastric disturbance. One patient developed cystitis. Leucopenia and a fall in platelets was, however, usual. The effective dose of the compound was large - 300 to 400 mg. per day - and the patients were hospitalised during the early part of treatment. Gardikas and Wilkinson (1951) and Wilkinson (1953) also reported their results with this compound. The daily dosage employed varied between 50 to 800 mg. given orally in divided doses, the usual quantity being 100 - 300 mg. Symptoms suggestive of cystitis, viz. dysuria and haematuria also occurred in some of their patients, other toxic features being purpura and occasional nausea and diarrhoea. The amount of the drug prescribed did not seem to bear any relationship to toxic phenomena. Good responses were observed in three out of ten patients with chronic myeloid leukaemia, three showed only

moderate responses and four failed to benefit at all. Seven of twelve patients with chronic lymphatic leukaemia showed variable degrees of improvement, as did thirteen out of nineteen patients with Hodgkin's disease. Acute leukaemia and lymphosarcoma were unaffected. Wilkinson concluded that the results with R 48 were inferior to those obtained with the original intravenous nitrogen mustards. McWhirter (1951) observed improvement in six of fifteen patients with chronic myeloid leukaemia and four of eleven patients with "idiopathic reticuloses". Nabarro (1951) obtained disappointing results with R 48 given in doses of up to 400 mg. per day. Of a total of twenty-two patients with leukaemia, Hodgkin's disease, myelomatosis and mycosis fungoides only five showed a moderate degree of improvement. He concluded that it should be only used if intravenous nitrogen mustard for any reason was contra-indicated. Galton (1951) also considered R 48 to be less active than nitrogen mustard.



R. 48



R. 151

A similar derivative to R 48, known as R 151 (B-naphthyldi-2-chloropropylamine) (Everett and Ross 1949) has also undergone clinical trials. McWhirter (1951) considered that although similar toxic effects may occur, they were generally less marked than those with R 48. The results obtained with R 151 in a series of twenty-nine patients suggested that this new compound may be rather more effective than R 48, eleven out of twenty-nine patients responding favourably as compared with eleven out of fifty-two treated with R 48. The dosage of R 151 employed was 50 to 100 mg. daily initially, reduced later to 25 - 50 mg. Used in conjunction with urethane, R 151 has been shown to be of value in the palliative treatment of multiple myelomatosis (Innes and Rider 1955). Although it might be argued that urethane alone may benefit patients with myelomatosis, the daily dose used (1 Gm. urethane and 50 mg. R 151) by these investigators, was so small that it suggested a synergistic action between the two drugs.

B. 1:4 Dimethanesulphonyloxybutane - Myeleran; GT 41

Haddow and Timmis (1953), as a result of investigating the tumour inhibiting effects of a

series of sulphonic acid esters, discovered one member, 1 :4 - dimethanesulphonyloxybutane ('Myeleran' or 'GT 41') to have an intense inhibitory effect on the growth of the Walker rat carcinoma 256, and a depressant effect on granulopoiesis, but not lymphopoiesis, both in animals and man, at a lower dose than other members of the series. Preliminary clinical trials (British Empire Cancer Campaign Annual Report, 1950) demonstrated it to be much less toxic than nitrogen mustard and that it had a favourable effect upon patients with chronic myeloid leukaemia.

Galton (1953) in a more detailed account showed that acute leukaemia was unaffected but nineteen patients with chronic myeloid leukaemia all showed an initial response. Nine relapsed within six months and eight patients obtained clinical and haematological remissions varying from six to twenty-one months.

Toxic symptoms were not encountered but the danger of thrombocytopenia was stressed and platelet counts below 100,000 per c.mm. were considered to be a contra-indication to Myeleran therapy. The dose of Myeleran suggested for adults was either (a) a course of 4 to 10 mg. daily up to a total of 200 to 500 mg., (b) one or more short courses in which 100 to 150 mg. was given in one to six days.

In a later paper (Galton and Till, 1955), the short intensive course was considered unwise because of the danger of bone marrow aplasia, and the dosage advised was 0.06 mg. per Kg. of body weight daily, i.e. approximately 4 mg. In some a maintenance dose of 0.5 to 4.0 mg. daily was prescribed, and the results compared with interrupted therapy. No definite conclusion was arrived at as to which was the best method of treatment. In a four year trial thirty-one patients with chronic myeloid leukaemia were treated with relief of symptoms, splenic regression, a reduction in leucocytes and a rise in haemoglobin, of various degree and duration. Patients on maintenance therapy were maintained satisfactorily for up to a period of two years. An important finding was that Myeleran may be beneficial when radiotherapy has become ineffective. Galton was of the opinion that Myeleran was superior to Urethane, benzene and arsenic because of the absence of side-effects, the response being less erratic and the remissions longer. Ledlie (1953) has referred to the effectiveness of Myeleran in chronic myeloid leukaemia and stated that it was now the only chemotherapeutic agent used at the Royal Cancer Hospital, London. Petrakis et al. (1954) considered Myeleran a promising therapeutic agent in this disease. In their series of eleven patients

given maintenance therapy, eight showed clinical and haematological improvement, and one patient continued in remission nineteen months after the start of treatment. Acute leukaemia and multiple myeloma were not benefited by treatment. Wilkinson (1953) giving 25 mg. orally for three to five days followed two to three weeks later when the full effects had developed by a maintenance dose of 2 to 4 mg. daily or on alternate days, found a good initial response in eighteen patients with chronic myeloid leukaemia and remissions varying from five to fifteen months in fifteen. The danger of bone marrow depression with consequent aplastic anaemia, thrombocytopenic purpura and agranulocytosis was pointed out. Bollag (1953), Hansen (1954) and Turesson (1953) also considered Myeleran an effective palliative treatment in chronic myeloid leukaemia.

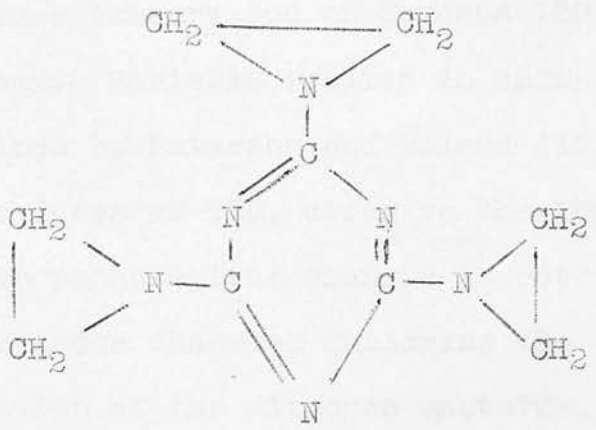
There is no doubt in the author's mind that Myeleran is a highly satisfactory chemotherapeutic agent. It is of low toxicity and not associated with unpleasant side-effects such as may occur with intravenous nitrogen mustard or triethylene melamine, it has a selective action on myeloid cells and may result not only in prolonged remissions but may sometimes benefit patients who have become resistant to therapeutic doses of x-rays. If radiotherapy is unavailable, as is the

case in many countries, Myeleran would at least be a satisfactory substitute. Whether Myeleran can, however, replace radiotherapy as a first choice of treatment remains to be seen, for only by a properly controlled trial can this answer be achieved.

C. Triethylene melamine: (2, 4, 6 triethylenimino-s-triazine: T.E.M.)

The encouraging results of Haddow et al. (1948) in the search for a compound with nitrogen mustard-like activity which could be administered orally led to research in this field by other groups of workers. At the Sloan Kettering Institute, New York, several compounds were synthesised and tested against animal tumours including mouse leukaemia (Burchenal et al. 1948). One, a methoxypyridoxine derivative of nitrogen mustard was found to produce inhibition of a variety of transplantable mouse and rat tumours (Stock 1950) but in human subjects disagreeable side-effects including nausea, vomiting and a foul taste, led to it being discarded as a possible therapeutic agent. Another compound, triethylene melamine with a structural formula shown in Fig. 8 was investigated independently at the Sloan Kettering Institute and in England at the Research Laboratories of the Imperial Chemical Industries (Rose et al. 1950).

FIG. 8.



TEM

It was found to be more satisfactory, being effective both orally and intravenously in man, less toxic than the previous compound, but liable to result in similar side-effects as the original nitrogen mustards. The pharmacological characteristics of TEM have been described by Philips and Thiersch (1950). The ethylenimino groups will be observed to be similar to the ethylenimonium transformation product of the tri and di - (2-chlorethyl) methylamines, which as has been pointed out earlier is the reactive form of the molecule. Various investigators have described its effect upon a wide variety of biological materials. Lewis and Crossley (1950) remarked on its ability to cause tumour retardation in mice when added to their diets in amounts of 0.001 - 0.15 per cent. by dry weight. Burchenal *et al.* (1950c and 1950d) observed that TEM when given intraperitoneally prolonged the survival time of mouse leukaemia. Suguira (1950) and Buckley (1950) noted its inhibitory effect on a variety of

rat and mouse tumours and on Sarcoma 180 respectively. Variable results in animal tumours were obtained by Paterson and Boland (1951).

Toxic doses of TEM, close to the LD_{50}, resulted in pathological changes in rats and dogs similar to those observed following the administration of the nitrogen mustards, viz. pancytopenia in the peripheral blood, atrophy of haematopoietic elements in the bone marrow and in all lymphoid tumours, and mucosal oedema with desquamation of intestinal epithelium and ulceration (Philips and Thiersch 1950). When given by mouth to dogs it was found that TEM was approximately half as toxic as when given by intravenous injection. A considerable variation in response to the drug occurs when administered orally compared with the intravenous route, due probably to inactivation by the acid gastric content. This may explain the erratic response and diminished toxicity to oral dosing in animals (Rose et al. 1950; Philips and Thiersch 1950) and in part to the variable response in man.

The first clinical reports relating to the effectiveness of TEM in the treatment of human neoplastic disease were by Karnofsky et al. (1951) and in the United States of America, and Paterson and Boland (1951) in England. The former group observed its effect when administered orally in

fifty-eight patients with a variety of malignant diseases, and in a further thirty-six patients when given intravenously. When administered intravenously in doses of 6 to 8 mg., in daily doses of 2 to 3 mg. diluted in normal saline to a concentration of 0.5 mg./ml., the results in patients with Hodgkin's disease, lymphosarcoma and chronic leukaemia were found to be no better than those expected to follow nitrogen mustard therapy. No benefit was recorded in patients refractory to irradiation or nitrogen mustard. Although nausea and vomiting were less common following intravenous TEM, depression of the bone marrow was liable to occur and bleeding as a consequence of thrombocytopenia was recorded. They concluded that nitrogen mustard was still the drug of choice for intravenous use. The dosage scheme advised for oral use was 5 to 10 mg. (2.5 or 5 mg. as a single dose per day) during the first week. If no reduction in the leucocyte count was observed a further 5 to 10 mg. was given followed by further courses at weekly intervals, depending on the white cell count, haemoglobin level, the platelet count and the patient's clinical condition. Improvement usually appeared within two to three weeks and there seemed little indication to continue treatment after three to four weeks. Occasional nausea and vomiting was observed and less

commonly diarrhoea. Bone marrow depression also occurred similar to that following intravenous use, and on the whole seemed more severe and more prolonged. This latter observation is in contrast to the findings of Philips and Thiersch (1950) in dogs and is probably related to the variability in response to the drug.

Karnofsky and his colleagues suggested that the most useful application of TEM was in the treatment of advanced Hodgkin's disease where it produced relief of itching, fever, weakness and regression of enlarged lymph nodes. Twenty out of twenty-five patients with Hodgkin's disease were reported as obtaining temporary benefit from TEM lasting two to fourteen weeks. Improvement was observed in patients with chronic lymphatic leukaemia and remissions in chronic myeloid leukaemia occurred lasting four to ten weeks. Some cases of mycosis fungoides were reported as benefiting from TEM. Advanced lymphosarcoma failed to respond to oral TEM, and various other forms of cancer, including bronchial carcinoma, also showed no improvement.

Paterson and Boland (1951) published their results of treating seventeen patients. They gave the drug intravenously in doses ranging from .09 to .22 mg. per Kg. of body weight, the total dose being spread in the majority of instances, over

three days. All patients had advanced disease or were unsuitable for x-ray treatment. In patients with leukaemia, polycythaemia and Hodgkin's disease and in one case of multiple myeloma improvement was noted, "about equal to the established methods of treatment". The absence of troublesome gastric upsets following treatment suggested that it might be a pleasanter alternative to nitrogen mustard.

Since these early papers, many reports of other investigators have been published. Hansen and Bichel (1951) observed oral TEM to be an effective, although temporary palliative in seven out of nine patients with Hodgkin's disease and in one of two patients with chronic lymphatic leukaemia. Bayrel (1952) treated thirty-eight patients, using a similar oral dosage as Karnofsky et al. (1951). Good results were obtained in chronic lymphatic leukaemia and chronic myeloid leukaemia, with symptomatic improvement, a fall in the leucocyte count, improvement in haemoglobin concentration and regression of the enlarged spleen, lymph nodes, etc. Remissions lasted from three to eleven weeks following the initial course of therapy. Four patients with Hodgkin's disease showed considerable improvement, the remissions varying from three to twelve weeks duration. One third of patients were troubled with vomiting and twenty-five per cent. developed leucopenia or temporary bone marrow

aplasia, while in two instances TEM probably contributed to death. Rundles and Barton (1952) in a comprehensive study of one hundred and thirty-four patients given oral TEM, reported excellent or good results in twenty-four of forty-five patients with Hodgkin's disease, eighteen of twenty-three with chronic lymphatic leukaemia and four of six with chronic myeloid leukaemia. Other neoplastic diseases were unaffected. Using a maintenance dose they considered TEM as possibly the treatment of choice in chronic lymphatic leukaemia. Bond et al. (1953) also obtained good results with TEM when administered orally at intervals in patients with chronic leukaemia but their results with Hodgkin's disease were less promising than suggested by other investigators. Symptomatic haemolytic anaemia was controlled in two patients by TEM. Meyer et al. (1952) thought that Hodgkin's disease was benefited but the best results occurred in chronic leukaemia. Acute leukaemia was unaffected and five cases of multiple myeloma showed no improvement after treatment. Wilkinson and Gardikes (1951) treated twenty patients with TEM with disappointing results, only one patient out of six with chronic myeloid leukaemia responding well. Three patients developed aplastic anaemia. Wilkinson considered TEM to be highly toxic and he did not recommend it

for clinical use on this account, and because of its unpredictable effect and variation in individual tolerance. In the published papers mention is made of the variability of response to oral TEM of individual patients and it is pointed out that dosage must be calculated according to the individual's needs. Gellhorn et al. (1952) in an attempt to obtain a more uniform response from oral administration have reccomended the use of sodium bicarbonate with TEM to neutralise gastric acid. Paterson et al., for similar reasons, have prescribed enteric coated tablets of TEM to dogs and showed that such a preparation had a more consistent effect. The same authors described the effect of this preparation in forty-four patients. A dose of 0.2 to 0.3 mg./Kg. was given to twenty-two patients with Hodgkin's disease and in over two-thirds a satisfactory remission of symptoms was obtained with diminution in objective evidence of disease. They stressed that treatment should only be repeated when the polymorphonuclear leucocyte count was restored. A dose of 0.1 to 0.2 mg./Kg. was given to fourteen patients with chronic leukaemia of either myeloid or lymphatic type, with definite benefit in seven. The response in lymphoid leukaemia was better than in the myeloid type. The numbers were, however, small and the patients with myeloid leukaemia had advanced disease. They

concluded that the simplification of dosing by enteric coated tablets seemed worthwhile but adequate haematological control was still essential.

Walsh *et al.* (1954), Silverburg and Damashek (1952), Gellhorn *et al.* (1952) and Wright *et al.* (1952) agree with other reported observations that TEM is a useful agent in treating Hodgkin's disease, chronic leukaemia and lymphosarcoma, and again point out the dangers of irreversible pancytopenia. Walsh *et al.* (1954), Ellison *et al.* (1953) and Rosenthal and Rosenthal (1952), have reported on the effectiveness of TEM in polycythaemia vera. The latter authors followed thirty patients with this disease, for over a year. Twenty showed satisfactory symptomatic and haematological remissions with a mean duration of eight to nine months, following an average course of 30 mg. TEM.

The advent of TEM has been another contribution to the palliative treatment of certain chronic malignant diseases. Its effects as most agree, and as one would expect from its chemical structure, are similar to those of the nitrogen mustards. Its advantage lays in the fact that it may be administered orally and patients may therefore be treated as out-patients. A drawback is the variability of response in individual patients which may lead to serious toxic manifestations. The attempt to produce more consistent effects by

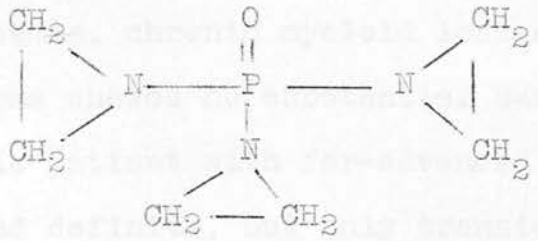
prescribing alkalies with TEM, or using enteric coated capsules has not, in the author's experience, altogether corrected this difficulty. Although toxic symptoms are less than the nitrogen mustards, TEM is a powerful bone marrow depressant, and in inexperienced hands is likely to result in marrow aplasia, with consequent infection and bleeding. Regular blood examinations must be carried out during treatment and further courses of TEM must not be given until the total leucocyte count is normal.

In view of the existence of equally satisfactory and less toxic compounds, it is unlikely that TEM will be used in the treatment of chronic myeloid leukaemia. Similarly the treatment of polycythaemia vera with x-rays, including radio-active phosphorus (P_{32}) is not likely to be displaced by TEM. Whether or not patients with advanced Hodgkin's disease, lymphosarcoma or chronic lymphatic leukaemia should be treated with TEM or the original nitrogen mustards depends upon the preference of the individual physician. The author suggests that the methyl-bis nitrogen mustard given intravenously is probably still the treatment of choice in advanced Hodgkin's disease, especially when a rapid therapeutic response is required. Chronic lymphatic leukaemia failing to respond to irradiation satisfactorily, in the presence of weight loss, anaemia, and a rising leucocyte count may be benefited by TEM AND this may

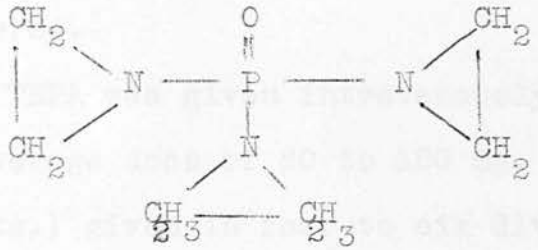
well be where the main usefulness of TEM lies.

D. Diethylene phosphoramide (DEPA), triethylene phosphoramide, and triethylene thiophosphoramide.

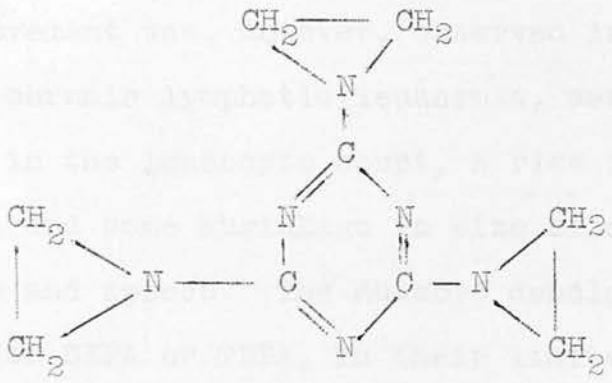
The encouraging results obtained with triethylene melamine in the treatment of leukaemia and related disorders led to the synthesis and testing against animal tumours of a number of other ethylenimine derivatives with nitrogen mustard-like activity. Included in this series were the phosphoramides, diethylene phosphoramide (DEPA), and triethylene phosphoramide (TEPA). Because of their ability to inhibit the growth of transplantable mouse leukaemia (Burchenal et al. 1952 a) and mouse and rat tumours (Buckley et al. 1951; Stock et al. 1952; Suguira and Stock 1952), and as the growth of these animal tumours were inhibited at smaller fractions of the LD_{50} as compared with triethylene melamine, they were selected for trial against human neoplasma. Preliminary pharmacological and pathological studies of the effect of these phosphoramides in various animals demonstrated a close similarity between their effects and those produced by triethylene melamine viz. bone marrow depression, lymphoid hypoplasia and damage to the intestinal mucosa. In view of their similarity in structure to triethylene melamine this is not altogether surprising. (Fig. 9)



Triethylene phosphoramide (TEPA)



Diethylene phosphoramide (DEPA)



Triethylene melamine (TEM)

Fig. 9.

Sykes et al. (1953) have described their clinical experiences with these two compounds. DEPA was given to nine patients with a variety of neoplastic diseases. The preparation was given intravenously in doses of 10 mg. daily up to an average total of 90 mg. (mg./Kg.) over a period of approximately three weeks. Patients with acute

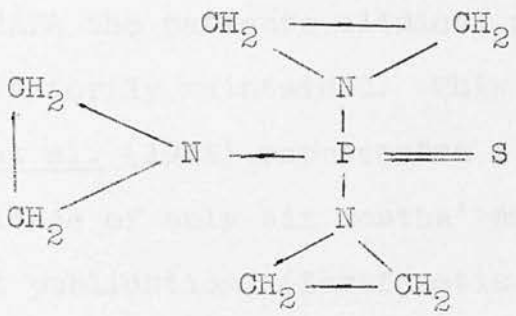
leukaemia, chronic myeloid leukaemia and lymphosarcoma showed no substantial benefit and only a single patient with far-advanced Hodgkin's disease showed definite, but only transient, improvement. The limiting factor in treatment was haematological and in one patient severe bone marrow depression occurred.

TEPA was given intravenously in pea-nut oil at an average dose of 80 to 120 mg. (1.2 to 2.0 mg. per kg.) given in four to six divided doses over a two week period, to twenty-eight patients. Results were generally disappointing. Definite subjective improvement was, however, observed in two patients with chronic lymphatic leukaemia, associated with a fall in the leucocyte count, a rise in haemoglobin level and some shrinkage in size of enlarged lymph nodes and spleen. The authors concluded that neither DEPA or TEPA, in their limited series, showed any advantage over the nitrogen mustards or TEM.

Farber et al. (1953 a) treated sixty-nine patients with TEPA. Those with acute or chronic leukaemia were not materially benefited and only one patient with Hodgkin's disease was improved. Occasional temporary improvement was recorded in patients with neuroblastoma and malignant melanoma.

The clinical results with DEPA and TEPA have shown them to be disappointing as chemotherapeutic

agents. More encouraging results, however, have been reported recently using another phosphoramide, triethylene thiophosphoramide (Thio-TEPA). This compound with a chemical structure shown below (Fig. 10) was found to suppress the activity of experimental rat leukaemia (Sparks et al. 1953, Shay et al. 1954) and to have a similar pharmacological action as nitrogen mustard, TEM, DEPA and TEPA. In a preliminary report Shay et al. (1953) showed thio-TEPA to produce effective remissions in both chronic myeloid and chronic lymphatic leukaemia.



Triethylene thiophosphoramide (Thio-TEPA)

Fig. 10

No nausea or vomiting or local reaction followed its intramuscular or intravenous administration. The usual parenteral dose was 2 to 10 mg. daily diluted in 10 mls. sterile saline when given intravenously, the total dose depending on the condition being treated as well as upon the haematological response. Over dosage resulted in bone marrow depression comparable to that observed

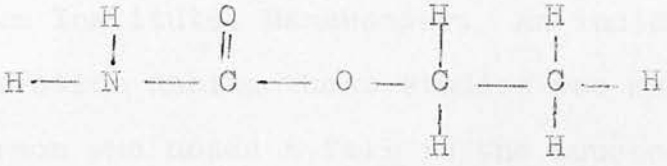
following treatment with other ethylenimines. Shay and his colleagues, however, found that such depression was readily reversed by withholding the drug. Thio-TEPA did not bring about remissions in acute leukaemia and lymphosarcoma was not materially benefited. One patient with Hodgkin's disease was much improved following Thio-TEPA. In both chronic myeloid and chronic lymphatic leukaemia a fall in leucocyte count with diminution in size of the spleen together with symptomatic improvement was shown to occur in ten out of the fourteen patients treated. By giving occasional maintenance doses of Thio-TEPA the patients clinical condition was satisfactorily maintained. This original report by Shay et al. (1953) represented a clinical experience of only six months' duration. In a recent publication, (Zarafonetis et al. 1955) the same group of workers described the effect of Thio-TEPA in a further nine cases of chronic myeloid leukaemia and two cases of chronic lymphatic leukaemia. One patient with chronic myeloid and one with chronic lymphatic leukaemia had been maintained in remission for approximately twenty-months, while th three additional cases of chronic myeloid leukaemia had been under treatment for over a year with excellent clinical and haematological results. The remainder showed good initial responses but three terminated in an acute myeloblastic phase within a

few months of the start of treatment.

One of the drawbacks of Thio-TEPA at the outset was that it had to be administered parenterally. Zarafonetis et al. (1955) have now described their results using an oral preparation in doses of 5 to 10 mg. daily as being just as effective. Results on a larger series of patients and the opinion of others using this new compound are awaited with interest. These early studies suggest it may be a useful compound in the management of patients with chronic leukaemia, and the absence of side-effects in contrast to the other members of this group of compounds, is an important additional recommendation.

URETHANE

Urethane (ethyl carbamate)



Urethane, first synthesised by Dumas in 1834, belongs to a group of carbamic esters known collectively as urethanes because of their structural similarity to urea. The term urethane has, however, been generally applied to ethyl carbamate itself. The commonly known pharmacological properties of urethane are those concerned with anaesthesia. Schmeideberg (1885) originally studied its narcotic properties in dogs, while Jolly was apparently the first to note the effectiveness of urethane as a hypnotic in clinical medicine. It, however, only enjoyed brief popularity as a hypnotic towards the end of the nineteenth century and was later replaced by the more dependable barbiturate group of drugs.

Urethane appeared as a chemotherapeutic agent as a consequence of experimental work on the growth inhibiting effects of various carbamic esters on animal tumours carried out by Haddow and Sexton (1946). Urethane having the most potent tumour inhibiting properties was selected for therapeutic trial in advanced cases of human malignant disease.

These clinical trials were begun in 1943, and were carried out initially at the Royal Cancer Hospital, London, and later at the Christie Hospital and Holt Radium Institute, Manchester. An incidental observation during these studies was made by Paterson who noted a fall in the leucocyte count during urethane therapy. This suggested its trial in patients with leukaemia. It should be mentioned, however, that the effect of urethane on the peripheral blood had been observed some years previously by Hawkins and Murphy (1925), during preliminary studies prior to determining the effect of X-rays on the leucocytes of rats and rabbits, using urethane as the anaesthetic. They demonstrated a fall in the circulating lymphocytes and an increase in polymorphonuclear cells following its injection into these animals, and suggested this effect of urethane may have been responsible for the difference of opinion concerning the effect of X-rays on lymphocytes, since many investigators at that time used urethane anaesthesia. In contrast to these observations in animals, Paterson et al. (1946) found in human subjects with breast carcinoma that the cells of the polymorphonuclear series were mainly affected.

The mode of action of urethane remains unknown. Paterson et al. (1947) has described it in the broadest sense as being related to "mitotic

disturbances induced in the leukaemic cells." Haddow and Sexton (1946) suggested it interfered with purine metabolism while Kirschbaum and Lu (1947), experimenting with leukaemic mice, suggested that urethane interfered with mitosis in the blast cells. Johnston (1942) had earlier noted that urethane had an anti-sulphanilamide effect similar to that of para-aminobenzoic acid and suggested the growth inhibiting properties of urethane were due to interference with utilization of some natural amine in cellular metabolism. Interference with cellular dehydrogenases or other enzyme systems has also been suggested, while Osgood and Chu (1948) studied the action of interference in marrow culture. An early increase in mitosis followed by nuclear fragmentation in the granular cells was observed.

Urethane has been shown to produce prolongation of survival time in both mouse and rat leukaemia (Engstrum et al. 1947; Murphy and Sturm 1946; Burchenal et al. 1948).

Paterson et al. (1946) first published the results of urethane therapy in human leukaemia. The initial dosage of the drug varied but was generally 3 - 4 Gm. daily, given orally, in a solution of chloroform water and syrup of orange. Nineteen cases of myeloid leukaemia were described of which eighteen were chronic, the symptoms having

been present from one to thirty-three months. The other case was regarded as one of acute myeloblastic leukaemia. The mean period of observation was six months and six patients subsequently received x-ray treatment. The leucocyte count generally fell to 20,000 per c.mm. in eleven to thirty-six days. The differential leucocyte count improved as the total white cells diminished in number and the haemoglobin value rose by an average of 16.6 per cent. in ten out of thirteen patients. Marked diminution in size of the spleen was observed, but anorexia, nausea, drowsiness, vomiting and occasional diarrhoea were unpleasant side-effects. Bone marrow depression was mentioned as the most serious complication of urethane therapy, and one patient was reported as dying with signs and symptoms of aplastic anaemia. Clinical and haematological remissions were maintained for periods of two to six months. The response of chronic lymphatic leukaemia to urethane was less satisfactory and much more variable. This variation was shown not only in the dose required, but also in the time necessary for an adequate reduction in the white cell count. The smallest total dose given was 8 G., which lowered the white cell count from 48,000 to 7,000 per c.mm. in nine days. At the other extreme, however, 360 G. failed to reduce a white cell count of 750,000 per c.mm. to normal limits after sixty-three days. On the

whole the average dose of urethane was larger and the time necessary for an adequate reduction in white cells varied from nine to forty-seven days. Five of thirteen patients were, however, classed as showing considerable improvement following urethane therapy. By comparing the effect of urethane with X-rays they concluded that the response of the disease to urethane was similar to that following irradiation. Previous X-ray therapy did not interfere with the later effectiveness of urethane, and X-rays were found to be active after urethane therapy. Thirteen patients with various types of malignant neoplasms treated with urethane were also described by Paterson and her colleagues. Only three, with carcinoma of the breast, showed any effect of treatment, a temporary diminution in tumour masses being observed. Four patients with various malignant lymphomas showed a slight temporary improvement following treatment. In a later publication, Paterson et al. (1947) analysed the results of treating thirty-eight patients with chronic myeloid leukaemia. Two-thirds were considered to obtain immediate benefit and the majority showed a rise in haemoglobin concentration following treatment. Approximately half the twenty-six patients with chronic lymphatic leukaemia showed general clinical improvement and a rise in haemoglobin, while three-quarters showed a fall in

white cells with an associated improvement in the distribution of white cells in the peripheral blood. Excessive dosage in either disease was again pointed out as liable to lead to bone marrow aplasia.

The initial observations by Paterson and her colleagues were verified by several other workers. Creskoff *et al.* (1948) administered 4 G. of urethane daily to twenty-four patients with leukaemia. Chronic myeloid leukaemia was benefited more than the lymphatic variety and acute leukaemia was found to be uninfluenced by the drug. Nausea was the commonest toxic symptom, occurring in 50 per cent. of patients. Vomiting, drowsiness, sweating and diarrhoea also occasionally occurred. Two patients developed bone marrow aplasia. Intravenous administration of urethane was described as being both highly effective and more agreeable in that nausea was prevented.

Watkins *et al.* (1948) also noted temporary remissions in patients with chronic myeloid leukaemia, similar to those occurring after radiotherapy. Maintenance therapy was recommended in an attempt to prolong remissions, the daily dose being highly variable but in some 0.5 to 1.0 G. was found adequate. The longest period of observation was eight months. Gastro-intestinal symptoms were the main toxic side-effects, the severity of which were found to be associated with the size of dose administered.

Hirschboeck et al. (1948) prescribed urethane

0.5 G. in gelatin capsules, and also, in an attempt to reduce alimentary upsets, as enteric coated capsules each containing 0.5 G. urethane. All four patients with chronic myeloid leukaemia showed clinical and haematological improvement, with reduction in spleen size, fall in leucocytes and improvement in haemoglobin level. Five out of eight patients with chronic lymphatic leukaemia showed variable degrees of improvement. Acute leukaemia and Hodgkin's disease were uninfluenced by treatment.

An analysis of ninety patients with various neoplastic diseases recorded in the literature as having been treated with urethane, was made by Berman and Axelrod (1948). They concluded that the best results were obtained in chronic myeloid leukaemia followed by chronic lymphatic leukaemia and androgen-independent carcinoma of prostate. Huggins et al. (1947) were also of the opinion that urethane was beneficial in prostatic cancer. The most important toxic manifestations were hypoplastic or aplastic anaemia, although they considered hepatocellular damage may result from over dosage with urethane. Goodman and Lewis (1946) noticed a dramatic disappearance of skin secondaries in a patient with an anaplastic carcinoma, probably arising in a bronchus, as a result of urethane therapy. Webster (1947) although agreeing that

urethane was of value in myeloid leukaemia stressed the dangers of aplastic anaemia and recorded a fatality due to same. Leucutia (1948), Bedinger et al. (1947), Heilmeyer (1948) and Moeschlin (1947) have also made contributions to the literature on the subject of urethane treatment of leukaemia. Heilmeyer claimed occasional good responses in patients with acute leukaemia and observed a remission lasting two years in one patient with acute myeloblastic leukaemia. Wilkinson (1953) considered that urethane was inferior to either irradiation treatment or nitrogen mustard in the treatment of chronic leukaemia, and stressed that adequate haematological control was necessary on account of the dangers of aplastic anaemia, agranulocytosis and thrombocytopenic purpura.

Loge and Rundles (1949) treated four patients with multiple myeloma with urethane for eight to ten weeks in total doses of 120 to 290 G., with striking benefit. A decrease in myeloma cells occurred and the characteristic biochemical abnormalities became less pronounced or disappeared. Serial x-rays of the skeleton shoed no progression in the destructive lesions but there was little evidence of recalcification during the four months following treatment. In a later report, Rundles and Reeves (1950) described the effect of urethane in a series of twenty-four patients observed over a two year

period. In addition to a disappearance of pain, fever and acute symptoms and a decrease in anaemia, recalcification in bones was occurring in some patients by the sixth month after institution of therapy. Harrington and Moloney (1950) found urethane to be more beneficial in patients with a more chronic type of multiple myeloma. In six of their eleven cases both clinical and biochemical improvement occurred coinciding with a diminution of myeloma cells in the bone marrow. They recommended a daily intake of 3 to 6 G. of urethane for the majority of patients although individual variability accounted for a dose range of 1 to 12 G. daily. Wilkinson (1953) has also reported beneficial effects in multiple myelomatosis after urethane therapy and more recently Innes and Rider (1955) have obtained good results using R 151 in conjunction with urethane, and suggested a synergistic action between the two compounds.

The main usefulnessof urethane has undoubtedly been in the chemotherapy of chronic myeloid leukaemia, and to a lesser degree in chronic lymphatic leukaemia. With initial doses of 3 to 4 G. per day followed when the leucocyte count has fallen to 40 to 50,000 per c.mm. by a maintenance dose of 0.5 to 2 G. daily, prolonged remissions have resulted in many patients with chronic myeloid leukaemia. An important advantage of urethane is its ready availability and

low cost. It has, however, several disadvantages. The remissions are of course only temporary and urethane is of little value in the late, terminal stage of the disease. It causes nausea in at least 50 per cent. of patients and may give rise to upsetting vomiting and other alimentary disorders. More serious are the effects on the bone marrow and several fatalities due to marrow aplasia are recorded. On this account, regular blood examination is essential during treatment. Urethane administered intravenously as a 20 per cent. solution may result in fewer side effects, but is generally impracticable. Although urethane is still widely used it seems likely that newer and less toxic preparations such as myeleran and possibly 6-mercaptopurine will gradually replace it.

Only isolated reports of the merits of urethane in carcinoma have appeared and these mainly relate to prostatic cancer. It is now accepted that urethane does not hold any significant place in cancer therapy.

One place in therapeutics urethane is likely to hold, however, is in the treatment of multiple myeloma. Subjects with disseminated disease, generally so refractory to treatment, have been shown to obtain relief from large doses of urethane. Symptoms disappear, fever settles, and biochemical abnormalities may revert to normal and myeloma cells

diminish in number. In some recalcification of bone has been described (Rundles and Reeves 1950). There seems little doubt that urethane has obtained a place along with radiotherapy, stilbamidine and pentamidine in the management of patients with multiple myeloma.

DEACETYLMETHYL-COLCHICINE
(Demecolcine; Colcemid)

One of the most recent chemical agents to be used with some success in the treatment of myeloid leukaemia is a derivative of colchicum, deacetylmethyl-colchicine, now marketed in Great Britain as Colcemid.

Colchicine has for several years been known to arrest mitotic division of cells in general in the metaphase (Lits 1934; Brues and Cohen 1936; Ludford 1937) and to inhibit the growth of certain animal tumours (Lits et al. 1938; Ludford 1945). The action on normal cell division and the upsetting side effects associated with its administration have gone far in preventing the therapeutic use of colchicine in human malignant disease. In 1950 Santavy and Reichstein isolated a series of new alkaloids, from the seeds, blossoms and bulbs, of the meadow saffron =Colchicum) which were less toxic. This decrease in toxicity they observed was also accompanied by a diminution of the antimitotic action. One of these compounds, named by Santavy and Reichstein as "Substance F", was investigated by Bock and Gross (1953) who noticed that it had a selective action on myeloid cells and in chronic myeloid leukaemia resulted in a fall in white cells associated with an increase in the number of metaphases. Moeschlin, Meyer and Lichtman (1953) showed in animal experiments that this compound,

"Substance F", or now known as Demecolcine, was thirty times less toxic than colchicine and supported the observations of Bock and Gross (1953) that it had a marked elective depresser effect on granulocytes. They also found it to be effective in chronic myeloid leukaemia and by the use of a daily maintenance dose were able to maintain the blood picture satisfactorily. Chronic lymphatic leukaemia did not respond so well and no success was obtained in patients with multiple myeloma, Hodgkin's disease, lymphosarcoma, carcinoma and sarcoma. Bock and Gross (1954) reported their results of treatment in twenty-seven patients with leukaemia or malignant tumours. Chronic myeloid leukaemia was the only disease to show a good response to treatment and acute leukaemia and chronic lymphatic leukaemia were not benefited. They observed no subjective complications but transient loss of hair, and, in five patients, a temporary herpetiform stomatitis developed following a marked drop in the leucocyte count. Piney (1955) reported a good response in one patient. He also observed a loss of hair during treatment but regrowth occurred in spite of continued therapy. Wilkinson (1955) also observed good results in five patients out of seven.

The usual dosage of Demecolcine or Colcemid recommended is 5 to 10 mg. daily at the outset

followed later as the leucocyte count approaches normal, by 2 to 5 mg. daily as maintenance therapy. The improvement noted has been not only haematological but also clinical. The general condition improves, the size of the spleen diminishes and other evidence of the disease also regresses. Larger groups of patients must be studied, however, before the value of this new compound can be fully assessed.

SUMMARY.

1. The historical background to the chemotherapy of leukaemia has been reviewed.

2. A comprehensive review of the chemotherapy of acute leukaemia has been presented. The folic acid analogues, ACTH and cortisone, and other analogues of nucleic acid precursors have been discussed.

3. The subject of spontaneous remissions with reference to incidence, age, type of leukaemia, duration and the possible relationship of predisposing factors has been investigated. The possibility that spontaneous remissions may follow adrenal stress was suggested. The occurrence of spontaneous remissions is rare and therefore should not interfere with the evaluation of chemotherapeutic agents.

4. Possible mechanisms of the development of resistance to the folic acid analogues have been discussed.

5. Recent trends in the search for new agents effective in acute leukaemia have been reported.

6. The chemical compounds available for the treatment of chronic leukaemia and allied disorders have been reviewed.

7. The toxic effects of the nitrogen mustard group of compounds on haemopoiesis have been studied in eighty patients with Hodgkin's disease. X-rays and the disease itself were found to produce terminal bone marrow depression, but the nitrogen mustards were the main cause.

8. From a personal study of thirty patients with polycythaemia vera, treatment with radio-active phosphorus yielded good results, and the absence of side effects suggested that P32 was still more satisfactory than present day chemotherapy.

METABOLIC STUDIES.

STUDIES IN THE METABOLISM OF 4 AMINO-N^{10} METHYL PTEROYL-GLUTAMIC ACID (AMETHOPTERIN) IN NORMAL AND LEUKAEMIC MICE

A. Tissue distribution of Amethopterin in Normal Mice following Intravenous Injection.

Probably as a result of the initial lack of a satisfactory simple method for assaying amethopterin directly little has been reported on the fate of this compound following its administration either to human subjects or to the experimental animal. Swendseid (1952) using a back titration modification of the standard method for microbiological assay of folic acid (PGA) has reported on the excretion of aminopterin (4-amino-pteroylglutamic acid) during treatment of leukaemic patients. The development by Burchenal et al. (1951a) of a simple disc method for determining the concentration of amethopterin in blood and urine suggested that such a technique might be of assistance in the study of the metabolism of this compound in animal tissues. As a preliminary to these studies, it was decided to attempt to determine the distribution of amethopterin in the tissues of the normal mouse following intravenous injection.

Materials and Method

Young mice of the inbred AkM stock, weighing approximately 20 G. each, were used. All the mice were maintained on a standard diet of Purina

Laboratory Chow prior to and during the experiment. Water was allowed ad libitum throughout the experiment. Each mouse was given a single injection of amethopterin at a dose of 0.5 mg./kg. by the intravenous route (into the tail vein). This was considered an optimum dose, producing, as demonstrated later, readily assayable amounts in the tissues and yet being well below the maximum tolerated daily dose, and approximately 1/200th of the acute LD_{50} dose (Ferguson et al. 1950). Mice were sacrificed at 15, 30, 60, 120, 180 and 240 minutes after injection. Under ether anaesthesia, blood was collected in a heparinised syringe by severing the axillary vessels, the animal being finally killed by decapitation. In those mice injected one, two, and four hours previously the liver, spleen, kidneys and lungs were then removed and a sample of muscle taken from the hind leg. Each specimen was weighed and homogenised in distilled water (10 or 20 mls. depending on size) immediately after removal.

Assay of Homogenate for Amethopterin Content.

The samples were divided into two groups, (A) and (B); Sample (A) was unboiled, and (B) was boiled immediately for five minutes to destroy autolytic enzymes. The samples from group (B) were centrifuged and the supernatant assayed. In Group (A) the whole homogenate was assayed. Suitable

dilution was carried out in both groups, following which 0.09 ml. was delivered to paper discs (Schleicher and Schuell penicillin assay discs, 12.7 mm. diameter) placed on Difco folic assay medium containing 1 mγ/ml. of PGA and inoculated with Streptococcus faecalis (ATCC 8043) in a Petri dish. Following incubation over-night the zones of inhibition were measured and calculated against standard zones of known concentration of amethopterin.

Details of Preparation of Assay Medium, Amethopterin Standards, and Assay of Test Solutions. (After Burchenal et al. 1951a)

75 G. of Difco Folic Acid Assay medium, 30 G. agar, and 2μg. of crystalline pteroylglutamic acid were added to 2,000 ml. of distilled water. This was heated to dissolve the agar, autoclaved 15 minutes at 15 lb. pressure, cooled to 45°C. in a water bath, and inoculated with 2 ml. of a twenty-four hour old broth culture of Streptococcus faecalis (ATCC 8043). After shaking thoroughly to mix the organisms throughout the media, 20 ml. aliquots were pipetted into 100 specially pressed flat bottom Petri dishes, arranged on a level surface, and allowed to solidify. These plates were then stored in the refrigerator at 4°C. and used when required one to ninety-six hours later. Using a specially purified sample of amethopterin (Seeger et al. 1949), standard tubes were prepared

containing 5 ml. each of a solution of the compound at a concentration of 1,000 mγ/ml. These stock tubes were frozen at -40°C., and a tube removed when new series of standards were required. By diluting this stock solution with distilled water standards containing 5, 10, 15, 20, 25, 30 and 35 mγ/ml. were prepared. New standards were prepared for each experiment as follows. Four Petri dishes containing the assay medium inoculated with Streptococcus faecalis were taken and on each were placed in turn four penicillin assay discs. 0.09 ml. of one of the prepared standard dilutions was delivered with an automatic penicillin pipette to each disc immediately it was placed on the assay medium. Standard dilutions of 5, 10, 15 and 20 mγ/ml. were placed on discs on each of two plates, and dilutions of 10, 25, 30 and 35 mγ/ml. on the remaining two plates. The standard was duplicated for accuracy. For further accuracy, on to a single disc on each test plate was delivered 0.09 ml. of a standard solution of 10 mγ/ml. amethopterin, three other discs being inoculated with the test solutions of various dilutions - ranging from 1-5 to 1-40 depending on the tissue and its expected amethopterin content as determined by preliminary experiments. Discs were inoculated with full strength serum and dilutions of 1-3 and 1-10. The standard and test plates were then

incubated for eighteen hours and the diameter of the inhibition zone read with a Bermuda Colony Counter (American Optical Co.) containing an etched glass scale graduated in millimeters. A standard curve was obtained by plotting the diameter of the zone against the concentration of the standard solution. The content of amethopterin in the test solutions was then calculated from the standard curve.

Results

Serum Levels. Fig. 11 shows the level of amethopterin in the serum in six series of normal mice. It will be observed that after the first thirty minutes following injection the levels are closely related. In all cases the serum concentration has reached or approximated zero in four hours.

Tissue Concentration. Table 9 shows the concentration of amethopterin in the tissues. It will be noted that much higher concentrations of amethopterin were assayable in the boiled specimens (B) in the case of the liver and to a lesser extent in the spleen. This can be readily explained by the fact that enzymatic freeing of folic acid and citrovorum factor (C.F.) from their conjugates was prevented by boiling. In the unboiled specimens (A) on the other hand, incubation resulting in freeing of folic acid and C.F. which reversed some or all

SERUM LEVELS OF AMETHOPTERIN IN NORMAL, AK4 AND AK4R MICE AFTER I.V. INJECTION AT 0·5mg./kg

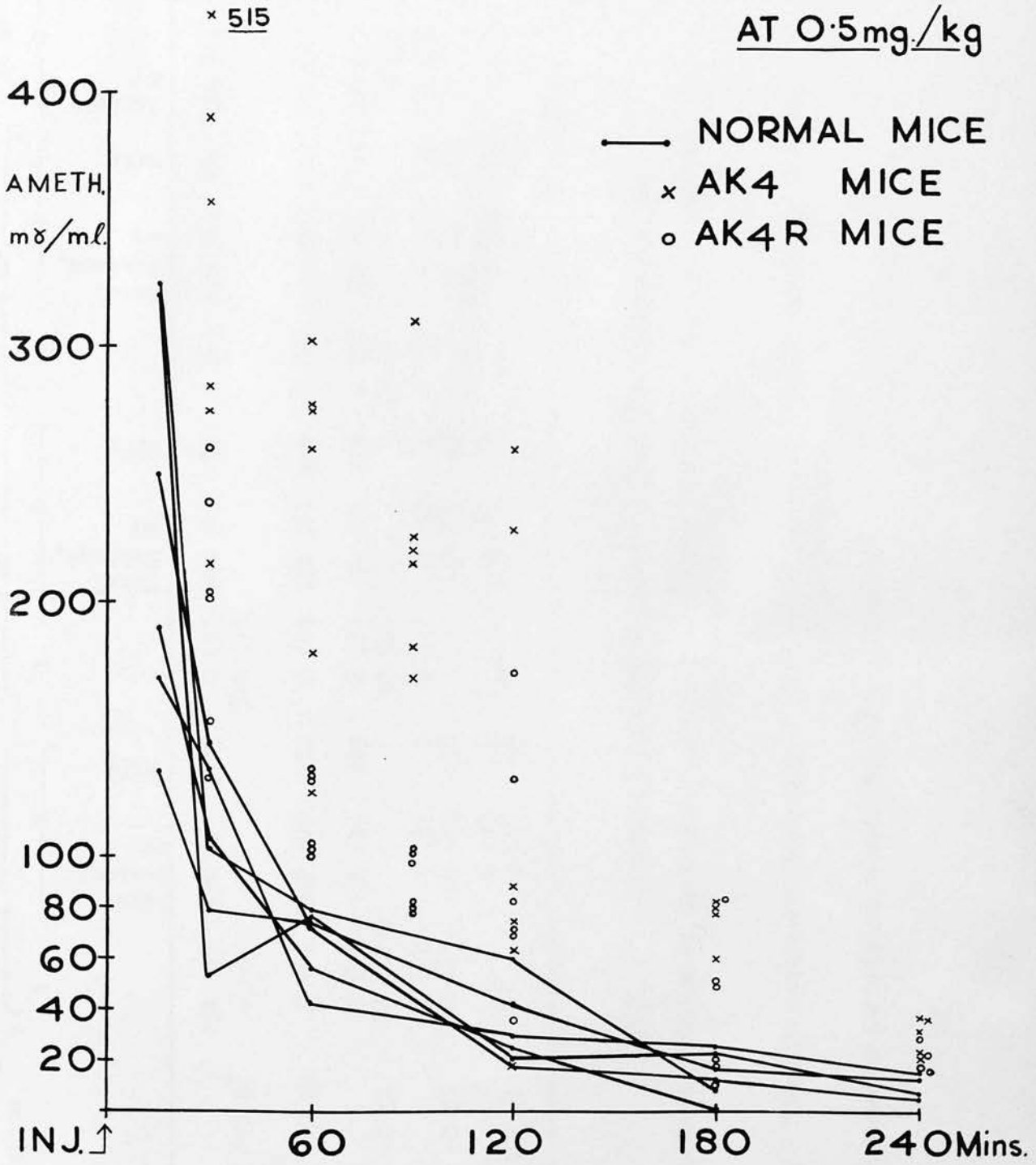


FIG. 11.

Time following injection	1 hr			2 hr			4 hr			24 hr	
	A	B		A	B		A	B		B	
		Total Content, mγ	mγ/g		Total Content, mγ	mγ/g		Total Content, mγ	mγ/g	Content, mγ	mγ/g
Liver (1.0 - 1.3 g)	424 (6)	1400 (6)	1215	0 (3) 369 (3)	868 (6)	734	0 (6)	628 (6)	594	449 (4)	410
Kidneys (.25 - .35 g)	0 (4)	227 (6)	712	0 (4)	162 (6)	562	0 (4)	120 (6)	412	127 (4)	384
Spleen (.07 - .1 g)	0 (3) 33 (1)	20 (6)	225	0 (3) 22 (1)	26 (6)	273	0 (3) 25 (1)	20 (6)	200	200 (1) 0 (3)	210 0
Lung (.15 - .2 g)	0 (3) 90 (1)	0 (3) 16 (3)	0 96	0 (4)	0 (5) 15 (1)	0 72	0 (4)	0 (5) 18 (1)	0 84	0 (4)	0
Muscle (.2 - .25 g)	—	0 (3) 5 (1)	0 25	—	0 (4)	0	—	0 (3) 10 (1)	0 40	0 (4)	0

TABLE 9. Amethopterin in Normal Tissues after Intravenous Injection

Average Concentration of Amethopterin in Tissues of the Mouse Following i.v. Injection at .5 mg/kg Expressed in mγ.

A - Unboiled specimens (mγ/organ). B - Boiled specimens (total content in mγ/organ and conc. in mγ/g).

Figures in parentheses denote No. of mice tested.

of the amethopterin activity. This was significant as it was an indication of the folic acid and CF content of the tissue and suggested that the highest concentration was present in the liver and kidneys. Owing to the small quantity of free folic acid and CF present in tissues (Dietrich et al. 1952) it was presumed that the boiled specimens gave an accurate estimate of the amount of amethopterin present in the tissues. It can therefore be observed that the majority of the compound was present in the liver followed in sequence by the kidney and spleen, while no significant quantities were found to be present in the lung after one hour and at no time in the muscle.

In view of the retention of relatively large quantities of amethopterin in the liver and kidneys after four hours at which time it had approximated zero in the serum, the amount of the compound present twenty-four hours after injection was determined. As may be seen, readily assayable quantities, little different from those found after four hours, remained.

B. Tissue Distribution of Amethopterin in Leukaemic Mice.

Young mice of the inbred Akm stock (as used in the previous investigation) were injected intraperitoneally with 0.1 ml. of a saline suspension of leukaemic spleen so diluted that 0.1 ml. contained

one million cells. Leukaemia AK_4, an acute strain causing the death of the animal in an average time of twelve days was used as amethopterin was known to have a definite therapeutic effect against this particular strain of leukaemia (Burchenal et al. 1949 b). In addition other mice of the Akm stock were given AK_4R leukaemia, a strain of leukaemia made resistant to amethopterin by passing leukaemic cells generation after generation through amethopterin treated mice (Burchenal et al. 1950 a). Two groups of mice were therefore prepared, one with amethopterin sensitive leukaemia and the other with amethopterin resistant leukaemia. The possible analogy is the human subject with leukaemia which is sensitive to treatment, and the one which has developed resistance to treatment with amethopterin. Both groups of mice had an advanced degree of leukaemia when used in this experiment, as evidenced by a markedly enlarged and easily palpable spleen, and a high leucocyte count.

The experimental procedure was the same as for normal mice, each mouse being given 0.5 mg./kg. amethopterin intravenously and thereafter sacrificing the animals at various intervals and assaying the tissues for their amethopterin content. Total concentration of the antimetabolite in the tissues was obtained by boiling the homogenates to prevent freeing of PGA and CF. from their conjugates.

Results.

1. Ak_4 Leukaemic Mice (Sensitive).

As may be observed in Fig. 11 the serum levels of amethopterin in the Ak_4 mice is consistently higher over the four hour test period than those of normal animals.

Amethopterin could be detected in all tissues assayed throughout the four hour period (Table 10) In contrast to the normal animals higher concentrations of the antimetabolite were present in the spleen and readily assayable quantities were present in the lungs.

2. Ak_4R Leukaemic Mice (Resistant Strain).

The findings in this group of mice were similar to those of the Ak_4 strain (Table 10)(Fig. 11) The serum levels were generally higher than the normal animals and the antagonist was demonstrable in all tissues over the four hour period. Although the level of amethopterin in the serum during the first two hours appeared lower in the mice with Ak_4R leukaemia, no obvious difference in concentration of amethopterin in the tissues of mice with the sensitive and amethopterin resistant strain of leukaemia could be detected.

Discussion

It has been shown by the experiment on normal mice that following the administration of amethopterin it became concentrated in folic acid

	1 Hour		2 Hours		4 Hours	
	Ak_4	Ak_4R	Ak_4	Ak_4R	Ak_4	Ak_4R
Liver (2 - 2.5 G.)	1397 (4)	1170 (4)	814 (4)	748 (4)	542 (4)	599 (4)
Kidneys (.35 - .45 G.)	213 (4)	197 (4)	168 (4)	163 (4)	167 (4)	126 (4)
Spleen (.35 - .6 G.)	142 (4)	143 (3)	139 (3)	127 (4)	127 (4)	116 (4)
Lung (.2 - .3 G.)	97.5	106 (4)	31 (3)	46 (4)	24 (3)	63 (4)

TABLE 10 Average concentration of Amethopterin in tissues of Ak_4 and Ak_4R mice following i.v. injection at 0.5 mg./kg. expressed in mg/organ (boiled specimens).

Figures in parenthesis denote number of mice tested.

and CF containing tissues. In addition it was observed that the highest concentration of amethopterin was found to be present in the liver followed in order by the kidney and spleen. This was of interest in view of the fact that a similar distribution of folic acid and CF has been demonstrated in the rat, guinea pig and chick tissues by direct microbiological assay (Dietrich et al. 1952). This tissue selectivity of amethopterin was not unexpected in view of the known properties of this compound, and supported the view that the antimetabolite replaces the metabolite, in this case amethopterin, and folic acid and CF respectively, in the enzyme systems in which they are associated. It also was additional evidence that the main action of the folic acid analogues in suppressing the acute leukaemic process is by interfering with the utilisation of folic acid and CF. It was suggested from this study that in normal mice, following an injection of amethopterin, most of the compound is rapidly excreted or metabolised within four hours, the serum level by that time having reached or approximated zero. The remainder is concentrated in folic acid and CF containing tissues. The continued presence of amethopterin activity in these tissues after twenty-four hours or more indicates a failure of the mouse tissues to complete the

metabolism of amethopterin.

The intravenous administration of amethopterin in mice with a strain of leukaemia (Ak_4) known to be sensitive to amethopterin resulted in a more widespread distribution of the compound in the tissues and higher concentrations in the serum as compared with the normal tissues. Although the amethopterin content of the liver and kidney showed little change from that in the normal animal - where the concentration was found to be the highest - the concentration in the leukaemic spleen was six times that observed in the normal. Similarly, readily assayable quantities of amethopterin were present in the leukaemic lung after four hours. The suggested inference was that in the leukaemic animal a more widespread distribution of amethopterin occurred possibly as a result of the affinity of the leukaemic cell for amethopterin. It should, however, be remembered that the mice had advanced leukaemia and the possibility of the higher serum levels of amethopterin being due to impaired renal function cannot entirely be excluded. Burchenal et al. (1951a) have demonstrated the effect of renal function on serum levels of amethopterin in human subjects. In the present study, however, if retention of the drug had occurred due to renal dysfunction one would have expected to find a higher concentration in the liver and kidneys,

where as previously mentioned the total content of amethopterin was no higher than in the normal animal.

Amethopterin was found to concentrate in the tissues of mice with Ak_4R (amethopterin-resistant) leukaemia in a manner similar to that observed in the sensitive leukaemia. No indication that amethopterin was altered in any way by the resistant cell was observed.

C. Retention of Amethopterin in Animal Tissues.

In the previous experiment it was shown that following the intravenous administration of amethopterin in normal mice at a single dose of 0.5 mg./kg. the majority of the compound was rapidly excreted or metabolised within approximately four hours. The remainder was found to be concentrated mainly in those tissues (liver and kidneys) containing relatively high concentrations of folic acid and citrovorum factor. The detection of the anti-metabolite in significant quantities (approx. 6 per cent. of the injected dose) in these organs twenty-four hours following injection suggested a delay or failure in excretion, or in completion of its metabolism. As a consequence of this finding it was considered of interest to attempt to determine for what period of time following the administration of amethopterin, using various dosage schemes, assayable amounts of antimetabolite remained in the tissues in the normal mouse. Also by implantation of amethopterin resistant leukaemic cells intraperitoneally into normal mice, previously given amethopterin, whether the resistant cell affected the concentration and retention of the antagonist in the tissues. Finally metabolic studies in human subjects were performed in an attempt to detect any differences in the metabolism of amethopterin in the non-leukaemic,

leukaemic, and leukaemic patient who had become resistant to amethopterin therapy.

(1) Normal Mouse Tissues.

Materials and Methods.

The mice used in this experiment were of the inbred Akm stock weighing approximately 20 G. All were young mice of approximately the same age. Groups of mice of different sexes were used. Previous to and throughout the experiment the mice were fed the standard Purina Laboratory Chow diet.

For convenience of description the mice used in this study are best divided into five groups.

Group 1 (31 mice) received amethopterin intraperitoneally at a dose of 3 mg./kg. on alternate days for a total of three injections. Thereafter mice were sacrificed at intervals as shown in Table up to twenty-one days and blood collected and homogenates of the liver, kidneys, spleen and lungs prepared in a manner described in the previous experiment. All homogenates were boiled immediately for five minutes to inactivate conjugase. Each specimen was thereafter centrifuged and the supernatant assayed for amethopterin activity by the plate assay technique also described previously.

Group 11 (13 mice) received 1 mg./kg. intraperitoneally on alternate days for a total of three injections.

Group 111 (10 mice) received a single intra-

peritoneal injection of 3 mg./kg.

Group 1V (12 mice) was given 1 mg./kg. intravenously on alternate days for three injections.

All mice in Groups 11, 111, and 1V, were sacrificed at intervals over the twenty-one day period and the tissues assayed for amethopterin as for Group 1.

Group V (18 mice) were each given amethopterin as for Group 1. Seventy-two hours after the last injection two mice were sacrificed and the tissues assayed for the presence of anti-metabolite. Thereafter at daily intervals all the remaining mice were given citrovorum factor intraperitoneally at doses ranging from 0.5 to 30 mg./kg. Mice were killed daily, twenty-four hours after the last injection of CF, and tissues assayed for amethopterin as outlined previously.

Results.

Control mice not injected with amethopterin showed no evidence of any inhibitory substance in the tissues.

Table 11 shows the concentration of amethopterin in mγ/G. of wet tissues in the organs of mice of Group 1 - 1V. Throughout the twenty-one day period mice in these groups showed the presence of amethopterin in high concentration in the liver and kidneys. The levels remained remarkably constant, and although there was some diminution in

	Group	Days after last injection 1	2	3	4	7	10	12	14	17	21
Liver	I	338(3)	345(4)	410(3)	305(3)	384(2)	—	482(2)	427(2)	268(2)	426(3)
(mγ/g)	II	294(4)	—	512(2)	—	408(2)	275(2)	432(1)	410(3)	—	375(2)
(1.0 - 1.3 g)	III	332(2)	315(2)	—	309(1)	330(1)	368(1)	—	315(1)	—	284(2)
	IV	380(2)	—	412(2)	—	—	340(1)	350(1)	387(1)	261(1)	320(1)
Kidneys	I	430(1)	453(2)	405(2)	430(1)	279(2)	—	327(2)	233(2)	218(2)	238(3)
(mγ/g)	II	380(2)	320(2)	—	—	331(2)	260(1)	—	212(2)	—	101(2)
(.25 - .35 g)	III	—	—	—	290(1)	219(1)	231(1)	—	156(1)	—	126(2)
	IV	—	—	—	—	—	—	—	285(2)	380(1)	226(1)
Spleen	I	0(1)	219(2)	140(1)	0(1)	0(2)	—	0(2)	0(2)	0(2)	420(1)
(mγ/g)				0(1)							0(2)
(.07 - .1 g)	II	0(2)	—	0(2)	—	0(2)	0(1)	—	0(3)	—	0(2)
	III	—	—	—	0(1)	0(1)	0(1)	—	0(1)	—	0(1)
	IV	—	—	—	—	—	—	—	0(2)	630(1)	0(1)
Lungs	I	240(1)	80(2)	0(2)	0(1)	0(2)	—	0(2)	0(2)	0(2)	150(1)
(mγ/g)											0(2)
(.15 - .2 g)	II	150(1)	—	0(2)	—	0(2)	0(1)	—	0(3)	—	0(2)
		0(1)									
	III	—	—	—	0(1)	0(1)	0(1)	—	0(1)	—	0(1)
	IV	—	—	—	—	—	—	—	0(2)	475(1)	0(1)

TABLE II. Amethopterin Levels in Normal Mice

Concentration of Amethopterin (mγ/g) in the Liver, Kidneys, Spleen and Lungs of Mice over a 3 Week Period.

Group I. Amethopterin 3 mg/kg X 3 i.p. alternate days. Group II. 1 mg/kg X 3 i.p. alternate days. Group III. 3 mg/kg i.p. single inj. Group IV. 1 mg/kg X 3 intrav. alternate days.

the concentration in the kidney over the test period, there was little evidence that the compound was being metabolised or excreted. The size of the dose and mode of administration bore no relationship to the amethopterin concentration. Similar results were observed following a single injection of 0.5 mg./kg. and also with a single dose of 20 mg./kg.

Apart from small quantities of amethopterin of doubtful significance detected in the spleen and lung within seventy-two hours after the last injection, no assayable quantity was present in the lung or spleen during the twenty-one day period.

The serum was of interest in that amethopterin was recovered in small amounts in all groups at intervals throughout the period of the experiment. The serum levels of Group 1 mice are shown in Fig. 12 and similar levels were detected in Groups 11 to 1V. Following the administration of CF to mice in Group V the serum level of amethopterin rose to significantly higher levels. In order to be assured that the serum level was relatively free of CF it was not tested for amethopterin in the majority of instances until twenty-four hours following the injection of CF. The actual amount of CF administered did not appear to bear any relationship to the height of the amethopterin serum level.

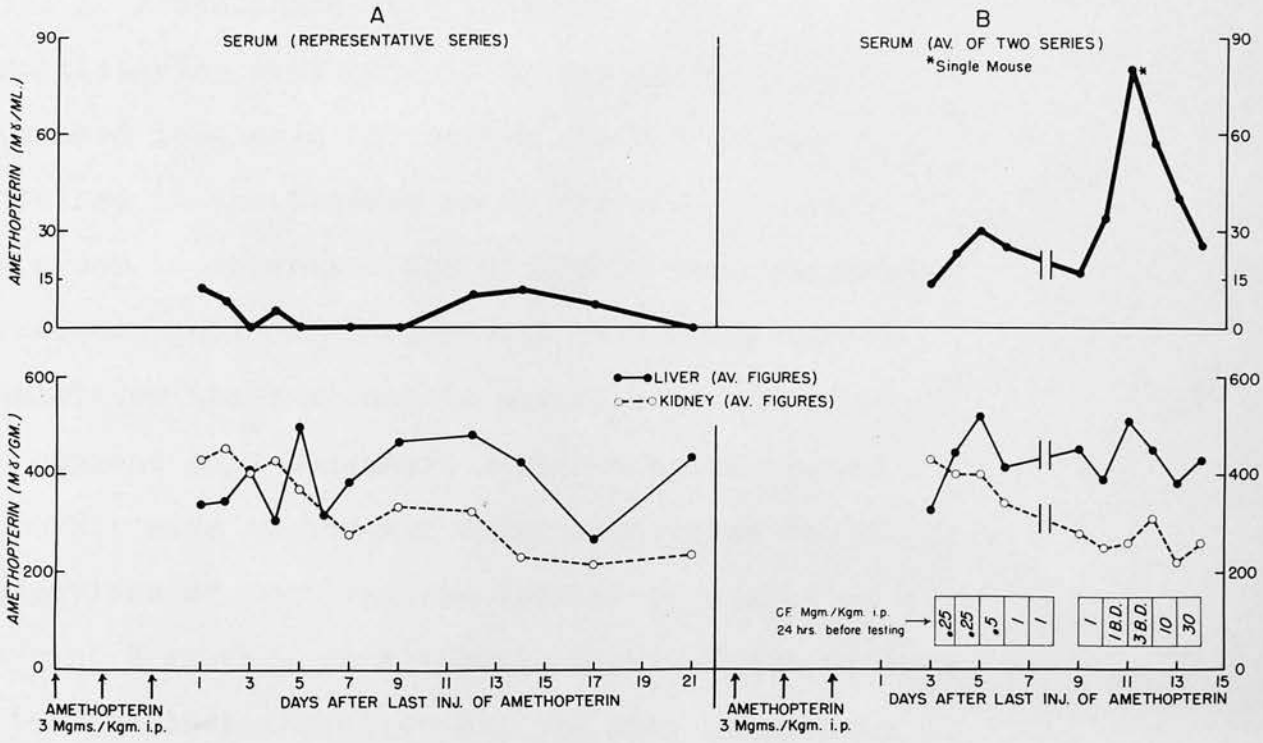


FIG. 12. Concentration of amethopterin in the serum, liver and kidneys of two groups of mice. (A) Amethopterin 3 mg/kg X 3 i.p., alternate days. (B) As for (A), followed by daily i.p. inj. of citrovorum factor.

(2) Tissues of Mice with amethopterin-resistant leukaemia (Ak_4R).

Materials and Method.

In view of the short survival time of mice with Ak_4R leukaemia it was impracticable by administering amethopterin to the animal with advanced leukaemia to observe whether it was retained in the tissues as in the normal animal. In order to determine the effect of the resistant leukaemic cell on amethopterin it was decided to administer the compound to normal mice and later transplant Ak_4R leukaemia to these animals.

Six mice of the Akm breed were given three injections of amethopterin intraperitoneally at a dose of 3 mg./kg. on alternate days. Fourteen days after the last injection all the mice were given a suspension of leukaemic spleen intraperitoneally from a mouse with advanced Ak_4R leukaemia, in a manner similarly to that for transmitting sensitive Ak_4 leukaemia as described previously. When the mice had developed evidence of advanced leukaemia as shown by considerable splenic enlargement, they were sacrificed, homogenates of the liver, kidneys, spleen and lung prepared, boiled for five minutes, and after centrifuging and diluting assayed for their amethopterin content.

Results.

	Av. Concn. Amethopterin 6 Mice (mγ/G.)
Liver (Av. wt. 2 - 3 G.)	145
Kidney (Av. wt. .4 - .6 G.)	145
Spleen (Av. wt. .35 - .65 G.)	167
Lung (Av. wt. .26 - .28 G.)	120 (1) 0 (5)

Table 12

It will be observed that assayable quantities of amethopterin were still present in the liver, kidneys and spleen in all mice (Table 12). Taking into account the increased weight of the organs the concentration of amethopterin in the liver and kidneys was similar to that found in normal mice. That in the spleen was, however, considerably raised which again indicated the affinity of leukaemic tissue for the antimetabolite. There was thus no evidence to suggest the transplantation of Ak_4R leukaemia into mice previously given amethopterin had any effect on the concentration of amethopterin in the tissues, which appeared to be retained in the mouse with resistant leukaemia in a manner similar to the normal animal. The resistant leukaemic cells did not appear to utilise or metabolise, at least detectable quantities, of the antimetabolite. The possibility that deamination to a weaker antagonist,

N^{10}-methyl pteroyl-glutamic acid (methopterin), occurred could not however be ruled out as this compound like amethopterin inhibits the growth of S. faecalis.

(3) Amethopterin Excretion Studies in Leukaemic and Non-Leukaemic Subjects

In conjunction with Drs. Burchenal, Murphy, Ellison and Hutchison, a study of the excretion of amethopterin in human subjects was carried out at the same time as the animal studies were in progress.

Materials and Methods.

Twenty-seven patients with acute leukaemia (of various ages, size and weight), some of whom later developed remissions on amethopterin treatment and some who were resistant to the anti-folics, and ten patients with advanced carcinoma, were given 0.05 mg./kg. amethopterin intravenously, after ensuring that renal function was normal. The significance of impaired renal function had previously been pointed out by Burchenal et al. (1951a). Venous blood samples were thereafter collected at 5, 15, 30 and 60 minutes intervals and the blood allowed to coagulate. Serum was removed with a Pasteur pipette and diluted. Full strength serum and 1-3 and 1-10 dilutions were found to be satisfactory for assay purposes. The

amethopterin content of the serum was then measured by means of the plate assay technique described previously (page

In twenty-four leukaemic and non-leukaemic patients urine was collected throughout the six hour period following the administration of the drug, the patient being asked to empty the bladder at the end of this period. This short, six hour excretion test was performed because Burchenal et al. (1951a) had previously demonstrated that when a 5 mg. dose of amethopterin was given orally to a normal human subject on an empty stomach, over 90 per cent. of the total amount excreted was passed in the urine within six hours. Preliminary studies extending over twenty-four hours showed this also to be the case when amethopterin was given intravenously. The intravenous route was preferred in order to overcome any possible variations in absorption and to ensure a rapid uptake by the tissues and rapid excretion.

The quantity of urine passed in the six hour period was measured after which suitable dilutions (1-50 to 1-500) were prepared and the amethopterin content per ml. determined. The total amount of amethopterin excreted and the amount expressed as a percentage of the dose administered was then calculated.

The assay was performed on the day of the test

in each case, the urine and blood samples being kept in a cold room at 4°C. until required.

Results.

The serum levels, and percentage excretion at six hours are demonstrated graphically (Fig. 13). It will be observed that the serum levels in the leukaemic patients closely followed the pattern observed in the control group. At the end of one hour in only three out of the total of thirty-seven patients were the serum levels still above 50 mγ/ml. In twenty-one of the total of twenty-four patients from both groups, the total excretion of amethopterin was found to be less than 60 per cent. of the injected dose. Considerable variation in the amount excreted was noted in both the leukaemic and non-leukaemic patients, ranging from 14 to 75 per cent. No obvious difference in rate of excretion between the leukaemic and control group was observed, nor did there appear to be any obvious difference between the antifolic-resistant and antifolic-sensitive acute leukaemias. For conclusive evidence a larger series should be studied, and normal individuals used as controls. The possible toxic-side effects associated with amethopterin however precludes the latter, and the wide individual variation in excretion in both the leukaemic and control subjects indicates that either no significant difference exists, or, if present, a

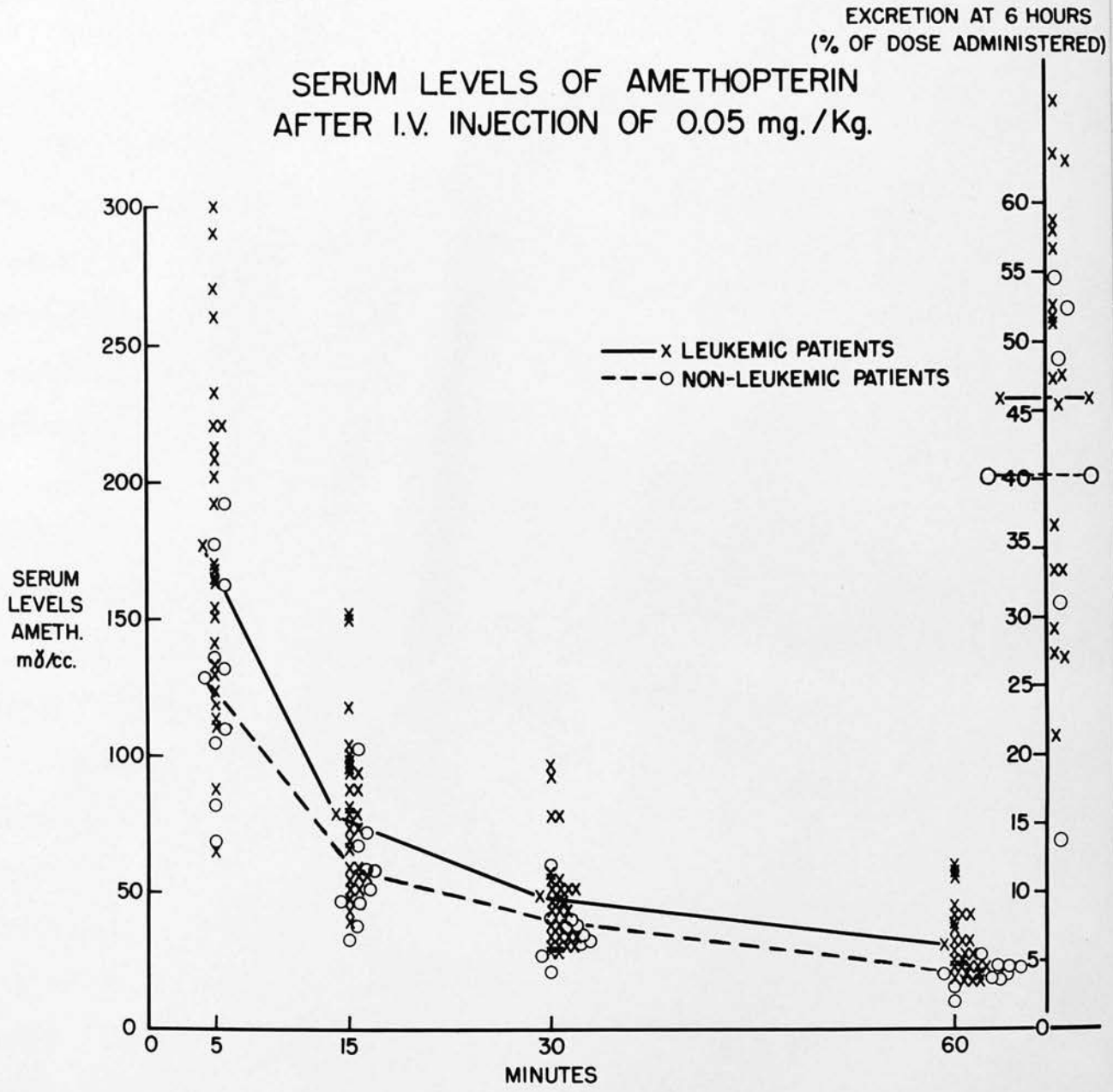

SERUM LEVELS OF AMETHOPTERIN
AFTER I.V. INJECTION OF 0.05 mg./Kg.
EXCRETION AT 6 HOURS
(% OF DOSE ADMINISTERED)
SERUM LEVELS AMETH. mδ/cc.
300
250
200
150
100
50
0
60
55
50
45
40
35
30
25
20
15
10
5
0
0
5
15
30
60
MINUTES
x LEUKEMIC PATIENTS
o NON-LEUKEMIC PATIENTS

FIG. 13.

very large series would have to be tested in order to demonstrate it.

Discussion.

It has been found that in normal human volunteers (Burchenal et al. 1951a) only 40 to 57 per cent. of an oral dose of 5 mg. amethopterin was excreted in twenty-four hours, the majority within four to six hours. Studies in a series of thirty-seven leukaemic and non-leukaemic subjects showed a similar delay in excretion, thirty-four excreting less than 60 per cent of the injected dose. Swendseid (1952) also noted that variable amounts of aminopterin were unaccounted for by renal excretion alone. It seems probable that the explanation for these findings may be similar to those found in the laboratory animal.

It was evident from the previous experiments that a relatively high concentration of amethopterin persists in tissues of the normal mouse for a period of three weeks. Moreover, it appeared that within the dosage range used in these experiments the mouse could rapidly eliminate within twenty-four hours all the amethopterin in excess of a threshold amount which was retained in the liver and kidneys. The effects of very large doses of amethopterin on tissue retention were not studied as it was considered desirable to keep the dosage below the range which produces toxic manifestations

(Ferguson et al. 1950) and within the chemotherapeutic range (Burchenal et al. 1949). The largest single dose given (20.0 mg./kg.) was approximately one fifth of the acute L. D 50 dose while the mice in groups I and V received a dose (3 mg./kg.) comparable to that when given three times weekly prolongs the survival time of mice with transplanted leukaemia.

Although small quantities of amethopterin were found to be frequently present in the serum there was little indication that any was being metabolised or excreted after the first twenty-four hours and with the results of the previous experiments in mind (Table 9) probably after a period of four hours following administration. Urine samples taken on several occasions at the time of sacrificing the animals failed to show the presence of amethopterin.

In keeping with the hypothesis that anti-metabolites function by excluding a structurally related metabolite from combination with its enzyme (Woolley 1952) and thus preventing the formation of an enzyme substrate complex, it was probable that the amethopterin retained for such long periods of time was incorporated up to a relatively constant concentration in enzyme systems ordinarily associated with folic acid and CF and any excess was rapidly excreted. This incorporation appeared stable but not entirely irreversible as

was shown by the rise in the serum level following the administration of C.F. (Fig. 12)

The tissue concentration of amethopterin was however not affected to any extent by high doses of C.F. which would explain the unsuccessful attempts to overcome the toxic effects of the folic acid antagonist in experimental animals (Higgins, 1949; Philips and Thiersch, 1949) and the leukaemic patient (Farber, 1949). The vitamin would appear unable to enter into body metabolic processes and is rapidly excreted. The work of Swendseid et al. (1952) in human subjects supports this concept.

It was observed that none of the animals in Groups I - IV developed any evidence of folic acid and CF deficiency although retaining relatively high concentrations of amethopterin in the liver and kidneys for a period of three weeks. Studies in mice (Ferguson et al. 1950) have shown that the chronic L.D. 50 for amethopterin to be 1.9 mg./kg. when given daily for five doses. This was of interest in view of the finding that a fortyfold variation in dosage of amethopterin (0.5 - 20.0 mg./kg.) when given in a single injection resulted in almost identical concentrations of amethopterin in the liver and kidney twenty-four hours later. Similar concentrations were noted following divided doses given on alternate days. Thus for folic acid and CF deficiency to occur an adequate increase

above the tolerated tissue concentration must presumably be obtained by administration of amethopterin at daily intervals. Another possible explanation might be the deamination of amethopterin to N^{10}-methyl pteroylglutamic acid (methopterin) which is known to be a weaker antagonist ineffective as a therapeutic agent in leukaemia, but readily inhibits the growth of S. faecalis. Such a concentration of methopterin together with an adequate dietary intake of CF would possibly not lead to CF deficiency.

By transplanting Ak_4R leukaemia to mice previously injected with amethopterin it was shown that the amethopterin resistant leukaemic cell had no effect on the concentration of the anti-metabolite. This was of some importance for it suggested that the resistant cell did not apparently utilise amethopterin for its metabolism, which has previously been considered as a possible property of the amethopterin resistant cell. Whether or not the resistant cell may convert amethopterin to a weaker antagonist must however still be demonstrated.

D. Identification of the Anti-metabolite in Mouse Tissues following administration of Amethopterin.

The substance present in mouse tissues, after administration of amethopterin, with growth inhibitory properties for Streptococcus faecalis has, to the present, been presumed to be amethopterin. Because of the suggestion by many investigators that resistance to amethopterin in leukaemia may be due to conversion of the drug to a weaker antagonist (Page 61) it seemed of some importance to determine whether or not the anti-metabolite present in normal mouse tissues after injection of amethopterin, and similarly later in amethopterin resistant leukaemic mice was in fact amethopterin. It is well recognised that the antagonist activity of the folic acid analogues is increased by substitution of an amino group in position 4 of the pteridine ring and it seemed possible that deamination of amethopterin may occur in the mouse liver. The deaminated derivative of amethopterin, methopterin (N^{10} methyl-pteroylglutamic acid) can inhibit the growth of Streptococcus faecalis but has no effect on the survival time of Ak_4 mice (Burchenal et al. 1949b) nor is it able to modify the course of human acute leukaemia.

It was therefore decided to attempt to identify the anti-metabolite present in the liver of mice previously given amethopterin, using a combination

of chromatography and bio-autography. As a preliminary to this, the following two studies were carried out.

1. Determination of Rf values of various Folic Acid Analogues.

Materials and Methods.

Solutions of various analogues of folic acid were prepared as listed below.

1. Amethopterin (4-amino-N^{10}-methyl pteroyl glutamic acid)
2. Aminopterin (4-amino pteroyl glutamic acid)
3. Methopterin (N^{10}-methyl pteroyl glutamic acid)
4. Iodo - amethopterin
5. Dimet-fol (9, 10-dimethyl pteroyl glutamic acid)
6. A-Ninopterin (4-amino-9-methyl pteroyl glutamic acid)
7. A-Denopterin (4-amino-9,10-dimethyl pteroyl glutamic acid)
8. Bremfol (Pteroyl 9-methyl glutamic acid)
9. Amino-Anfol (4-amino pteroyl aspartic acid)
10. Anfol A (pteroyl aspartic acid)
11. Amino-teropterin (4-amino triglutamic acid)

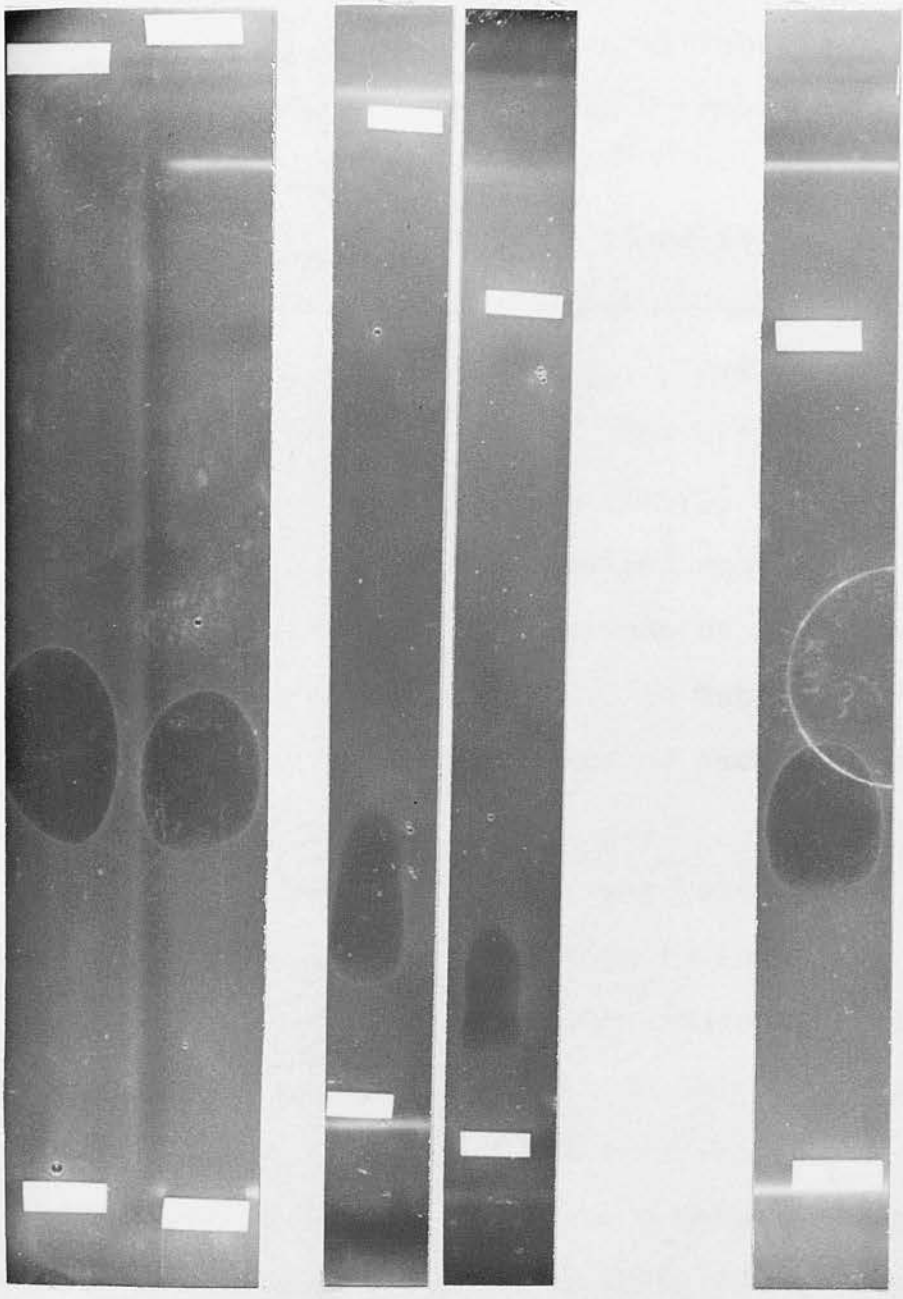
Solutions 1, 2, 3, 4, 5, 6, 7, were prepared at concentrations of 10 mγ/.01 ml., solution 8 at 30 γ/.01 ml., solution 9 at 100 mγ/.01 ml., solution 10 at 3γ/.01 ml. and solution 11 at 1γ/.01 ml.

Solvent System. Butyl Acohol (Butanol) and Acetic Acid, prepared by saturating butanol with water in a separating flask, running off the butanol and to it adding 10 per cent. by volume of glacial acetic acid. The pH was taken and found to be 3.0.

Paper Strip. Approximately $\frac{3}{4}$" No. 1. Whatman paper.

Procedure. A fine pencil line (A) was drawn 5 cms. from one end of the paper strips, and on the centre of each was placed .01 ml. of the above solutions. The strips were then placed 'spot-end' lowermost in separate glass cylinders containing the solvent which reached a level of approximately 5 cms. from the bottom of the cylinder so that the lower end of the strips were just immersed in the solvent. The upper end of the strips were folded and fastened with a paper clip over glass rods resting over the mouths of the cylinders.

The paper strips were allowed to remain for a period of twenty-four hours until the solvent had moved high up the strips but had not reached the clip at the upper end. The strips were then removed and the upper level of the solvent flow marked immediately by pencil (B). After drying the strips were cut across at A and B and placed for five minutes on large Pyrex plates which contained Difco Folic Acid Assay agar plus PGA at 1 mγ/ml. and seeded with S. Faecalis. The position of each spot and solvent flow was marked on the agar by



(A) (B) (C)

FIG. Bio-autographs of the three principal folic acid analogues:-
(A) Amethopterin;
(B) Aminopterin;
(C) Methopterin.

SOLVENT SYSTEM - Butanol and Acetic Acid.

small pieces of paper and after removal of the strips the plates were placed in an incubator for a period of sixteen to twenty-four hours.

Results and Comments. (Table 13)

All the test solutions were found to produce zones of growth inhibition. The Rf value for each folic acid analogue was determined by measuring (C) the distance from the spot to the upper level of the zone of growth inhibition and (D) the distance from the spot to the maximum point of solvent flow. $Rf = \frac{C}{D}$. The Rf values of the eleven folic acid analogues are presented in Table together with the approximate size of each zone of growth inhibition.

It will be observed that it was possible to determine Rf values for the various analogues of folic acid by a combination of chromatography and bio-autography. The inference, from this experiment was that, using a butanol-acetic acid solvent system, those analogues containing a methyl group in their molecule (samples 1, 3, 4, 5, 6, 7, 8) have similar Rf values much higher than those not containing a methyl group (samples 2, 9, 10, 11). Two separate zones of inhibition were noted in the case of Bremfol (Sample 8) suggesting the presence of an impurity.

2. Separation of Amethopterin from Citrovorum Factor in Simple solution and from livers of mice previously injected with Amethopterin.

	Sample	Conc$^{n.}$/0.01 ml.	Solvent Flow (cms.)	Spot to Distal Pt. of Inhibition Zone (cms.)	Size of Zone (cms.)		Rf
					Ht.	Width	
1	Amethopterin	10 mγ	29.0	16.2	3	3.5	.56
	"	10 mγ	28.2	15.9	3.4	3.0	.56
	"	10 mγ	28.8	16.5	4.8	3.0	.57
	"	10 mγ	30.4	15.5	4.4	3.2	.51
	"	10 mγ	23.4	13.6	4.9	3.5	.58
	"	10 mγ	25.9	14.9	4.8	3.4	.58
2	Aminopterin	10 mγ	26.5	7.9	4.3	3.5	.29
	"	10 mγ	29.7	7.5	4.0	3.0	.25
	"	10 mγ	27.8	6.7	4.3	3.0	.24
	"	10 mγ	29.1	7.9	5.1	3.0	.27
3	Methopterin	10 mγ	27.0	16.5	5.2	3.0	.61
	"	10 mγ	30.4	16.6	4.8	3.1	.55
4	Iodomethopterin	10 mγ	27.5	15.9	4.0	2.9	.58
5	Dimetfol	10 mγ	28.5	19.3	6.0	3.0	.68
	"	10 mγ	29.1	19.3	5.1	3.0	.66
6	A-minopterin	10 mγ	27.3	15.5	6.2	3.3	.57
	"	10 mγ	29.5	16.6	5.7	3.5	.56
7	A-denopterin	10 mγ	27.2	19.6	7.3	3.8	.72
	"	10 mγ	29.1	18.4	7.4	3.9	.63
8	Bremfol	30γ	27.5	17.5	6.1	3.1	.63
				5.8	1.6	1.6	.21
9	Amino-Anfol	100 mγ	27.0	7.1	4.3	3.3	.26
10	Anfol A	3γ	26.2	6.6	6.6	3.5	.25
11	Aminoteropterin	1γ	27.1	6.0	4.5	3.0	.22

TABLE 13.

Another preliminary experiment was to determine whether or not amethopterin could be separated chromatographically from growth factors present in mouse liver.

Materials and Methods.

Solutions containing amethopterin and CF were prepared at concentrations tabulated below. In addition a normal mouse injected twenty-eight days previously with amethopterin at a dose of 3 mg./kg. X 3, on alternate days, was sacrificed, the liver removed, weighed and muddled in 5 ml. distilled water, and boiled immediately and centrifuged. The concentration of antimetabolite in the supernatant of the liver sample was then assayed by the plate technique.

Three normal mice given amethopterin fourteen days previously at a dose of 3 mg./kg. X 3, on alternate days, and thereafter given Ak_4R leukaemia by intraperitoneal transplantation of Ak_4R leukaemic cells were sacrificed when showing evidence of advanced leukaemia. The livers were removed, weighed, muddled and the homogenate boiled immediately and centrifuged as before and the antimetabolite content of the supernatant assayed.

The chromatographic technique was similar to that described in the previous experiment. .01 ml. of the solutions containing amethopterin or mixtures of amethopterin and CF were placed on paper

strips and after drying developed for twenty-four hours in the butanol and acetic acid solvent system.

With a knowledge of the content of anti-metabolite in each of the liver extracts it was possible to determine the volume containing an amount of between 7 to 10 mγ, a concentration known to give a good zone of inhibition. This amount was placed on the paper strip, .01 ml. at a time with cool air drying between each application (hot air via a hairdrier resulted in a 'hard spot' and interfered with the chromatogram). Thereafter the strips were developed for twenty-four hours, using the butanol and acetic acid solvent system.

After removal of the strips and drying, bio-autograms were prepared and the necessary calculations made as described.

Materials.

1. Amethopterin at 10 mγ/.01 ml.
2. Amethopterin at 10 mγ/.01 ml. + CF at 30 mγ/.01 ml.
3. Amethopterin at 10 mγ/.01 ml. + CF at 60 mγ/.01 ml.
4. Normal Mouse Liver containing 308 mγ antimetabolite/5 mls. distilled water.
5. Ak_4R Mouse Liver containing 355 mγ antimetabolite/5 mls. distilled water.
6. Ak_4R Mouse Liver containing 526 mγ antimetabolite/5 mls. distilled water.
7. Ak_4R Mouse Liver containing 380 mγ antimetabolite/5 mls. distilled water.

Results and Comments.

From this study it was evident that the presence of citrovorum factor did not interfere with the chromatographic and bio-autographic identification of the antimetabolite present in mouse liver after inoculation with amethopterin. The antimetabolite was found to have a similar Rf value to amethopterin and it was of considerable interest that the antimetabolite present in both the normal liver and the livers of mice with amethopterin resistant leukaemia had similar Rf values, using the butanol-acetic acid solvent system, (Table 14). As it had been observed previously, however, that folic acid analogues containing a methyl group had similar Rf values the possibility that the antimetabolite was a weaker antagonist could not at this stage be excluded. In this connection deamination of amethopterin to methopterin seemed possible and it was now decided to attempt to differentiate between these two substances, by the use of different solvent systems. From experience gained various modifications of technique were to be employed in order to lead to a high degree of accuracy. One difficulty with regard to testing of the anti-metabolite in liver samples was the need under the present arrangements of putting a relatively large volume of extract on the chromatogram paper which

Sample	Amt. on Spot (ml.)	Solvent Flow (cms.)	Spot to Distal Point of Inhibⁿ Zone (cms.)	(Approx.) Size of Zone		Rf.
				Ht.	Width	
1. Ameth. 10 mγ	.01	31 31.4	15.5 15.6	5.8 5.2	3.6 3.2	.50 .51
2. Ameth. 10 mγ + CF 30 mγ	.01	32.7	14.8	4.7	3.5	.46
3. Ameth. 10 mγ + CF 60 mγ	.01	22.7	12.2	4.3	3.4	.53
4. Normal Liver	0.15	31.8 27.5	13.9 14.0	6.0 12.0	3.0 1.8	.44 .52
5. Ak_4R Liver	0.1	26.5	12.9	3.4	2.0	.49
6. Ak_4R Liver	0.1	27.7	16.7	3.2	2.6	.60
7. Ak_4R Liver	0.1	27.8	12.6	3.7	2.5	.45

TABLE 14.

tended to lead to a 'hard spot' and occasional impairment of flow. By concentrating the liver extract it was thought that this might be overcome. A larger series of controls were also thought desirable.

Identification of Anti-Metabolite in Normal Mouse Tissues Following injection of Amethopterin using using three different solvent systems.

Materials and Methods.

A series of mice was injected intraperitoneally with amethopterin at a dose of 3 mg./kg. These animals were sacrificed at half an hour intervals up to three hours then at four, five, seven, seventeen, twenty-four, forty-eight, seventy hours, four, eight and fourteen days. Other mice not in this series were tested up to eight months after injection. The liver was removed from each animal and treated as previously mentioned, after which the supernatant was concentrated in vacuo to approximately 1 ml.

As controls for this experiment homogenates of four normal livers were prepared. Amethopterin (0.5γ/G-) was added to the first preparation, methopterin (2γ/G-) to the second, aminopterin (1γ/G-) to the third and to the fourth control was added a single preparation containing the three folic acid antagonists in the concentrations given above. These controls were boiled, clarified and concentrated like the test samples. .01 ml. of the

liver concentrates were placed on Eaton-Dikeman No. 613 paper strips after which the strips were developed for six to sixty-eight hours in three different solvent systems; (A) 1 per cent. K_2HPO_4 aqueous (Wieland et al. 1952), (B) 5 per cent. sodium citrate and iso-amyl alcohol (Wieland et al. 1952) and (C) 70 per cent. Iso-propanol, 10 per cent. NH_4OH, 20 per cent. distilled water. It should be mentioned that the paper strips were also buffered with 0.08 M.Na_2HPO_4.

After developing, the strips were removed and air dried and placed on Pyrex plates containing Difco Folic Acid Assay Agar plus folic acid at 0.5 mγ/ml. seeded with S. faecalis for five minutes. Thereafter the bio-autographs of the inhibition zones were observed after an incubation of sixteen hours.

Results and Conclusions.

With the technique described above it was possible to identify the antimetabolite which persisted in the mouse liver and to follow its course in a series of mice. A bio-autograph of a series of liver samples and of the clinical amethopterin (parenteral preparation) is presented in Fig. 14. Strip No. 1 shows the separation of aminopterin, methopterin and amethopterin. Strip No. 2 represents the particular sample of clinical amethopterin that was injected into this series of mice. In the

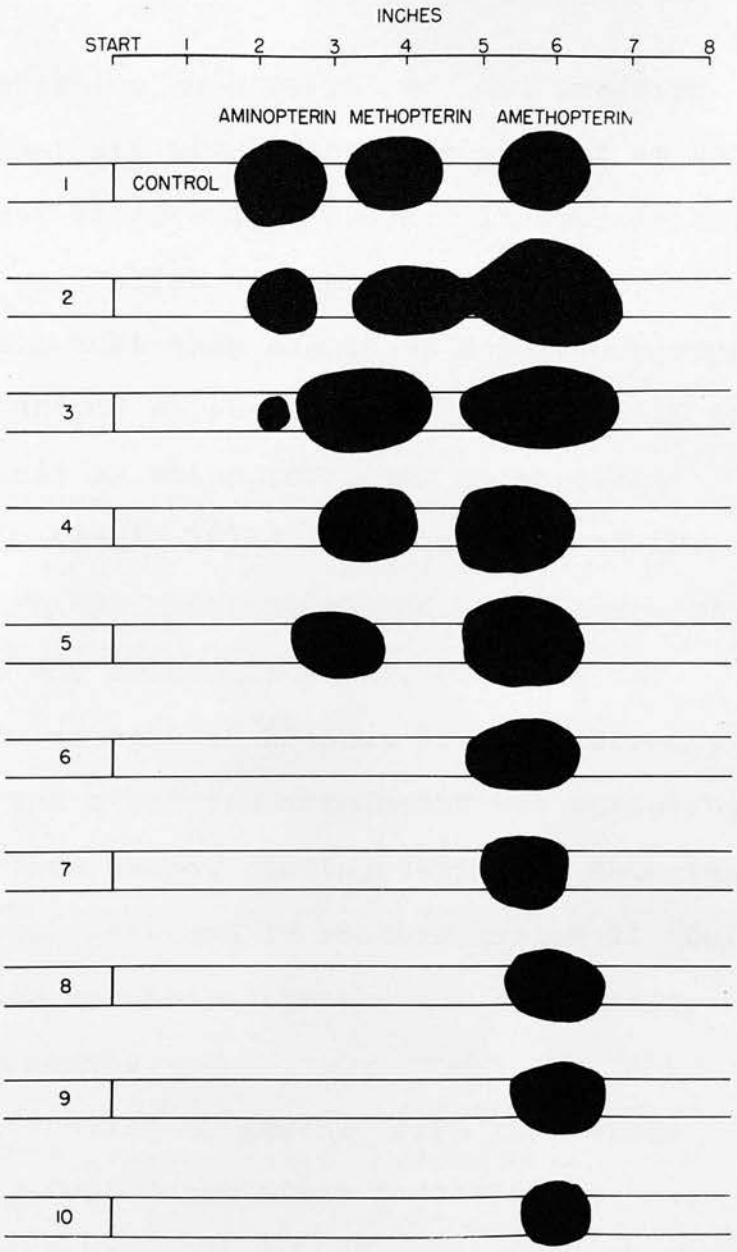


Fig. 14. Bioautographs of strips from alkaline iso-propanol system on S. faecalis (8043).

Strip 1. .01 ml. of control liver concentrate which contained amethopterin, methopterin, and aminopterin.

Strip 2. 100 mγ of amethopterin, Lederle Lot No. 7-8528.

.01 ml. of liver concentrates following administration of amethopterin (3 mg./kg.).

Strip 3. After 1 hr.
4. 2
5. 7
6. 48
7. 4 days
8. 8
9. 14
10. 8 mo

liver concentrates aminopterin was not detected after one hour and methopterin was present at seven hours but not at seventeen hours. It is possible that these antifolics were excreted, but it is also possible that they cannot be detected because folic acid and CF appear to have approximately the same Rf values as aminopterin and methopterin respectively (Table 18). The finding of three components in the amethopterin molecule preparations complicates any metabolic study, but only the amethopterin content of tissues was considered since neither of the other two components was detectable after seventeen hours. Amethopterin was detectable after fourteen days and in another series it could be identified by chromatography and bio-autography after seven months.

Identification of amethopterin in a liver taken twenty-four hours after injection is demonstrated in Fig. 15. Strip 1 shows the three spots representing aminopterin, methopterin and amethopterin seen when these compounds were added to normal liver. Strip 2 shows the single spot found in liver of a mouse twenty-four hours after injection of amethopterin. When amethopterin was added to this liver preparation, only one spot was noted (Strip 3) but when methopterin (Strip 4) or aminopterin (Strip 5) was added a second spot appeared in addition to the original one representing

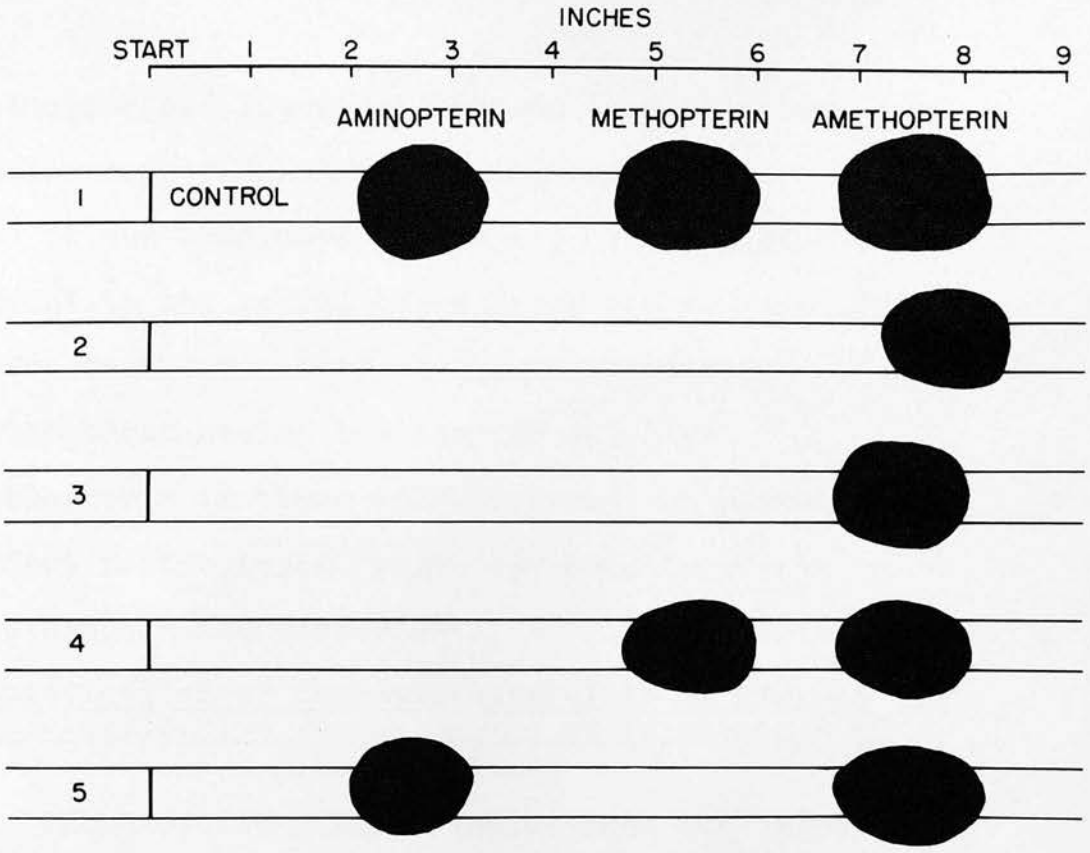


Fig. 15. Bioautographs of strips from alkaline iso-propanol system on S. faecalis (8043).

Strip 1. .01 ml. n.l.h.* + 0.5 γ/g amethopterin (control).
.015 ml. n.l.h. + 2 γ/g methopterin (control).
.01 ml. n.l.h. + 1 γ/g aminopterin (control).

Strip 2. .01 ml. l.c.** 24 hrs. after injection of amethopterin (3 mg./kg.).

Strip 3. .01 ml. l.c. 24 hrs. after injection of amethopterin (3 mg./kg.) + .01 ml. amethopterin control.

Strip 4. .01 ml. l.c. 24 hrs. after injection of amethopterin (3 mg./kg.) + .015 ml. methopterin control.

Strip 5. .01 ml. l.c. 24 hrs. after injection of amethopterin (3 mg./kg.) + .01 ml. aminopterin control.

*n.l.h. = normal liver homogenate

**l.c. = liver concentrate

amethopterin. Identical results were obtained in the iso-amyl alcohol and K_2HPO_4 systems.

It was concluded that the inhibiting substance present in the normal mouse liver twenty-four hours to fourteen days after the administration of amethopterin having the same Rf value as amethopterin in three different solvent systems was in fact amethopterin, which appeared to remain unchanged in the tissues.

Identification of the Anti-metabolite in Tissues of Leukaemic Mice following the administration of Amethopterin.

The previous study revealed that the injected 4-amino-N10-methyl pteroyl glutamic acid (Amethopterin) retained in the normal mouse tissues remained unchanged and if in fact any altered product of the antifolic was present it occurred in too small an amount to be detected by these methods, or it did not affect the growth of Streptococcus faecalis.

The natural consequence of the studies on normal mice was the investigation of the metabolic fate of amethopterin in the animal with sensitive and amethopterin resistant leukaemia, in the hope that it might lead to knowledge concerning the mechanism of resistance. It has been previously shown that in mice given Ak_4R, amethopterin resistant leukaemia, an antimetabolite was retained in the tissues following intraperitoneal injection of amethopterin, as in normal animals, and that this

antimetabolite had a similar Rf value to amethopterin (Table 14) in the butanol-acetic acid solvent system. However, as the analogues of folic acid containing methyl groups appear to have similar Rf values (Table 13) in this solvent system, the possibility that deamination of amethopterin occurred to form methopterin (N10-methyl pteroylglutamic acid), a weaker antagonist, could not be excluded. The main object of this present study was to show whether or not the resistant leukaemic cell had the ability to deaminate the antagonist, as several observers have considered this to be one of the possible mechanisms of resistance, and have suggested that aminopterin might be converted to utilisable folic acid by the resistant cell and amethopterin to the much weaker, and therepeutically inactive, methopterin.

Materials and Methods.

In this study L 1210 (sensitive) and L 1210/A (amethopterin resistant) leukaemia were used. These varieties of leukaemia obtained from Dr. Lloyd Law, of the National Institutes of Health, United States of America, result in the formation of a very large tumour at the locus of injection which offers a good mass of "pure" leukaemic cells with which to study. Compared with the AK_4 And AK_4R leukaemias there is not so much involvement of

the spleen.

Mice were injected with L 1210 and L 1210/A leukaemia and developed tumours on the eleventh and thirteenth day respectively. At this time, amethopterin 3 mg. per Kg. was injected intraperitoneally and twenty-four hours later the animals were sacrificed. The livers, spleens and tumours were removed, and were homogenised separately in water. There were five L 1210 mice and four L 1210/A mice in the experiment. The resulting suspensions were centrifuged and the supernatants concentrated under nitrogen to a volume of 0.5 ml. The samples, and standard solutions of amethopterin, methopterin and aminopterin were spotted on filter paper strips which had been soaked in 0.08 M. Na_2HPO_4 and dried. The solvent system consisted of 70 per cent. isopropyl alcohol; 10 per cent. NH_4OH and 20 per cent. H_2O. The strips were developed on agar plates containing the complete Flynn medium (Flynn et al. 1951), PGA and seeded with S. faecalis 8043.

The Study consisted of three experiments:-

A. In the first experiment, 0.01 ml. of the samples were spotted and run for forty-eight hours, and the strips developed on plates containing 1 mγ/ml. PGA. Only one inhibition zone was noted on each strip, corresponding to the amethopterin control, except in the case of one spleen sample from an amethopterin resistant leukaemic mouse, which

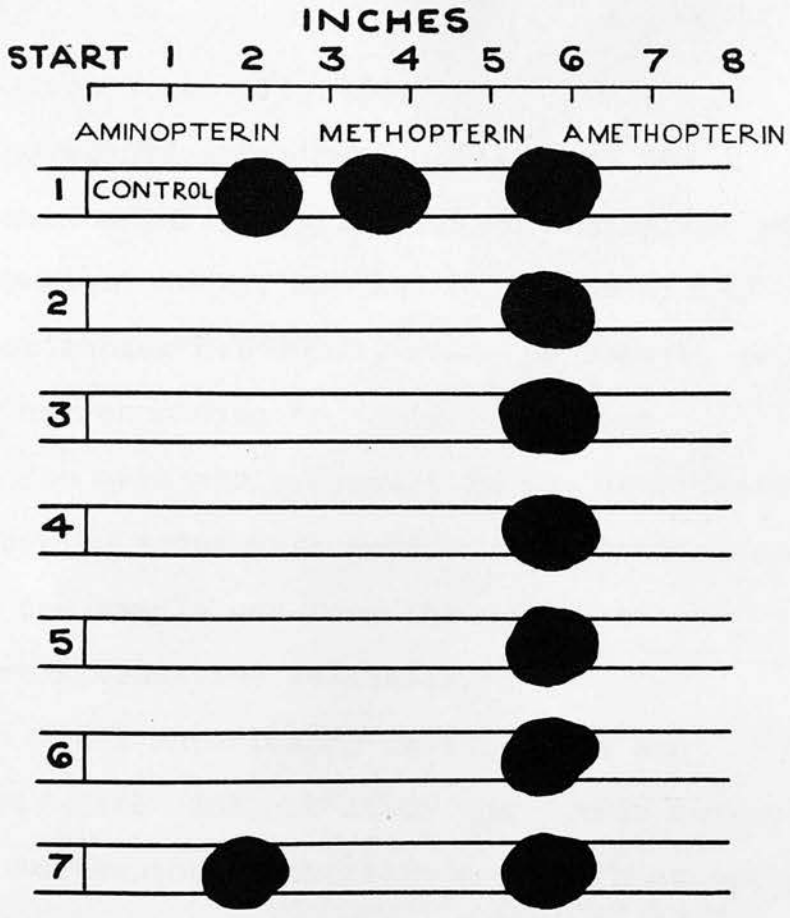


Fig.16(a) Bioautographs of strips from alkaline iso-propanol system on S. faecalis (8043).

Strip 1. Standard solutions of amethopterin, methopterin and aminopterin (control).

Strip 2. .01 ml. t.c.* 24 hrs. after injection of amethopterin (3 mg./kg.) - L 1210 (amethopterin sensitive) leukaemia

Strip 3. .01 ml. t.c. 24 hrs. after injection of amethopterin (3 mg./kg.) - L 1210/A (amethopterin resistant) leukaemia.

Strip 4. .01 ml. l.c.x 24 hrs. after injection of amethopterin (3 mg./kg.) - L 1210 leukaemia.

Strip 5. .01 ml. l.c. 24 hrs. after injection of amethopterin (3 mg./kg.) - L 1210/A leukaemia.

Strip 6. .01 ml. s.c.** 24 hrs. after injection of amethopterin (3 mg./kg.) - L 1210 leukaemia.

Strip 7. .01 ml. s.c. 24 hrs. after injection of amethopterin (3 mg./kg.) - L 1210/A leukaemia.

*t.c. = tumour concentrate

x l.c. = liver concentrate

** s.c.= spleen concentrate

contained two zones (Fig. 16a).

B. In the second experiment 0.03 ml. of the tissue samples were spotted, the chromatograms run for sixty-eight hours, and the strips developed on plates containing 0.5 mγ/ml. PGA. As before, only one zone corresponding to amethopterin was observed for each strip, except in one case where two inhibition zones were noted. In this instance, however, the sample was from the spleen of an amethopterin sensitive leukaemia.

C. In a final experiment, amethopterin and methopterin were added to liver and tumour extracts from one amethopterin sensitive and one amethopterin resistant leukaemic mouse which had been injected intraperitoneally with amethopterin, 3 mg./Kg., twenty-four hours before being sacrificed. The extracts were concentrated and volumes spotted as shown in Fig. 16b the chromatograms run for forty-eight hours, and the strips developed on plates containing 1 mγ/ml. PGA. Where amethopterin was mixed with the extracts only one zone was observed, while the methopterin mixture gave two distinct zones, corresponding to those of amethopterin and methopterin.

Comments.

The results of this experiment indicated that most, and probably all, of the injected amethopterin remained unchanged in the livers, tumours and spleens

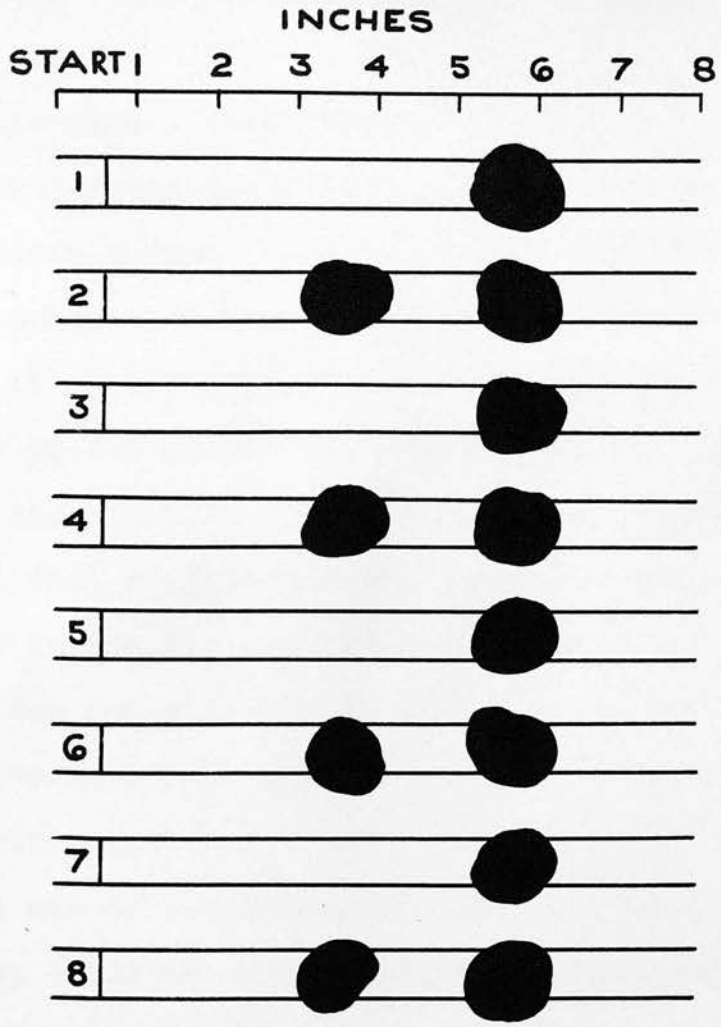


Fig. 16b Bioautographs of strips from alkaline iso-propanol system on S. faecalis (8043). All mice given amethopterin (3 mg./kg.) intraperitoneally 24 hrs. previously.

Strip 1. .01 ml. t.c.* + 0.5 γ/G. amethopterin - L 1210 leukaemia (amethopterin sensitive).

Strip 2. .015 ml. t.c. + 2 γ/G. methopterin - L 1210 leukaemia.

Strip 3. .01 ml. t.c. + 0.5 γ/G. amethopterin - L 1210/A leukaemia (amethopterin resistant).

Strip 4. .015 ml. t.c. + 2 γ/G. methopterin - L 1210/A leukaemia.

Strip 5. .01 ml. l.c.x + 0.5 γ/G. amethopterin - L 1210 leukaemia.

Strip 6. .015 ml. l.c. + 2 γ/G. methopterin - L 1210 leukaemia.

Strip 7. .01 ml. l.c. + 0.5 γ/G. amethopterin - L 1210/A leukaemia.

Strip 8. .015 ml. l.c. + 2 γ/G. methopterin - L 1210/A leukaemia.

*t.c. = tumour concentrate

x l.c. = liver concentrate

of animals with either amethopterin sensitive or resistant leukaemias. The method was probably not quantitative enough to say for sure, but if any altered product of the folic acid analogue was present it occurred in too small an amount to be detected by the present methods, or on the other hand it did not affect the growth of S. faecalis. The fact that a considerable amount of amethopterin was left in the tissues, was more than suggestive that it was not altered, or if it was altered enough amethopterin remained so that it could have been inhibitory.

The use of two biological systems, mice and bacteria, leads to some difficulty in evaluation of these experiments, but the weight of evidence strongly favours the conclusion that the amethopterin resistant leukaemic cells do not in any way result in an alteration of the anti-metabolite. The importance of such a conclusion cannot be underestimated, for it indicates that resistance of leukaemic cells to folic acid analogues is not due to an increased ability of the resistant antagonist by way of deamination or demethylation, to form either a weaker antagonist or to convert the antagonist to utilisable folic acid.

The second zone of inhibition which was noticed in two instances, in a spleen sample from L 1210/A

mouse in Experiment A, and a spleen sample from an L 1210 mouse in Experiment B, was probably aminopterin, which is known to be present in small amounts in the amethopterin used for injecting the mice.

FOLIC ACID AND CITROVORUM FACTOR IN NORMAL, AND LEUKAEMIC MOUSE LIVER.

Many observers consider that the folic acid activity (FA) of normal mouse liver tissue is largely due to its content of citrovorum factor (CF). Swendseid *et al.* (1951a) using mouse liver concluded that "----- under standard dietary conditions, folic acid is absent from the liver or present in very small amounts." May and his associates (May, M. *et al.* 1951) using a combination of paper chromatography and bio-autography came to similar conclusions. Others, however, provided evidence of the presence of FA in animal tissue (Dietrich *et al.* 1952, Wieland *et al.* 1952). It is generally recognised that the folic acid vitamins are present in liver largely in conjugated forms (Mims *et al.* 1944; Dietrich *et al.* 1952) although small quantities of free vitamin are also present. Swendseid *et al.* (1951a) considered this to be largely CF, whereas Dietrich *et al.* (1952) observed small quantities of free FA in rat, guinea pig and chick livers, frozen immediately upon removal to prevent conjugase activity, but under similar conditions no free CF.

This study was undertaken in an attempt to determine any difference in the total amounts and distribution of FA and CF in the normal and leukaemic mouse liver.

Microbiological assay was the method for quantitative determination of vitamin present in the liver tissue. An attempt has been made to determine the relative proportions of FA and CF present in both free and bound forms, a matter which always gives rise to doubts and difficulties. It has been demonstrated that the growth of Streptococcus faecalis on a medium deficient in FA is stimulated by the presence of either FA or CF, whereas the growth of Leuconostoc citrovorum responds only to CF. The assay with S. faecalis may therefore be used as a measure of the sum of the activities of FA and CF whereas the L. citrovorum assay yields information solely relating to CF activity. Calculation of the FA activity alone would then appear straight-forward but in view of the stimulation of growth of S. faecalis by FA and CF being variable, such an estimation of FA activity alone becomes difficult. Swendseid et al. (1951a) approached the problem by using CF to produce the standard curves and measuring the concentrations of vitamin in terms of CF activity. In such an assay procedure, in which CF was used as a standard, if the activity in the tissue was greater in stimulating growth of S. faecalis than L. citrovorum, FA was considered to be present, whereas if the activities were similar only CF was present. Girdwood (1951) used a similar procedure in estimating FA and CF

activity in human liver tissue. Under the conditions of assay reported here preliminary studies (Table 15) showed that the stimulation of growth of S. faecalis by FA was approximately equal to that produced by the same amount of synthetic CF (leucovorin, Lederle). It was therefore decided that the S. faecalis assay using FA for the standard curve should be used to determine the sum of the CF and FA activities. Undoubtedly the validity of the data from such differential assays rests to a great extent on the relative activity of folic acid and CF for the two test organisms. Swendseid et al. (1951a) reported that the growth stimulation of S. faecalis produced by 1 mg. of folinic acid was approximately equivalent to that produced by 1.5 mg. of folic acid. Bond et al. (1949) stated that folinic acid did not appear to be less active than folic acid in promoting growth of S. faecalis while Wieland et al. concluded that leucovorin was only 50-60 per cent. as active as FA, which was more in agreement with Girdwood's (1951) finding that 1.0 mg. of citrovorum factor was equivalent to 0.58 - 0.78 mg. of folic acid in stimulating growth of S. faecalis. Hitchings et al. (1952a) observed that folic acid and citrovorum factor (synthetic "Leucovorin") had approximately the same growth stimulating properties for S. faecalis, which corresponded to the finding

CF		FA
S. faecalis assay		S. faecalis assay
(γ/Gm)		(γ/Gm)
1	(a) .85	.84
	(b) 1.58	1.64
	(c) 2.71	2.54
2	(a) .92	.88
	(b) .86	1.16
	(c) 1.60	1.54
	(d) 2.85	2.70
3	(a) .63	.61
	(b) .59	.56
	(c) .71	.76
	(d) 2.90	2.64

TABLE 15.

Comparison of assays using CF and FA to produce standard curves. Similar results indicate equal growth stimulating properties of synthetic CF (leucovorin) and F.A.

(Homogenates of three separate mouse livers incubated for various periods of time).

of the author in the present study. Although erroneous conclusion may have been arrived in differential assays for FA and CF owing to a lack of exact knowledge concerning the relative activity of CF and FA for the growth of S. faecalis, it is probable that the variable results obtained by several investigators may at least in part be due to individual conditions of assay.

Another difficulty encountered in assay procedures such as this is the use of reference standards consisting of synthetic material of doubtful purity. In the present experiment, however, it was shown by chromatography and bio-autography that the synthetic CF contained no FA and had the same Rf value as naturally occurring CF. Indeed the synthetic and natural material appeared identical.

Another possible source of error is the presence in liver of growth factors other than the folic acid vitamin. Thymidine can promote maximal growth of L. citrovorum but growth produced by thymidine is slow and unlikely to interfere with assays made after twenty hours. Thymine or thymidine may also promote the growth of S. faecalis and Wieland et al. (1952) have shown by chromatography several other factors which may stimulate S. faecalis and L. citrovorum.

Experimental.

A. Microbiological Assay of FA and CF.

Preliminary studies were carried out to determine the best assay procedure and it was concluded that assay of the clear supernatant after boiling the liver homogenate and centrifuging gave the best results. Chicken pancreas was found to be a good source of conjugase and liberated bound forms of vitamin satisfactorily. This method was therefore used in the estimation of 'total' growth factor, although autolytic enzymes by comparison seemed to be equally effective. Recovery of known quantities of CF and FA were found to lead to accurate results. (Table 16).

The mice used in this experiment were (1) normal mice of the inbred Akm stock weighing approximately 20 Gm. and (2) Akm mice with advanced Ak_4 or Ak_4R leukaemia. The mice were made leukaemic in a manner similar to that described in a previous experiment (p. 164). All animals were maintained on a standard Purina Laboratory Chow Diet.

The animals were anaesthetised with ether, decapitated and bled. The livers were quickly removed, weighed and homogenised in 10 mls. disodium phosphate - citric acid buffer at pH 7.0 in a Potter-Elvehjem homogeniser (Potter and Elvehjem, 1936). The homogenate was transferred to a test tube and the muddling tube rinsed with 10 mls.

Sample	CF	CF	FA
	L. citrovorum assay	S. faecalis assay	S. faecalis assay
	(mγ)	(mγ)	(mγ)
1	102	116	109
2	No Growth	117	114
3	49	108	108

TABLE 16.

Assay of solutions of known concentration of CF and FA.

1. CF ⟶ 100 mγ/Stock.
2. FA ⟶ 100 mγ/Stock.
3. Mixed equal vols. CF and FA i.e. 50 mγ each.

buffer. The rinsings were then added to the homogenate yielding a 5 per cent. homogenate. The homogenate was then boiled immediately for five minutes to destroy autolytic enzymes. 10 mls. was transferred to another test tube and chicken pancreas added at a concentration of 100 mg. per Gm. of liver tissue. After covering liberally with toluene this sample was incubated for approximately twenty hours at 37°C. Thereafter it was autoclaved, centrifuged and the supernatant assayed for vitamin content. A further tube containing chicken pancreas alone in phosphate-citric acid buffer was incubated, autoclaved and centrifuged in a similar manner and assayed for its vitamin content, in order that the folic acid and CF content of a given quantity of chicken pancreas be known for purposes of calculating the amount of bound vitamin present in the liver.

The tube containing the remaining 10 mls. of 5 per cent. boiled homogenate was centrifuged and the supernatant assayed. The assay values obtained for this sample was interpreted as representing the amount of the folic acid and CF present in the liver in free form.

The assay procedures for folic acid and CF are outlined as follows:-

Folic Acid. A series of test tubes were partly filled with a measured quantity of Difco folic acid

assay medium and graded dilutions of the treated liver homogenates. After autoclaving the tubes were inoculated with the test organism - in the case of folic acid - S. faecalis (ATCC 8043) and incubated for eighteen hours at 37°C. The turbidity of the tubes was then estimated in a Coleman photoelectric absorptionometer and compared with a standard curve prepared from readings of the turbidity caused by growth of the test organism in tubes containing known quantities of folic acid - 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 4.0, 6.0 mγ folic acid per tube up to a concentration of 20 mγ. The galvanometer reading was set initially, and after each estimation, at zero with the aid of an inoculated reagent blank. The combined folic acid and CF activity present in the liver samples could then be calculated.

Citrovorum Factor. The assay procedure was essentially the same. The medium used was that suggested by Sauberlich and Baumann (Sauberlich and Baumann 1948) with Leuconostoc citrovorum (ATCC 8081) as the test organism. The standard curve was prepared from tubes containing from 0.2 to 2.0 mγ synthetic CF (leucovorin, Lederle). The CF activity in the liver preparations was then calculated.

The assay with S. faecalis was used as a measure of the sum of the activities of FA and CF in the liver preparations as it had previously been demonstrated that the growth of S. faecalis on

medium deficient in FA is stimulated by the presence of either FA or CF. CF alone was estimated as indicated previously by using L. citrovorum this responding only to CF. It was thus possible to calculate the amount of total (conjugate + free) and free FA and CF present in the liver.

Results. (Table 17)

Assay of normal mouse liver indicated that folic acid vitamins were present almost completely in conjugated forms and that growth activity in the incubated specimens was due almost completely to its CF content. The presence of traces of free FA and CF were detected. In the leukaemic mice conjugate CF was similarly predominant. The interesting finding was, however, the definite increase in free vitamin, both FA and CF and particularly FA, as compared with the normal. The mean total vitamin content of the normal liver was found to be 2.25 γ/Gm. (standard error $\pm$ .306). A statistical analysis using Students "t" test showed that in a comparison of the vitamin concentration in the normal and leukaemic livers $t = 2.111$, $n = 18$ $p < .05$. This indicated a barely significant increase in total growth factor concentration per Gm. in the Ak_4 liver as compared with the normal, but in view of the leukaemic liver being approximately twice the weight of the normal mouse liver the total concentration of

Bioassay of Normal and Leukaemic Mouse Liver for Folic Acid (FA) and Citrovorum Factor (CF)

Mouse	CF γ/Gm.		FA γ/Gm.		Total Growth Factor (γ/Gm.)
Normal (Akm)	Free	Conj.	Free	Conj.	
Average liver weight = 1.23 Gm.					
1	.04	1.52	.01	.84	2.41
2	.02	3.16	.05	0	2.90
3	.03	1.81	.07	.02	1.93
4	.09	.72	.07	.01	.89
5	.02	2.84	.01	.18	3.05
6	.07	2.16	.04	0	2.20
7	.01	3.45	.04	0	3.53
8	.09	1.53	.07	0	1.53
9	.20	1.15	.07	.36	1.73
10	.08	2.10	.05	.05	2.28
					Mean = 2.25 γ/Gm. Standard error ± .246
Amethopterin Sensitive (Ak_4)					
Average liver weight = 2.49 Gm.					
1	.10	2.96	.28	.87	4.21
2	.12	1.98	.05	.46	2.62
3	.09	2.37	.14	.89	3.48
4	.19	3.48	.48	.23	4.38
5	.19	2.91	.47	0	3.00
6	.27	3.23	.47	.32	4.30
7	.16	1.80	.32	0	2.11
8	.24	1.14	.38	.79	2.55
9	.22	2.32	.37	0	2.41
10	.12	1.25	.26	.03	1.70
					Mean = 3.08 γ/Gm. Standard error ± .306
Amethopterin Resistant (Ak_4R)					
Average liver weight = 2.14 Gm.					
1					3.4
2					3.1
3					3.7
4					5.4
5					3.8
					Mean = 3.88 γ/Gm. Standard error ± .398

TABLE 17.

growth factor in the leukaemic liver was twice that of the normal, the increase being presumably due to the FA and CF present in the infiltrating leukaemic tissue.

Discussion.

The findings for normal mice corresponded closely to that of Swendseid et al's. (1951a). They disagreed, however, with later workers, Dietrich et al. (1952), and Wieland et al. (1952) who detected a higher degree of FA activity in animal liver tissue. Wieland and his co-workers presented excellent evidence including bio-autographic studies indicating the presence of FA in the incubated specimens. Using similar techniques the author (Fountain 1952a) obtained similar evidence of the presence of FA activity in liver tissue. In the non-incubated specimens the growth stimulating activity seemed to be largely, if not wholly, due to FA. (Table 18)

The interesting finding from this study was that although the total vitamin concentration per Gm. of the leukaemic liver showed only a minimal and barely significant increase to that in the normal liver a definite increase in free CF and FA was present. Using F_1 mice (from C 58 male and Bagg albino female) and Line 1 leukaemia, Hutchinson and Burchenal (1954) later showed that whereas the CF concentration in the normal and leukaemic liver were similar, the total CF content of the leukaemic spleen was approximately three times that of the

A. Plate with No PGA - S.faecalis (8043)

	Sample	Amount on strip ml.	Solvent Flow cms.	Distal Point cms.	Rf
1.	CF - 10 mγ	.01	27.8	11.0	.40
2.	CF - 10 mγ	.01	29.0	11.8	.41
3.	PGA - 10 mγ	.01	25.2	5.2	.21
4.	PGA - 10 mγ	.01	29.3	7.2	.25
5.	Normal liver (Boiled)	.1	26.6	5.0	.19
6.	Normal liver (Incubated)	.01	25.8	4.9 9.5	.19 .37
7.	Normal liver (Boiled)	.05	28.9	5.5	.19
8.	Normal liver (Incubated)	.01	28.6	6.3 11.6	.22 .41
9.	Normal liver + Amethopterin 1γ (Incubated)	.01	28.6	10.3	.36
	B. Plate with No CF - L.citrovorum (8081)				
10.	PGA - 10 mγ	.01	28.5	NO GROWTH	
11.	CF - 10 mγ	.01	29.4	12.6	.43
12.	Normal liver (Boiled)	.05	28.6	NO GROWTH	
13.	Normal liver (Incubated)	.01	28.6	9.6	.34
14.	Normal liver + Amethopterin 1γ (Incubated)	.01	28.6	12.5	.44

TABLE 18.

(See over page)

TABLE 18.

Rf values of synthetic folic acid and CF, and an attempt to demonstrate the FA and CF activity in normal liver tissue, using a combination of paper chromatography and bio-autography. Experimental details similar to those described previously (page 189). Solvent system:- Butanol and acetic acid. Two sets of plates (A) containing Difco folic acid assay agar, inoculated with S. faecalis but not containing folic acid and (B) containing CF assay medium inoculated with L. citrovorum but no added CF. In the incubated specimens two areas of growth were observed giving a 'dumbell' appearance to the growth zone, the upper with an Rf value corresponding to synthetic CF and the lower to synthetic folic acid.

KEY

Samples 1, 2 and 11; CF - 10 mγ/.01 ml.

3, 4 and 10; PGA - 10 mγ/.01 ml.

5, 7 and 12; Normal liver - boiled immediately after removal and homogenising to destroy autolytic enzymes.

6, 8 and 13; Normal liver - incubated for 24 hours to liberate conjugated vitamins.

9 and 14; Normal liver to which amethopterin 1γ added after homogenising. Incubated then for 24 hours.

normal tissue and free CF was higher in the leukaemic tissue. In view of the low content of vitamin in the normal spleen, as compared with the liver, assay of splenic suspensions probably gives a more accurate assessment of the relative concentrations of growth factor in leukaemic tissue. If, however, the increased size of the leukaemic liver in the present study (approximately twice normal) was due to its content of leukaemic tissue, the fact that the concentration per Gm. of vitamin was the same as for normal liver would suggest the FA and CF activity in leukaemic tissue was approximately equivalent to that in normal liver.

Hutchinson and Burchenal (1954) comparing the CF content of Line 1 leukaemic tissue with that of Line 1/A (amethopterin resistant leukaemia) have shown that the free CF in the resistant leukaemic spleen was significantly higher than in the sensitive. Assay of five Ak_4R livers showed a mean concentration of 3.88 γ/G. (standard error $\pm$ .398) total growth factor, which using the Student "t" test showed a significant increase over the concentration in normal liver but comparing it with Ak_4, sensitive, leukaemic tissue the Student "t" test failed to reveal any significant difference in concentration of total vitamin.

The evidence of a high content of free and total vitamin in leukaemic tissue compared

favourably with the findings of Swendseid et al. (1951) who showed that leucocytes of patients with acute leukaemia contained five times the concentration of CF than the normal. The inference was that the increase in CF reflected the high metabolic activity in these tissues and it has been suggested that on this account they become more susceptible to the action of the folic acid antagonists. The possibility that resistance to the therapeutic action of the folic acid antagonists may in part be related to an increased synthesis of CF gained some support from the work of Hutchinson and Burchenal (1954). These workers went further and suggested that one of the actions of amethopterin was to block the conjugase activity of the leukaemic cells and that the conjugase of the resistant cells was less susceptible to the antagonist. Nichol (1954b) has shown, however, that although the resistant strain of S. faecalis had the ability to synthesise more than a hundred times the amount of CF from folic acid than the parent sensitive strain, resistant leukaemic tissue was unable to synthesise CF from synthetic folic acid more readily than the sensitive. The present study has also failed to demonstrate any increased CF activity in the resistant leukaemic tissue as compared with the sensitive. Further work is obviously necessary before any final conclusions

can be drawn relating to the possible role of increased CF synthesis as a mechanism of resistance to the folic acid antagonists.

UTILISATION OF CITROVORUM FACTOR (CF) IN THE LEUKAEMIC AND NON-LEUKAEMIC SUBJECT.

The report by Swendseid et al. (1951) that in patients with acute leukaemia rapidly proliferating leucocytes have a content of CF five times that of the normal white cell and the findings of Swendseid et al. (1952a), and at a later date Girdwood (Girdwood, 1953), that leukaemic patients excreted less of a test dose of folic acid than normal individuals suggested an increased utilisation of folic acid and CF by the leukaemic cell as a consequence of their high metabolic requirements. With this in mind, in conjunction with Drs. Burchenal, Ellison, Murphy and Hutchinson, it was decided to investigate the utilisation and excretion of a test dose of CF in the leukaemic and non-leukaemic child.

Materials and Methods.

A series of thirty-four leukaemic children of which the majority had received no treatment with the folic acid antagonists or other chemotherapeutic agents were tested. A small percentage had developed resistance to amethopterin after previous haematological remissions. Fourteen non-leukaemic children with simple tumours, e.g. lipomata, were selected to act as control subjects. After drawing an initial venous blood sample CF at a dose of 0.05 mg./kg. was given by the intravenous route. Thereafter further specimens were obtained at five, fifteen, thirty and sixty minutes following

injection. The blood was allowed to coagulate and the serum separated by centrifuging at 5,000 revs. per minute for ten minutes. All urine passed following the injection of CF was collected for a period of six hours, the patient being asked to empty the bladder at the end of this time. All urine specimens were stored in brown bottles containing toluene and a phosphate buffer at pH 6.8.

Assays for CF were carried out within the next seventy-two hours, the specimens being stored in a cold room at 4°C. until required.

Serum and urinary estimations of CF were carried out microbiologically in a manner similar to that described previously (page 218) using Leuconostoc citrovorum as the test organism.

Results and Conclusions.

The results are expressed graphically in Fig. 17.

The 'fasting' serum levels of CF in both leukaemic and non-leukaemic subjects showed no significant difference and were within the range of 10 to 30 mγ/ml. The rapidity of uptake of CF following intravenous injection was found to be similar in both leukaemic and control subjects, and after one hour the serum concentration of CF had returned to the 'fasting' level. Patients whose disease had become resistant to treatment showed no variation in pattern.

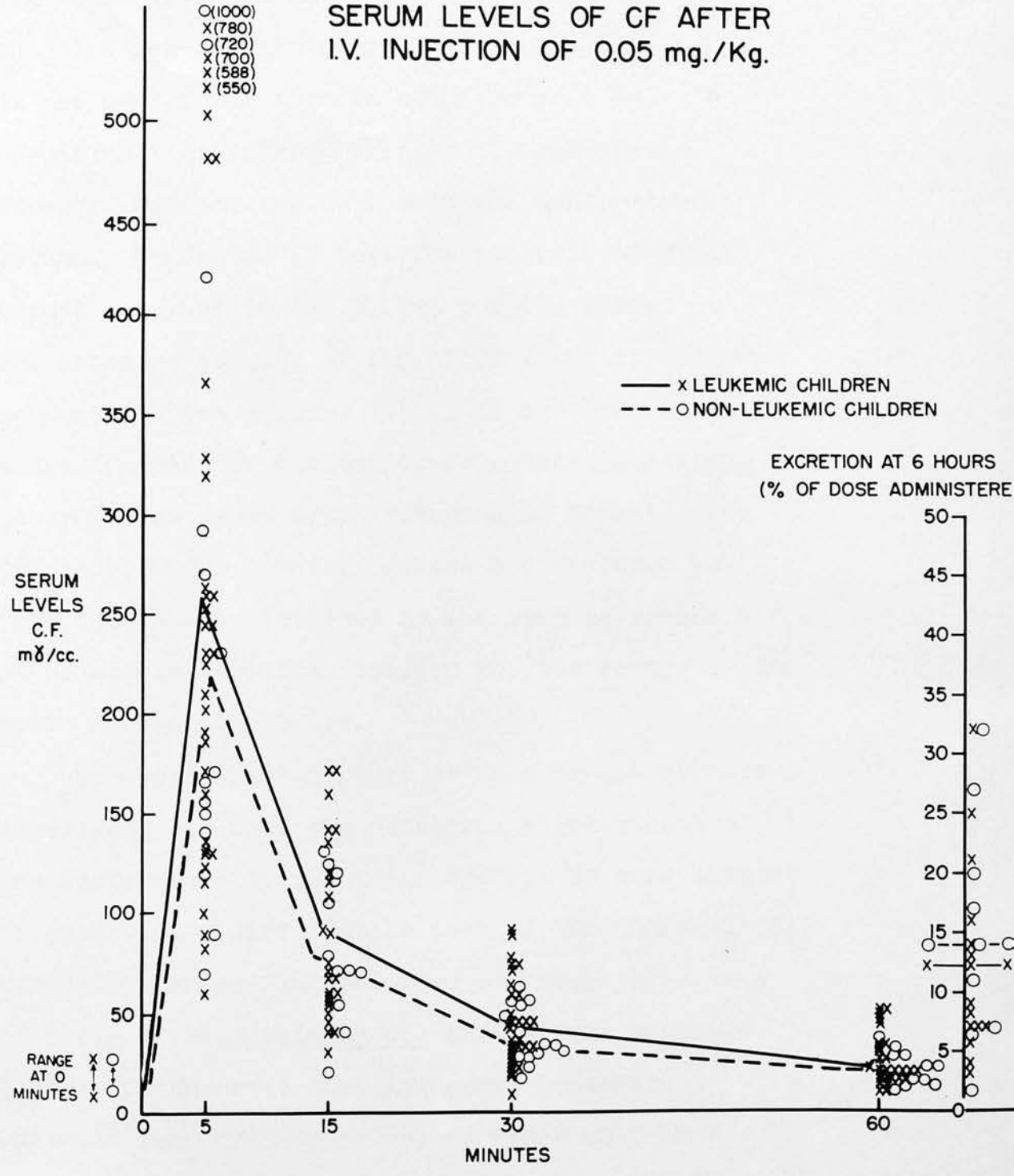

SERUM LEVELS OF CF AFTER
I.V. INJECTION OF 0.05 mg./Kg.
O(1000)
X(780)
O(720)
X(700)
X(588)
X(550)
x LEUKEMIC CHILDREN
O NON-LEUKEMIC CHILDREN
EXCRETION AT 6 HOURS
(% OF DOSE ADMINISTERE
SERUM LEVELS C.F. mγ/cc.
RANGE AT 0 MINUTES
MINUTES
0
5
15
30
60
500
450
400
350
300
250
200
150
100
50
0
50
45
40
35
30
25
20
15
10
5
0

FIG. 17.

Estimation of the urinary excretion of CF six hours after administration in fifteen leukaemic and nine non-leukaemic children showed less than 32 per cent. excretion in both groups. No significant difference in excretion of CF was observed between the leukaemic and non-leukaemic groups. Students "t" test for analysis of small groups resulted in $t = 0.772$, $p < .5$, which indicated an absence of any significant difference between the two groups. Study of a larger series and estimation of the twenty-four hour excretion, although the serum level returned to normal after one hour, might possibly reveal a difference but from the results obtained in the present series any considerable variation between the two groups would appear improbable.

The conclusion arrived at as a result of this experiment was that the metabolic requirements of the leukaemic patients for exogenous CF were little or probably no different to that of the non-leukaemic subjects. These findings were of interest in view of those of Swendseid et al. (1952a) and Girdwood (1953) who observed that leukaemic patients excreted less of a test dose of folic acid than normal individuals. One can only deduce from the present study that if the leukaemic cell utilises more CF than the normal leucocyte the increased demands are obtained from endogenous sources and

that any tissue depletion is balanced by an increased synthesis from folic acid.

EXPERIMENTAL SECTION : SUMMARY

1. The concentration of amethopterin in the serum, liver, kidneys, spleen, lungs and muscle of normal mice was ascertained at intervals following intravenous injection of 0.5 mg./Kg. of the compound.
2. The relative concentration of amethopterin in the tissues appeared to parallel the folic acid and CF content viz. liver, kidneys and spleen in descending order. No significant quantity of amethopterin was found in the lungs or muscle.
3. Amethopterin was found to be still present in the liver and kidneys in relatively large quantities twenty-four hours after injection, although the serum level had reached or approximated zero in four hours.
4. A similar dose of amethopterin given intravenously to Ak_4 and Ak_4R mice resulted in higher concentrations in the blood and a more widespread distribution in the tissues than in normal mice suggesting an affinity of the leukaemic tissue for amethopterin. The possibility that this may, however, have been due to impaired renal function was mentioned.
5. No significant difference in the distribution and concentration of amethopterin in the Ak_4 and Ak_4R leukaemic tissues was observed.
6. Amethopterin given parenterally in various doses to normal mice resulted in the retention of a more

or less constant amount of amethopterin in the liver for a period of at least three weeks. A gradual slow decrease in the renal concentration of amethopterin was observed.

7. CF at doses ranging from 0.5 to 30 mg./Kg. intraperitoneally failed to have any detectable effect on the concentration of amethopterin in the liver and kidneys but caused a significant rise in the serum level of amethopterin. This stable incorporation of amethopterin was suggested as the likely explanation of the inability of large doses of the folic acid vitamins to overcome the toxicity of the folic acid analogues.

8. Ak_4R leukaemic cells did not appear to metabolise amethopterin in vivo, although the possibility that amethopterin might be converted to a weaker antagonist, N10-methyl pteroylglutamic acid (methopterin) was not eliminated at that stage.

9. Amethopterin when given intravenously in doses of 0.05 mg./Kg. to human subjects with acute leukaemia, including patients with amethopterin sensitive and resistant leukaemia, and to a control group with advanced cancer showed no difference in serum level pattern. In both groups less than 60 per cent. was excreted in a six hour period. No obvious difference in the rate of excretion between the two groups was observed, but a larger series was recommended before final proof could be obtained.

10. Rf values of various folic acid analogues were determined using a combination of paper chromatography and bio-autography. A butanol and acetic acid solvent system was used.

11. Using a butanol-acetic acid solvent system analogues containing a methyl group in their molecule were found to have similar Rf values.

12. Following preliminary studies the antimetabolite present in normal mouse tissues following the injection of amethopterin was identified by means of paper chromatography and bio-autography, using three different solvent systems. The antimetabolite was found to have the same Rf value as amethopterin in the three solvent systems, and it was concluded that amethopterin remained unchanged in the normal mouse tissues.

13. Using mice with L 1210 and L 1210/A leukaemia it was shown that amethopterin remained unchanged in the livers, tumours and spleens of both amethopterin sensitive and amethopterin resistant leukaemias.

14. It was concluded that resistance was not due to an increased ability to detoxify the antagonist by way of deamination or demethylation. Certain drawbacks of the technique employed were mentioned.

15. A method for estimating the folic acid and CF activity in mouse liver was described.

16. Assay of normal mouse liver indicated that

folic acid vitamins were present almost completely in conjugated forms. Growth activity in the incubated specimens was due almost completely to its CF content. Traces of free FA and CF were detected.

17. Using chromatographic and bio-autographic techniques evidence was obtained supporting the concept that FA is present in mouse liver.

18. An increase in free FA and CF, particularly FA, was observed in leukaemic liver (Ak_4) as compared with the normal.

19. The total FA and CF activity in the Ak_4 liver, expressed as γ/Gm. liver tissue was approximately the same as in the normal liver. The total expressed as γ/organ was approximately twice the normal. It was suggested that the increased total activity in the leukaemic liver was probably due to the high vitamin content of the infiltrating leukaemic tissue.

20. No evidence was obtained to support the suggestion that resistance to the therapeutic action of the folic acid antagonists may be due to increased synthesis of CF. CF activity in the Ak_4 and Ak_4R mouse liver showed no significant difference.

21. Serum levels of CF following an intravenous test dose of CF at 0.05 mg./Kg. were similar in both leukaemic and non-leukaemic children.

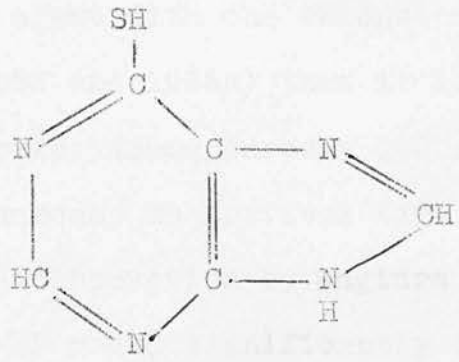
22. The six hour excretion of CF following the test

dose showed no significant difference in a limited series of leukaemic and non-leukaemic children. It was concluded that there was probably no increased demand for exogenous CF by leukaemic cells.

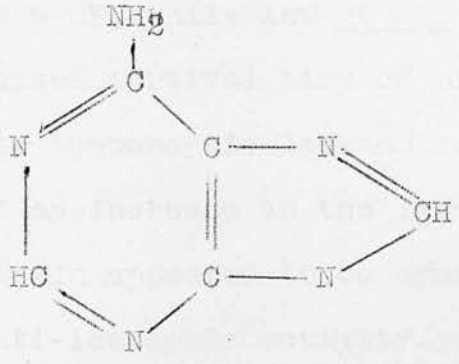
THE TREATMENT OF LEUKAEMIA AND SOME ALLIED DISORDERS WITH 6-MERCAPTOPURINE

The discovery that antagonists of folic acid were able to induce remissions in a proportion of children with acute leukaemia, resulted in an intensive search for therapeutically active analogues of other nucleic-acid precursors. Of the purine and pyrimidine derivatives discovered to date the most therapeutically successful has been the new compound, 6-Mercaptopurine (6-MP). This substance was synthesised and developed by Hitchings and his associates (Elion et al. 1951 and 1952, Elion and Hitchings 1953) during a comprehensive study of the roles of pyrimidine and purine bases in nucleic acid metabolism and at the same time in co-operation with the Sloan-Kettering Institute, New York, in an attempt to discover new compounds with anti-tumour activity.

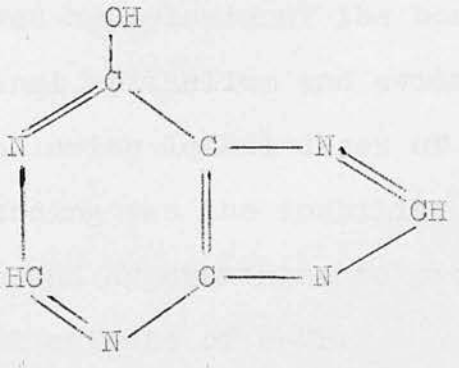
6-Mercaptopurine (Elion et al. 1952) is an analogue of the nucleic acid constituent adenine and the physiological purine base hypoxanthine and differs only in chemical structure (Fig. 18) by substitution of an SH group for the amino and hydroxyl groups respectively. It is one of a large series of such analogues studied by Hitchings and his colleagues (Hitchings et al. 1945, 1950, 1950a, 1952; Falco et al. 1951) and tested for chemotherapeutic activity in view of its property of interference with nucleic acid biosynthesis. It initially stimulated interest as a possible



6-Mercaptopurine



Adenine



Hypoxanthine

Fig. 18.

chemotherapeutic agent with the demonstration by Clarke et al. (1953 and 1953a) that it inhibited the growth of the Crocker mouse sarcoma 180 and resulted in an increase in survival time. An extension of this observation by Sugiura (1953) disclosed that 6-MP could significantly inhibit the growth of several other mouse tumours. Biesele (1954) reported growth inhibition of mouse tumours in tissue culture by 6-MP, while Law et al. (1954) observed an increased survival time of mice with transplanted acute lymphocytic leukaemia. Skipper (1954) also noted an increase in the life span of 6-MP and amethopterin appeared to be synergistic with regard to anti-leukaemic activity.

The toxic effects of 6-MP in animals have been studied by Clarke et al. (1953a) and Philips et al. (1953) who observed hypoplasia of the bone marrow, damage to intestinal epithelium and evidence of liver necrosis following lethal doses of the drug. An interesting finding was the inability of adenine guanine, xanthine and hypoxanthine to protect mice against the lethal effects of 6-MP.

The exact mechanism of action of 6-MP in patients with leukaemia remains unknown. It has been shown by Elion et al. (1951 and 1953) by microbiological studies that the growth of Lactobacillus casei is inhibited by 6-MP, and growth can be restored by any of the four physiological

purine basis - adenine, guanine, xanthine and hypoxanthine. It has also been demonstrated that growth of Streptococcus faecalis (ATCC 8043) is inhibited by 6-MP and that this can be reversed by adenine, guanine, xanthine and hypoxanthine (Sullivan 1953). The conclusion drawn is that 6-MP in bacterial systems acts as a purine antagonist, although definite proof of a similar mode of action in animal tissues has not been demonstrated.

Large amounts of folic acid or citrovorum factor are unable to prevent the toxicity of 6-MP in mice (Philips et al. 1953) or the therapeutic effect of 6-MP on transplanted leukaemia in mice (Burchenal et al. 1953a). While there is no direct evidence that the mode of action against the leukaemic cell in the human subject is the same as in bacterial systems or in animals, the weight of evidence, including the finding of activity in anti-folic resistant mouse and human leukaemias (Law 1953; Burchenal et al. 1953), supports an anti-purine rather than an anti-folic acid mode of action. The finding by Law et al. (1954) that 6-MP resistant mouse leukaemia has actually an increased sensitivity to amethopterin lends further support to this belief.

By using a 6-MP resistant strain of Lactobacillus casei Elion et al. (1953a) have

obtained information suggesting that a hypoxanthine containing metabolite may be an intermediate in the conversion of adenine to guanine and that 6-MP may act by interfering with the conversion of this substance to a corresponding guanine containing substance. Hutchison (1954) used three strains of S. faecalis, S. faecalis 8043, S. faecalis/A (amethopterin resistant) and S. faecalis/MP (6-MP resistant), in an attempt to obtain information concerning the mode of action of 6-MP in bacterial systems. The possibility that in these micro-organisms the metabolism of xanthine or a closely related compound was interfered with, was suggested.

The metabolism of 6-MP in animals and man has been investigated with the aid of radioactive isotopes. As a preliminary to studies in the human subject Elion et al. (1954) investigated the metabolic fate of 6-MP in mice using C 14 and S 35 labelled 6-MP. They observed that the antimetabolite was rapidly metabolised with the excretion of 6-thiouric acid and other unknown products. Following intraperitoneal injection the drug was found to be in highest concentration in the gut followed by kidney, liver, blood, lung, sternum, spleen and brain. Three hours after injection radioactive material was found to be incorporated into both the ribonucleic acid (RNA) and

desoxyribonucleic acid (DNA) fractions of the nucleic acids. Hamilton and Elion (1954) and Hamilton, Elion and Bases (1954) later studied the fate of 6-MP in human subjects with acute leukaemia and chronic myeloid leukaemia with the aid of intravenously administered S 35 labelled 6-MP. The results in both types of leukaemia were essentially similar. A rapid metabolism of the drug occurred, the urinary findings indicating a rapid and extensive transformation of 6-MP. As in the mouse 6-MP was found to be excreted partly as thiouric acid and unknown products but in contrast sulphate excretion was more prominent in man. From preliminary data these authors believe that, as in animals, 6-MP becomes incorporated into nucleic acids. This is suggestive but not proof of the mechanism of growth inhibition. Such studies as these are of considerable importance, for not only do they assist in determining the metabolism and site of action of chemotherapeutic agents, but may also increase our knowledge concerning the metabolism of nucleic acids.

The first report of the clinical use of 6-MP was by Burchenal et al. (1953) who showed that good clinical and haematological remissions occurred in fifteen out of forty-five children with acute leukaemia and in another ten patients, partial remissions and clinical improvement were observed. The same authors described occasional remissions

in adult patients with the disease, and encouraging results in patients with chronic myeloid leukaemia. Chronic lymphatic leukaemia did not appear to respond to 6-MP therapy and in thirty-five patients with Hodgkin's disease, reticulum cell sarcoma and miscellaneous forms of cancer no improvement, at doses which produced bone marrow depression, was observed. Fountain (1954, 1955) also reported remissions in acute leukaemia following 6-MP therapy and noted the beneficial effects of "maintenance therapy" in chronic myeloid leukaemia. At a conference on 6-Mercaptopurine held by the New York Academy of Sciences in 1954, many investigators reported the effects of treatment of acute leukaemia with this new antimetabolite (Conference on 6-Mercaptopurine, 1954). An analysis of the results show that of three hundred and twenty-four children treated with 6-MP by fourteen different investigators 60 per cent. showed clinical and haematological improvement, 35 per cent showing a complete disappearance of the clinical manifestations of the disease with a return of the blood picture to apparent normality. The effect of treatment on adults was less marked. Definite improvement was, however, observed in 40 per cent of two hundred and four patients, but only 12 per cent developed complete remissions. Few patients with chronic

RESULTS OF TREATMENT OF ACUTE LEUKAEMIA WITH 6-MERCAPTOPURINE

A. CHILDREN

Investigator	No. of Patients	Complete Remissions	Partial Remissions	No Response
Burchenal	87	41	16	30
Bernard	34	11	5	18
de Asúa	11	6	1	4
Farber	60	9	19	32
Pierce	22	6	7	9
Rundles	8	3	2	3
Hyman	22	7	6	9
Fountain	4	1	1	2
Rosenthal	12	5	2	5
Hill	12	7	4	1
Sawitsky	14	4	5	5
Gaffney	23	10	9	4
Petrakis	7	-	5	2
Wilson	8	3	-	5
	324	113 (35%)	82 (25%)	129 (40%)

60%

B. ADULTS

Burchenal	50	7	10	33
Hall	25	3	11	11
Bernard	8	-	1	7
de Asúa	5	1	1	3
Rundles	10	2	6	2
Hyman	12	2	2	8
Fountain	5	2	1	2
Rosenthal	35	1	12	22
Hill	16	6	3	7
Sawitsky	6	1	-	5
Gaffney	13	-	2	11
Petrakis	7	-	3	4
Wilson	12	-	7	5
	204	25 (12%)	59 (28%)	120 (60%)

40%

(Data compiled from a Symposium on 6-Mercaptopurine held in New York on April 30 and May 1, 1954, and published in the Annals of the New York Academy of Sciences. Vol. 60, Art. 2. 1954).

TABLE 19.

myeloid leukaemia treated with 6-MP were reported. Most observers agreed that temporary improvement followed its administration but relapse tended to follow withdrawal of the drug. No details of the effect of maintenance therapy were reported.

The present study is based on experience gained in the treatment of fifty patients between June 1953 and September 1955 (Table 20). Of these, twenty-eight had acute leukaemia, sixteen chronic myeloid leukaemia, two chronic lymphatic leukaemia, two multiple myeloma, one mycosis fungoides and one erythroderma secondary to an underlying reticulosis of undetermined type.

DIAGNOSIS	TOTAL NUMBER
ACUTE LEUKAEMIA	
ADULTS	11
CHILDREN	17
CHRONIC MYELOID LEUKAEMIA	16
CHRONIC LYMPHATIC LEUKAEMIA	2
MULTIPLE MYELOMA	2
MYCOSIS FUNGOIDES	1
RETICULOSIS of SKIN	1
	50

TABLE 20.

PLAN of TREATMENT

Patients were unselected and included all

those whom the author was requested to treat. Those with acute leukaemia received no other form of chemotherapy prior to or as a supplement to treatment with 6-MP in the first instance. Following relapse the effect of other forms of therapy either alone or in combination with 6-MP was observed in some instances. Of the sixteen patients with chronic myeloid leukaemia eleven had received no previous treatment, four had initially received deep x-ray therapy and one urethane. With the exception of one patient with mycosis fungoides who had several courses of x-ray treatment none of the other patients had received any other form of treatment prior to 6-mercaptopurine.

Blood transfusions and antibiotics were prescribed as the need arose.

The majority of patients received the initial course of treatment in hospital and clinical and haematological examinations were performed at frequent intervals, usually daily or on alternate days. In patients with acute leukaemia bone marrow examinations were performed initially and thereafter at intervals during treatment. Patients who improved sufficiently were later followed-up in the out-patient department and had blood examinations at weekly or fortnightly intervals.

DOSAGE OF 6-MERCAPTOPURINE.

The dosage of 6-MP was based on that suggested

by Burchenal *et al.* (1953), who recommended an initial dose of 2.5 mg. per Kg. body weight, calculated to the nearest 25 mg. amount. For purposes of simplification children around five years of age were started on 50 mg. daily, at ten years 100 mg., and adults 150-200 mg. The compound was administered as 50 mg. scored tablets in divided doses of 25 or 50 mg., given over the twenty-four hour period. The same dosage scheme was used both for patients with acute and chronic leukaemia apart from two instances (cases 9, 26) where an attempt was made to induce rapid responses with initial large doses of 1 G. in the first twenty-four hours.

A failure to show a response to 6-MP treatment resulted in the dose being increased. In view of the latent period before the drug becomes effective the dose was not as a rule increased during the first three weeks. A fall to leucopenic levels necessitated withdrawal of the drug, or reducing the dosage.

In patients with acute leukaemia who responded satisfactorily to treatment, it had to be considered whether or not they should be given a daily "maintenance" dose of the drug. Although much difference of opinion exists as to the value of continuous treatment during periods of remission, it has been the practice in this series in all but a

few patients to prescribe a small daily maintenance dose of 6-MP. From early observations there appeared little doubt that for continued benefit in patients with chronic myeloid leukaemia a daily maintenance dose of 6-MP was essential. It has, therefore, been the custom in chronic myeloid leukaemia to prescribe a daily maintenance dose at the outset, approximately half or a quarter the initial dose, usually about 50 to 100 mg. Thereafter the maintenance dose was adjusted until the optimum dose was reached which would maintain the clinical and haematological state as near to normality as possible.

TOXIC MANIFESTATIONS.

Clarke et al. (1953a) and Philips et al. (1953, 1954) have reported the toxicological and pathological effects in animals. In mice a decrease in granulocytes and reticulocytes in the peripheral blood was observed in addition to anorexia and loss of weight. The bone marrow was found to be hypoplastic at autopsy. Similar evidence of bone marrow injury was observed in rats and dogs following toxic doses of the drug in addition to damage to the intestinal mucosa and evidence of impairment of liver function.

Unlike the majority of chemotherapeutic agents used in the management of patients with leukaemia, 6-MP has a low degree of toxicity. In excessive

doses it is liable to produce severe depression of bone marrow activity with anaemia, leucopenia and thrombocytopenia and their obvious consequences. It should be remembered, however, that a fall in the leucocyte count in the leukaemic subject is a therapeutic response and in the author's experience a fall to leucopenic levels is an aim of treatment in patients with acute leukaemia, and should not give rise to undue anxiety. The leukaemic cell does not function as a normal cell and it appears that only by its removal can a return to normal bone marrow function take place. The dividing line between toxic and therapeutic effect in acute leukaemia therefore seems slender.

Thrombocytopenia, attributable to 6-MP, was observed in only one instance, in a patient with multiple myeloma (case 47). Again it should be mentioned that in the experience of the author a low platelet count in acute leukaemia with or without haemorrhage is not an indication to interrupt treatment, for improvement in platelet production is to be expected only when the leukaemic process is controlled. The possibility of over-dosage leading to thrombocytopenia and bleeding in patients with chronic myeloid leukaemia should, however, be guarded against.

Bone marrow examination, when the leucocytes in the peripheral blood were reduced as a result of

treatment, often showed a hypoplastic picture. Megaloblastic erythropoiesis, which is an occasional sequel of treatment with the folic acid antagonists and the diamino-dichlorphenlpyrimidines, was not observed.

Ulceration of the mouth, which commonly follows treatment with aminopterin and amethopterin, was not observed.

Burchenal et al. (1953) have stated that gastro-intestinal symptoms may occur, but whereas occasional nausea, vomiting and diarrhoea have been seen (cases 12, 16 and 24) on no occasion were these symptoms definitely attributable to the drug and treatment had not to be interrupted on their account.

Experience to date has revealed no toxic symptoms consistently associated with 6-MP, and the only danger to guard against is its depressant effect on the bone marrow. With experience and careful regulation of dosage this in the author's experience should not give rise to anxiety.

RESULTS OF TREATMENT.

A. ACUTE LEUKAEMIA.

It has become the custom to classify remissions occurring during the course of treated acute leukaemia, complete or partial. Such a classification is based largely on the degree of improvement of the bone marrow and peripheral blood picture. The term

'complete' remission in its strictest sense would seem incorrect as it is doubtful whether treatment, which can only at present produce temporary improvement, ever completely rids the body of leukaemic cells, although clinically there may be no obvious evidence of the disease. The criteria on which a complete remission was based in this series consisted of -

(a) a disappearance of all clinical evidence of disease and a return of the patient to full activity.

(b) a return of the peripheral blood picture to normal, and

(c) a return of the bone marrow to near normality with a disappearance of blast cells or a reduction to less than 10 per cent.

In the case of patients with acute lymphoblastic leukaemia, as a remission developed, it was difficult to determine the difference between lymphocytes normally present in the bone marrow and leukaemic cells. It was therefore decided to consider patients with this type of leukaemia to be in full remission when (a) and (b) were present and when the bone marrow showed less than 30 per cent. lymphocytes and blast cells.

A partial remission was said to be present when considerable clinical and haematological improvement occurred but which fell short of the criteria of a

complete remission.

In some instances, in the interest of the patient, repeated bone marrow punctures were not performed. Remissions in these cases were therefore based largely on clinical and peripheral blood findings. On no occasion, however, was a patient said to be in complete remission without observing changes in the differential bone marrow nucleated cell count in keeping with this classification.

Of the twenty-eight patients with acute leukaemia treated with 6-MP (Table 21), seventeen were children under the age of fifteen years and eleven were adults. Included was one infant with congenital leukaemia (case 19) and an adult with leukanaemia (case 23). Six of the children (cases 1, 2, 3, 4, 5, 6), between the ages of two and ten years developed complete remissions while another six (cases 10, 11, 12, 13, 14, 15) showed improvement in keeping with a partial remission. Five failed to respond to treatment (cases 7, 18, 19, 20, 21), two of which (cases 18 and 19) died within a few days of starting treatment.

Of the eleven adults, three developed complete remissions (cases 7, 8, 9), two partial remissions (cases 16, 28) while six (cases 22, 23, 24, 25, 26, 27) failed to show any clinical or haematological improvement as a result of therapy. Two of the

No. of Patients	Remissions		No Response	
	Complete	Partial	Adequate Therapy	Inadequate Therapy
Adults 11	3	2	4	2
Children 17	6	6	3	2
Total 28	9	8	7	4

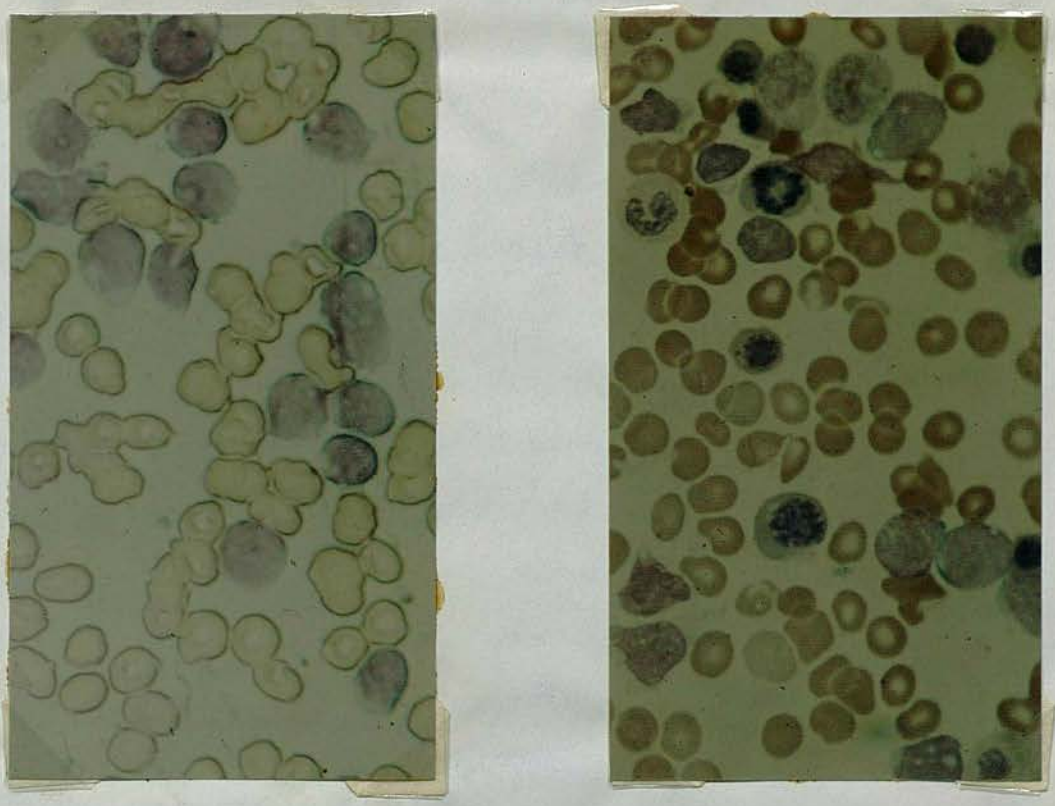
TABLE 21.

failures (cases 25, 26) had courses of treatment of only eight days and three days respectively and in view of the known latent period before the drug becomes effective, were probably uninfluenced to any extent by treatment.

The results in this relatively small series compares favourably with those obtained by other workers. The figures suggest that approximately 70 per cent. of children with acute leukaemia show clinical and haematological evidence of improvement, of varying degree and duration. Previously it has been mentioned that in a series of three hundred and twenty-four leukaemic children treated with 6-MP and reported at a Conference on 6-Mercaptopurine (1954), 60 per cent. were found to improve following treatment. The less satisfactory effect of 6-MP on the leukaemic process in adults was also voiced at the above Conference. The results in the present series support this finding, but suggest that 6-MP is an improvement over the folic acid antagonists in the treatment of adult acute leukaemia.

In both children and adults a delay was noted before the drug became effective. This latent period was of variable duration but usually between seven and twenty-eight days. The first indication of a response to treatment was a fall in the leucocyte count to a leucopenic level. In two instances of aleukaemic leukaemia (cases 8 and 15)

further reduction in the white cell count did not follow treatment but both patients developed remissions, the leucocytes increasing as improvement occurred. It was of interest that patients with high initial white cell counts showed responses earlier than those with normal or slightly raised counts. Thus, in case 16, the white cells fell from 60,000 to 6,800 per c.mm. after ten days treatment; in case 13, from 250,000 to 30,000 per c.mm. in five days; in case 12, from 137,600 to 40,800 per c.mm. in four days and after eleven days to 1,700 per c.mm., and in case 11, from 320,000 to 3,000 per c.mm. in eight days. All these patients received the routine dosage of 6-MP. Many haematologists believe that a high leucocyte count in acute leukaemia is a sign of a poor immediate prognosis and indicative of increased metabolic and mitotic activity. That this is probably the case is suggested by the susceptibility of these cells to 6-MP as indicated above. Early in this therapeutic trial a male patient (case 9) with extremely acute myeloblastic leukaemia was given an initial "loading" dose of 1 G. of 6-MP, in an attempt to increase the rate of action of the drug. The leucocyte count thereafter fell rapidly and after five days had fallen from 129,000 to 4,800 per c.mm. In the light of further experience it seems doubtful whether the initial high dose of 6-MP was any more beneficial



(A) (B)

FIG. 19. Sternal bone marrow of a child with acute lymphoblastic leukaemia (Case 2) (A) before treatment, and (B) 5 weeks after the start of treatment with 6-mercaptopurine.

than the routine dose. Another patient (case 26) was also given a "loading" dose of 6-MP, but she died two days later without any effect of treatment being observed.

A fall in the white cells to leucopenic levels indicated a reduction in the dose of 6-MP or a temporary cessation of therapy. In some instances (cases 1, 7) the leucopenia persisted for up to three weeks after stopping treatment.

A fall in the leucocyte count was almost invariably associated with a reduction in size of the spleen, liver, lymph glands and other manifestations of the disease. These changes were not, however, necessarily indicative of subsequent remissions, although frequently associated with symptomatic improvement. In patients developing remissions, haematological improvement also occurred. The onset of definite haematological improvement appeared at variable intervals after the start of treatment, ranging in children from fourteen to fifty-two days (cases 11 and 3 respectively) and in adults from sixteen to thirty-five days (cases 9 and 8). The average delay before both clinical and haematological improvement was observed in the seventeen patients - adults and children - who developed complete or partial remissions was approximately twenty-seven days.

The bone marrow during the leucopenic phase was

generally hypoplastic. As improvement in the blood picture occurred, the percentage of blast cells in the bone marrow diminished accompanied by increased erythroid and myeloid activity. This was reflected in the peripheral blood by a rising haemoglobin and erythrocyte count, and an increase in platelets, together with a rise in the proportion of mature polymorphonuclear cells in the peripheral blood at the expense of leukaemic cells. The persistence of blast cells in the bone marrow and peripheral blood and an associated rise in the leucocyte count was an indication to increase the dose of 6-MP.

Table 22 indicates the extent of improvement observed in the seventeen patients responding to treatment. In some instances partial and complete haematological remissions were indistinguishable clinically. Fourteen of the seventeen showed complete regression of lymph nodes, hepatomegaly and splenomegaly, etc. In three (cases 12, 14, 16) a persistent splenomegaly remained and in another (case 10) cervical lymph node enlargement remained obvious. All experienced a definite increasing feeling of well being and returned at least temporarily to full activity. Several of the children went back to school. All remissions were associated with haematological improvement and in all but three (cases 15, 16 and 28) the haemoglobin rose to over 13.0 G. The platelet count rose from

TABLE 22.

Patients with Acute Leukaemia showing a Response to 6-Mercaptopurine

Case No.	Age (years) Sex	Status before 6-MP Therapy: Clinical Type of Leukaemia L. Lymphoblastic M. Myeloblastic Mo. Monocytic (1)	Status before 6-MP Therapy: Haematological: Peripheral Blood Hb. (Gm.)	RBC x 10^6	WBC x 10^3	Polys (%) (2)	P's x 10^3	Bone Marrow % Blast Cells / % Erythroid Cells (3)	6-MP Therapy: Days before response (4)	Total Dose before response (4)	Status after 6-MP Therapy: Maximum Improvement: Clinical: General	Clinical: Regression of lymph nodes, spleen etc.	Haematological (5): Peripheral Blood Hb. (Gm.)	Polys (%)	P's x 10^3	Bone Marrow % Blast Cells / % Erythroid Cells (3)	Duration (wks.) (6)	Follow up after Relapse (7)
1	3½ M	Subacute (L)	6.0	1.9	4.8	1	60	100/0	35	1.0	+	+	14.5	67	214	23.6/30	9	16 wk. remission on 6-MP. No ACTH response. S.T. 18½ months.
2	4½ F	Subacute (L)	5.9	1.9	9	11	64	91.6/6	33	4.07	+	+	13.3	57	250	15/48	36	Sympt. improvement on 6-MP. No ACTH response. S.T. 20¼ months +
3	6½ F	Subacute (L)	5.2	1.51	12.4	5	-	95/1.5	52	1.525	+	+	14.8	70	260	26/28.4	38 +	(Continues in remission. S.T. 12½ months +
4	2½ F	Acute (L)	2.8	.945	3.8	2	-	100/0	33	1.95	+	+	14.4	68	270	20/36	52 +	(Continues in remission. S.T. 15 months +
5	2 M	Subacute (L)	4.48	1.4	3.2	24	90	88.5/0.5	35	1.325	+	+	16.8	83	350	19.5/15.5	13	No response to 6-MP. S.T. 6½ months.
6	10½ F	Subacute (L)	8.7	2.9	2.7	17	89	94.6/2.6	21	2.1	+	+	15.5	68	250	30/44.2	10	No response to 6-MP. S.T. 7½ months.
7	44 M	Acute (Mo.)	8.5	2.8	6.8	41	66	59/8.5	29	5.3	+	+	13.3	80	250	0/29	7	No response to 6-MP. S.T. 5 months.
8	62 F	Subacute (L)	7.8	2.8	1.9	36	119	66.4/16.8	35	4.2	+	+	14.6	67	275	20/34.8	33	No response to 6-MP. S.T. 15½ months.
9	41 M	Acute (M)	11.8	3.8	129	2	35	85.2/0.4	16	3.0	+	+	14.1	84	450	3.2/39.2	9½	No response to 6-MP. S.T. 5 months.
10	9 F	Subacute (L)	11.9	4.0	3.4	4	35	96/2.8	32	2.45	+	± (Lymph nodes +)	14.8	49	274	44/30	36	Response to ACTH S.T. 15½ months.
11	3 M	Acute (L)	7.6		320	1	10	-	14	0.7	+	+	11.8	65	220	-	22	No response to 6-MP or ACTH. S.T. 7¼ months.
12	3¾ M	Acute (L)	6.2	2.1	137.6	5	95	91.2/0.8	21	1.05	+	± (Spleen +)	14.8	75	145	20/34.5	2½	No response to 6-MP. S.T. 3¾ months.
13	3½ F	Acute (L)	5.8	1.9	250	1	15	99/0	17	.675	+	+	13.2	78	250	-	8½	No response to 6-MP. Improvement with 6-MP + ACTH. S.T. 7½ months.
14	14 M	Acute (L)	14.7	5.0	24.6	51	210	78.8/10.0	14	2.1	+	± (Spleen +)	14.7	68	300	21/32.5	6½	Improvement with 6-MP + ACTH. S.T. 5¼ months.
15	5 F	Subacute (L)	11.9	3.8	2.4	39	275	96.8/2	21	1.05	+	+	12.2	61	285	29/5	7½	No response to 6-MP. S.T. 6½ months.
16	30 F	Acute (Mo.)	6.9	2.26	69	21	101	84/2.4	21	2.3	+	± (Spleen +)	11.1	42	180	28.5/14.5	2	No response to 6-MP. S.T. 4 months.
28	34 F	Acute (M)	5.1	1.4	40.0	11	30	95/0	13	2.6	+	+	10.4	50	175	34/20	7	S.T. 4¾ months.

																			S.T. $6\frac{1}{2}$ months.
16	30	F	Acute	(Mo.)	6.9	2.26	69	21	101	84/2.4	21	2.3	+	± (Spleen +)	11.1	42	180	28.5/14.5	2	No response to 6-MP. S.T. 4 months.
28	34	F	Acute	(M)	5.1	1.4	40.0	11	30	95/0	13	2.6	+	+	10.4	50	175	34/20	7	S.T. $4\frac{3}{4}$ months.

KEY

(1) Acute indicates severely ill patient with bleeding.
Subacute indicates absence of severe symptoms, e.g. bleeding, and good general condition.

(2) Mature polymorphonuclear cells. Remaining white cells, mainly lymphocytes or blasts.

(3) In acute lymphoblastic leukaemia all lymphoid cells counted as leukaemic cells.

(4) From start of therapy to appearance of haematological improvement.

(5) Max. recorded improvement.

(6) Duration of remission since response.

(7) S.T. - Survival Time since onset of symptoms.

critical levels to within normal limits in all instances and in all but three patients the platelets rose to over 200,000 per c.mm. The proportion of mature polymorphs in the peripheral blood increased in all patients. In fifteen the percentage rose to over 50 per cent., in the other two to 42 per cent. and 49 per cent. respectively (cases 16, 10). Leukaemic cells in the bone marrow decreased in numbers associated with a re-appearance of polymorph and erythrocyte precursors. The maximum recorded improvement in bone marrow cytology is shown in Table 22.

Age and Remissions.

From the data available children appear more likely to respond to 6-MP therapy than adults (Table 21). This is in agreement with other workers (Burchenal 1953; Conference on 6-MP, 1954). Remissions of varying degree were observed, however, in over one third of adult patients.

Type of Leukaemia and Remissions.

In a small series it is not possible to reach a definite conclusion as to which cytological type of acute leukaemia responds best to treatment. Of the seventeen children, fifteen had lymphoblastic and two myeloblastic leukaemia. Neither of the latter responded to treatment. Of the eleven adults, four had monocytic, six myeloblastic and one lymphoblastic leukaemia. Of the three complete remissions observed

in adults, one occurred in the patient with lymphoblastic leukaemia and the remaining two in patients with myeloblastic and monocytic leukaemia respectively. The impression gained was that the lymphoblastic type of leukaemia may be more susceptible to 6-MP, and the higher incidence of this type in children may be a factor associated with the higher rate of remissions in children. On the other hand, the increased incidence of lymphoblastic leukaemia in children may explain the suggested higher incidence of remissions in this type of leukaemia.

Duration of Remissions.

The duration of the remissions was calculated from the time clinical and haematological improvement was first observed and was found to vary considerably (Table 22). The longest remission was in a child (case 4) who up to the present, has been in complete remission for fifty-two weeks while another (case 3) is still in remission after thirty-eight weeks. Two children (cases 2 and 10) had remissions lasting thirty six weeks, while one adult, aged 62 years, (case 8) showed a clinical and haematological remission of thirty-three weeks duration. Of the remaining patients, four out of nine children had remissions lasting longer than ten weeks, but none of the three adults had remissions exceeding ten weeks duration.

The effect of chemotherapy after relapse and in patients failing to show an initial response to 6-MP.

Only one patient (case 1) developed a second complete remission on 6-MP therapy. Cases 7, 8, 9, 10, 12, 15 and 16 all failed to respond to 6-MP alone a second time, while case 2 obtained only some symptomatic benefit. A combination of 6-MP and ACTH resulted in temporary improvement in case 13 and 14 while one (case 10) responded to ACTH alone after becoming refractory to 6-MP therapy.

Five children and six adults failed to obtain any benefit from 6-MP therapy. Two children (cases 18 and 19) and two adults (cases 25 and 26) died within a week of starting treatment and were considered to have had inadequate courses of treatment. One child (case 17) rapidly developed a complete remission following ACTH therapy after failing to respond to prolonged treatment with 6-MP. The possibility that 6-MP may have influenced the leukaemic process in this patient cannot altogether be ignored. ACTH was ineffective in one child and two adults (cases 20, 22 and 27). A total of nine patients, all of whom were resistant to 6-MP therapy, were treated with ACTH or cortisone and it was of interest to note that in four a response to ACTH or cortisone occurred.

Although eleven patients, six adults and five children, failed to develop remissions as a consequence of 6-MP therapy, some effect of treatment was noted in eight, viz. a reduction in leucocyte count and diminution in size of the spleen or other objective evidence of the disease. No symptomatic or haematological improvement was however observed.

Survival time of acute leukaemia in the present series.

Survival time may be measured from the onset of the first symptom or the date of diagnosis of the disease. As most of the large series recorded in the literature are measured in the former manner, for comparative purposes the survival time in the present series has also been measured from the date of the onset of symptoms.

The average survival time of the twenty-eight patients treated with 6-MP was seven and a half months, the range being one week to twenty-one months. 50 per cent. were dead by the end of seven months and 75 per cent. by the tenth month. Six of the twenty-eight patients survived longer than one year. Comparison of the group in which remissions developed with those who failed to respond to treatment is interesting. The average survival time in the former was 9.8 months as compared with 4.3 months in the latter. 45 per cent. of those

responding to treatment were alive for longer than nine months compared with none in the group failing to show improvement, while all six patients surviving longer than one year had developed remissions following treatment. In conclusion it may be said that although those patients who fail to show a clinical ahd haematological remission probably survive no longer than untreated subjects (see page 75), patients responding to chemotherapy may have several more months of useful, symptom-free life to look forward to.

B. CHRONIC MYELOID LEUKAEMIA.

Improvement following treatment with 6-MP was assessed subjectively, objectively and with regard to haematological changes (Table 23).

e.g. Subjective improvement: increased feeling of well being, increased activity, appetite and weight, etc.

Objective improvement: diminution in size of the spleen, liver, lymph glands and other objective evidence of disease.

Haematological improvement: a fall in the leucocyte count with diminution in proportion of primitive cells, a rise in the haemoglobin concentration and maintenance of a normal platelet count or rise from a subnormal level.

Dosage

The initial dosage of 6-MP was similar to that

employed in the treatment of acute leukaemia. As the leucocyte count fell to normal treatment was either stopped temporarily or reduced until the optimum maintenance dose of between 50 and 150 mg. daily was obtained. Early studies showed that maintenance treatment with 6-MP was essential in chronic myeloid leukaemia (cases 29 and 39).

Results of Treatment.

Of sixteen patients at various stages of the disease, including one in the terminal acute phase, a response of varying degree was observed in fifteen, the other patient (case 43) dying of cerebral thrombosis within a week of starting treatment. Four of the remaining fifteen patients had previously received deep x-ray therapy and one urethane, prior to 6-MP. The other ten had no previous treatment.

(a) Clinical improvement was observed in all but one of the fifteen patients. The patient failing to show any response (case 42) had previously been treated with x-rays and had reached a terminal refractory stage. The spleen and liver were reduced in size and the leucocyte count fell from 10,000 to 2,400 per c.mm. A refractory anaemia persisted, however, and there was no subjective improvement. Another patient (case 41), although showing temporary symptomatic improvement initially, relapsed quickly and has since required repeated

TABLE 2.3. EFFECT OF CONTINUOUS 6-MP THERAPY IN CHRONIC MYELOID LEUKAEMIA

Case No.	Age and Sex	Clinical Condition	PRE-TREATMENT: Blood Count Hb. (Gms.)	RBC's x 10^6	WBC's x 10^3 (7)	P's x 10^3	Previous Treatment	B.T. (1)	Dose of 6-MP (Gms.) (2)	Clinical Condition at 2 months	Blood Count at 2 months Hb. (Gms.)	WBC's x 10^3	Clinical Condition at 6 months	POST-TREATMENT: Blood Count at 6 months Hb. (Gms.)	WBC's x 10^3 (8)	P's x 10^3	A-Alive D-Dead (6)	Response to 6-MP Therapy: S.I. (3)	O.I. (4)	Fall in WBC's	Hb. (5)	Comment
29	49 M	Fair. Vomiting. Spleen 8 cms.	9.0	3.3	128	300	-	-	4.4 / .05-.1	Well, working. Spleen 1 cm.	12.7	22.4	Good, working. Spleen 1 cm.	13.6	10.5 P.68% L.32%	250	A/24+	+	+	+	+	Continues satisfactorily.
30	25 M	Good. Spleen 2 cms.	14.5	4.6	75	220	X-rays	-	3.5 / .05-.1	Well, working. Spleen 1 cm.	12.2	11.8	Good, working. Spleen 1 cm.	16.0	12.8 P.82% L.16% M. 2%	190	A/12+	+	+	+		Continues satisfactorily.
31	9 M	Poor. Weight loss, dyspnoea. Spleen to pelvis.	6.9	2.0	300	25	-	1	5.75 / .05	Good. Spleen 1 cm.	10.8	10.6	Good. At school Spleen 1cm.	13.0	7.0 P.74% L.22% M. 4%	150	A/13+	+	+	+	+	Continues satisfactorily.
32	18 M	Fair. Spleen to pelvis.	10.4	3.5	259	175	-	-	4.4 / .05-.1	Good, working. Spleen 1 cm.	13.4	14.6	Good, working. Spleen impalpable.	14.8	12.6 P.78% L.22%	225	A/13+	+	+	+	+	Continues satisfactorily.
33	42 F	Poor. Severe bronchitis. Spleen 12 cms. below costal margin.	11.8	4.2	96		Urethane	-	3.05 / .05-.1	Fair. Chest improved. Spleen 5 cms.	13.6	7.4	Well for 6 months. Pain in chest.	12.3	12.4 My.59% P.30% L.11%	-	D/7	+	+	+	+	Died of respiratory infection.
34	34 F	Poor. Haematemesis. Pain lt. abdomen Spleen to pelvis.	4.9	2.2	204	270	-	1	2.9 / .05-.1	Good. Spleen impalpable.	13.4	19.3	Good. Spleen impalpable.	13.6	6.3 P.81% L.19%	150	A/6+	+	+	+	+	Continues satisfactorily.
35	62 F	Poor. Pain lt. abdomen. Haematuria. Spleen 4 cms. above pubis.	8.3	3.3	275		-	-	5.15 / .05-.1	Good. Spleen 1 cm.	13.5	6.7	Good. Fully active.	14.6	11.2 P.75% L.25%	305	A/7+	+	+	+	+	Continues satisfactorily.
36	56 F	Poor. Acute phase. Pyrexia, vomiting, epistaxis. Spleen 15 cms. below costal margin. Blasts in bone marrow.	8.2	2.3	152	225	X-rays	2	4.1 / .05	Good. Spleen 1 cm.	11.8	4.4 P.80% L.20%	Relapsing.	12.3	27.3 Blasts ++ in marrow	-	D/6	+	+	+	+	Died. Preceded by relapse into acute stage.
37	58 F	Fair. Liver enlarged 12 cms. below costal margin. Abdomen pain.	12.0	3.7	128	210	X-rays	-	5.1 / .05-.1	Good. No symptoms. Liver impalpable.	11.0	35.0	Good. Liver not palpable.	13.6	24.1 My.3% P.96% L. 1%	260	A/19	+	+	+	+	Refractory anaemia. WBC's normal. 6-MP continued. T.B. glands.
38	48 F	Poor. Mitral stenosis. C.H.F. Spleen 5cms.below costal margin.	7.4	2.52	202	150	-	1	3.4 / 0.5-.1	Good. No complaints. Spleen 1cm. Liver impalpable.	11.1	7.1	-	-	-	-	A/5+	+	+	+	+	Continues satisfactorily
39 (Course i)	49 M	Fair. Ulcer on leg. Spleen in pelvis. Liver 4cms.	7.7	2.3	112	150	-	3	2.8 / -	Fair. Ulcer healed. Spleen below umbilicus.	8.1	50.4	-	-	-	-	-	+	+	+	-	Temporary benefit. No maintenance therapy. For x-rays.
(Course ii)		Poor. C.H.F. Spleen in pelvis.	3.2		198	100	X-rays	2	2.4 / .05-.15	Good. Symptom free Spleen at umbilicus, liver not palpable.	10.0	35.1	Good. Spleen 1 cm. Light work.	12.2	40.8 My.2% P.71% L..4%		D/9½	+	+	+	+	Relapsed 9½ mths. 6-MP and myeleran ineffective.
40	55 M	Good. Spleen halfway to umbilicus.	10.9	3.4	58		-	-	3.85 / .05-.15	Good. Spleen impalpable.	13.0	15.8	-	14.2	9.6 P.76% L.22% M. 2%	159	A/6+	*	+	+	+	Continues satisfactorily.
41	48 M	Fair. Spleen 2 cms. Liver at umbilicus.	7.5	2.5	364	200	-	1	4.8 / .1-.15	Good, following temporary relapse.	10.4	14.4					A/5+	-	+	+	-	Persistent anaemia. Requiring blood transfusions.
42	58 F	Poor. CHF. Spleen to pubis. Lymph	7.7	2.5	10	110	X-rays	1	2.4	Poor. Liver impalpable. Spleen	7.4	2.4	-	-	-	-	D/3	-	+	+	-	Died.

		Spleen 5cms.below costal margin.								Spleen 1cm. Liver impalpable.													satisfactorily
39 (Course i)	49 M	Fair. Ulcer on leg. Spleen in pelvis. Liver 4cms.	7.7	2.3	112	150	-	3	2.8 / -	Fair. Ulcer healed. Spleen below umbilicus.	8.1	50.4	-	-	-	-	-	+	+	+	-	Temporary benefit. No maintenance therapy. For x-rays.
(Course ii)		Poor. C.H.F. Spleen in pelvis.	3.2		198	100	X-rays	2	2.4 / .05-.15	Good. Symptom free Spleen at umbilicus, liver not palpable.	10.0	35.1	Good. Spleen 1 cm. Light work.	12.2	40.8 My.2% P.71% L.4%		D/9½	+	+	+	+	Relapsed 9½ mths. 6-MP and myeleran ineffective.
40	55 M	Good. Spleen halfway to umbilicus.	10.9	3.4	58		-	-	3.85 / .05-.15	Good. Spleen impalpable.	13.0	15.8	-	14.2	9.6 P.76% L.22% M. 2%	159	A/6+	*	+	+	+	Continues satisfactorily.
41	48 M	Fair. Spleen 2 cms. Liver at umbilicus.	7.5	2.5	364	200	-	1	4.8 / .1-.15	Good, following temporary relapse.	10.4	14.4					A/5+	-	+	+	-	Persistent anaemia. Requiring blood transfusions.
42	58 F	Poor. CHF. Spleen to pubis. Lymph glands +. Liver 6 cms.	7.7	2.5	10	110	X-rays	1	2.4	Poor. Liver impalpable. Spleen 10 cms. below lt. costal margin. No C.H.F.	7.4	2.4	-	-	-	-	D/3	-	+	+	-	Died.
43	69 M	Poor. CHF. Spleen below umbilicus. Liver 15 cms.	10.5	-	198	85	-	-	0.2 / -	-	-	-	-	-	-	-	-	-	-	-	-	Died before treatment effective.
48	32 M	Good. Spleen to umbilicus.	11.5	4.2	262	-	-	-	4.6 / .1-.15	Good. Spleen halfway to umbilicus.	13.3	54.4	-	-	-	-	A/4+	+	+	+	+	Continues satisfactorily.

KEY (TABLE 23)

1. B.T. - Blood Transfusions.

2. Dose of 6-MP - "Stabilising" / "Maintenance"

3. S.I. - Subjective Improvement.

4. O.I. - Objective Improvement.

5. Rise in Hb. or maintenance of normal level.

6. Duration of 6-MP response, from time treatment started.

7. For differential leucocyte counts see case reports.

8. P - polymorphs; L - lymphocytes; My - myelocytes; M - monocytes.

blood transfusions. He had received no previous treatment and was considered a therapeutic failure.

(b) Objective improvement was noted in all patients treated. Cases 31, 32, 34, 39 and 42 had massive splenomegalies, the lower pole of the spleen extending below the pelvic brim. Following treatment the spleen became impalpable in two (cases 32 and 34), the tips just palpable in one and considerable reduction in size in the remaining two. One patient (case 37) who had previously received x-ray treatment and whose spleen had been removed previously, presented with a huge hepatomegaly with associated pain and discomfort. After 6-MP therapy, the liver returned to a normal size, the symptoms cleared and she thereafter remained symptom-free with no clinical evidence of disease for a further nineteen months. Another patient (case 39) presented with an ulcer on the leg and was found to have a grossly enlarged spleen. Blood examination revealed the diagnosis of chronic myeloid leukaemia. After treatment with 6-MP the ulcer healed completely (Fig. 47), and the spleen regressed in size. Clinical improvement may be said to have been complete, apart from slight occasional splenic enlargement, in ten out of fifteen patients (cases 29, 30, 31, 32, 34, 35, 37, 38, 40, 48). All obtained the maximum expected benefit from treatment and returned to normal

activities. Case 29, a hospital porter, has not lost a day's work through sickness since 6-MP therapy was begun over two years ago, the blood picture has been consistently normal and the only clinical evidence of leukaemia has been an occasionally palpable spleen. Cases 30 and 32 have not been away from work for a year and case 31, a boy aged nine years, returned to school following treatment and has now been in remission for over thirteen months. Cases 34, 35 and 37, all females, returned to full domestic duties. Others who have obtained considerable benefit from treatment may not have returned to full activities on account of other infirmities. Thus, case 38, has rheumatic heart disease and case 40 osteoarthritis. In neither, however, have there been any symptoms referable to leukaemia. The duration of individual responses to treatment is given in Table

(c) Haematological Changes.

Leucocytes. 6-MP led to a fall in the white cell count in all fifteen patients. A delay in response of one to three weeks, comparable to that observed in acute leukaemia, was followed by a rapid fall in the number of leucocytes in the peripheral blood, a normal level being reached within a month of the start of treatment. The diminution in leucocytes coincided with a reduction in size of the spleen, liver and other objective evidence of

the disease. A fall to leucopenic levels occurred in some instances which led thereafter to the withdrawal temporarily, or reduction in dosage of the drug as the count fell towards normal. The white cells continued to fall for a period following the withdrawal of the drug but in instances where the drug was withheld too long (cases 29 and 39), the leucocytes were found to increase and evidence of leukaemia became more obvious. This resulted in the prescribing of a maintenance dose of the drug. The optimum dosage for maintenance of a satisfactory clinical and haematological state varied from 50 to 150 mg. daily. In three subjects (cases 37, 39 and 48) the most satisfactory improvement was observed when the leucocyte count was at higher than normal levels. At these levels (24 - 75,000 per c.mm.) the patients were maintained symptom-free and with good haemoglobin levels.

The proportion of immature granular cells in the peripheral blood was observed to diminish as the number of leucocytes fell, and in several prolonged disappearance of immature cells from the peripheral blood was noted (Table 23). In one (case 29) the differential count has been consistently normal for over two years.

Haemoglobin. One of the essential criteria of an effective therapeutic agent is its ability to

produce a rise in the haemoglobin level. In the present series definite improvement in erythropoiesis and haemoglobin synthesis followed treatment with 6-MP in twelve patients. In one (case 30) the initial haemoglobin level was within normal limits and was maintained at a normal level following treatment. In two further patients (cases 41 and 42) no improvement occurred. In ten patients the haemoglobin level rose to normal levels, i.e. over 13.0 G., while in two a rise above 12.0 G. was observed. Seven patients required initial blood transfusions but in all but two (cases 41 and 42) the haemoglobin level was improved following treatment with 6-MP. The rate of rise of haemoglobin varied and in some was difficult to estimate because of blood transfusions. In seven patients not receiving transfusions the increase averaged 3.2 G. during the first two months of treatment. In all, the maximum haemoglobin level was not reached until three to five months after the beginning of treatment. In two (cases 30 and 31) a temporary fall occurred within the first two months.

Platelets. Another essential property of a chemotherapeutic drug is that it should inhibit only myeloid tissues and should not depress either erythrocyte or platelet development. It has been shown that erythropoiesis is frequently improved

after 6-MP therapy and, in the present series, thrombocytopenia has not been observed to follow 6-MP therapy. After six months continuous therapy one patient (case 34) has shown a slight fall in the platelet count. Two (cases 31 and 39) showed a definite increase following treatment. In one patient particularly (case 31) was platelet formation improved by treatment, the count rising from 25,000 per c.mm. to within normal limits. Other patients retained a normal count.

C. CHRONIC LYMPHATIC LEUKAEMIA.

Two patients (aged 73 and 56 years) (cases 44 and 45) with chronic lymphatic leukaemia showed no clinical or haematological evidence of improvement following treatment with 6-MP.

D. MISCELLANEOUS GROUP.

Two patients (cases 46 and 47) with multiple myeloma, and one with erythroderma considered to be due to a reticulosis of undetermined type, failed to benefit from a month's treatment with 6-MP. One patient with mycosis fungoides appeared to be temporarily benefited.

Discussion.

A study of the results obtained in this more limited series supports the finding of Burchenal and his colleagues (1953) and of other workers in this field that 6-MP may produce temporary remissions in a percentage of patients with acute

leukaemia and modify the course of chronic myeloid leukaemia. Chronic lymphatic leukaemia, however, appears to be unaffected by it.

Adults, as well as children, may be improved by 6-MP, which is of interest when one considers the ineffectiveness of the folic acid antagonists in adult leukaemia. Five out of eleven adults with acute leukaemia were benefited in the present series which compares favourably with the figure of 40 per cent. obtained from an analysis of the results of others (Conference on 6-MP, 1954). A larger series of patients must be treated before definite information can be obtained as to the morphological type of acute leukaemia likely to be most responsive to treatment with 6-MP. All that can be said is that remissions have been observed in the lymphoblastic, myeloblastic and myelomonocytic types of leukaemia. Although the majority of remissions occurred in the acute lymphoblastic variety this may be explained by the higher incidence of this type in children, but the possibility that the lower incidence of lymphoblastic leukaemia in adults may be an explanation of their less successful response to treatment cannot at this stage be dismissed. There seems little doubt, however, that 6-MP is more versatile in its action than aminopterin and amethopterin, which are generally accepted as ineffective in adults and

most effective in acute lymphoblastic leukaemia.

As with other present day chemotherapeutic agents used in the management of acute leukaemia (Fountain, 1954b), the remissions in patients who respond are only temporary. Second remissions are unusual, and the disease gradually becomes resistant to the drug. The absence of cross resistant to ACTH, cortisone and amethopterin (Burchenal et al., 1953) is important, for patients who have become resistant to 6-MP may respond to other compounds. In the present series four out of nine patients who had developed resistance to 6-MP, showed varying degrees of clinical and haematological improvement following ACTH. It would therefore seem desirable at present to use these compounds in sequence. Because of its low toxicity, and the absence of associated added discomforts to the patient, an important consideration in the management of cases of leukaemia, 6-MP seems the choice of treatment at the outset, followed after resistance develops by amethopterin and ACTH and cortisone. The latter will probably find their main value in the very acute case associated with severe haemorrhage, and in producing symptomatic relief in adults who fail to respond to the antimetabolites, as well as in the later stages of the disease in children. A combination of antimetabolite and hormone therapy has been

advised by several investigators on the grounds that a higher incidence of initial remissions occur (see Page 71). Others disagree with this mode of therapy, believing that resistance to the two compounds is likely to occur at an earlier stage. By using the various drugs available in the above sequence Burchenal et al. (1954b) have demonstrated a definite increase in survival time. If a true synergistic relationship existed between any of these compounds it may, however, be an indication for combined therapy but no evidence of such has, however, been demonstrated (Burchenal, 1954). The various reports of the additive effects of combinations of drugs in animal leukaemia suggests that this mode of therapy may eventually lead to better results in human subjects and with this in mind effective combinations should be sought for.

In the hope that the rate of action of 6-MP would be increased, an initial "loading" dose of 1 G. in the first twenty-four hours was given to one patient (case 9) with a high leucocyte count. Dramatic clinical and haematological improvement followed within the next four days. Another patient (case 26), however, failed to respond rapidly to large doses and others with high initial leucocyte counts have shown a rapid response to routine doses of 6-MP. It therefore seems doubtful whether initial "loading" doses are any more

beneficial than routine therapy.

Whether patients who experience a complete remission should receive a daily maintenance dose of 6-MP remains unanswered. Although the majority of patients in this series continued to receive treatment when in remission, it seems doubtful whether maintenance therapy does in fact greatly prolong the remission. In this respect, it might be mentioned that case 8, an adult aged 62 years, had a complete remission lasting seven months during which time she received no treatment. Some patients, however, seem to benefit by regular daily treatment, especially those whose response to therapy is incomplete (e.g. case 10), and it is probably this observation that led to the use of maintenance therapy throughout this group of patients with acute leukaemia.

Whether or not 6-MP becomes accepted as a suitable agent for the treatment of chronic myeloid leukaemia remains to be seen. As with any other chemotherapeutic agent one must consider whether it is an effective substitute for the most widely accepted form of treatment at the present day, radiotherapy. Only a carefully controlled comparative study extending over several years would give an accurate answer to this question. However, comparison of the survival rates, and the degree, and duration of remissions in patients

treated with 6-MP with those reported in the literature following irradiation will yield most of the desired information. It is at present too early to compare the survival rates of patients treated with 6-MP alone with those treated by radiotherapy alone, and until this analysis becomes possible one cannot advocate 6-MP as a first line of treatment. The results reported do, however, suggest that 6-MP may yield prolonged remissions in a large number of patients during which time a satisfactory clinical and haematological state may be maintained. The longest response observed so far is in case 29, a male who has continued satisfactorily for over two years. Four other patients have now been doing well for over a year. The degree of improvement in the thirteen patients, out of a total of fifteen responding satisfactorily to 6-MP, appeared as good as any other form of treatment, the haemoglobin level rising spontaneously as the leucocyte count fell together with a disappearance of clinical evidence of the disease. 6-MP would therefore appear to be a satisfactory substitute for radiotherapy if unavailable or contra-indicated. An advantage it holds over external irradiation is its lack of toxic side-effects although administration of x-rays in the form of radio-active phosphorus has tended to overcome this difficulty. Other

advantages of 6-MP would appear to be its simplicity of handling and administration, the ease with which the disease may be controlled with proper dosage and adequate haematological supervision, and the absence of depression of platelet production. In addition, by the use of maintenance therapy, longer periods during which the patient may be clinically and haematologically normal, may be obtained. A definite advance over x-ray treatment would be reached when a chemotherapeutic agent is found that will produce a good response in patients who have either responded unsatisfactorily to x-rays from the start or who have reached the terminal refractory phase associated with unresponsive anaemia. It is true to say that no such compound has yet been found which will consistently bring about clinical and haematological improvement in the "radio-resistant" patient, and the discovery of such a drug would undoubtedly be a major advance in the treatment of chronic leukaemia. 6-MP, like others may occasionally yield good results in the patient responding poorly to x-rays (case 39) but at other times is quite ineffective (case 42).

The effectiveness of 6-MP must also be compared with that of other chemotherapeutic agents. Individual preference still plays a large part in the choice of agent and there is no doubt that

experience in the handling of a particular drug, whether treating acute or chronic leukaemia, leads to better results. The results obtained with the majority of present day agents are, in experienced hands, probably much the same. Certain compounds would, however, seem to have definite advantages over others, particularly when used by those not accustomed to handling them. Thus 6-MP and myeleran, both of which are relatively non-toxic, are superior to arsenic, benzene, urethane and nitrogen mustard including TEM because of the lack of upsetting side-effects and the more readily controlled effects on the bone marrow, in addition to the longer remissions which may follow the use of maintenance therapy. Certain advocates of these other forms of treatment have, however, at times reported excellent results including Wilkinson (1955a) who considers intravenously administered nitrogen mustard as the most satisfactory modern treatment of chronic myeloid leukaemia and able to produce results equal to x-rays including an equally long survival time. On the whole, however, it is the author's opinion that myeleran and probably 6-MP, when a longer trial has been studied, are the treatment of choice, other than radiotherapy, for the reasons previously mentioned, and particularly for the use of the general physician with no special knowledge of chemotherapy. At the

present stage, myeleran, which has undergone more extensive clinical trials (Galton 1953; 1955), in view of the prolonged improvement it may produce, its known effectiveness in some patients responding poorly to x-ray therapy, its apparently specific action on myeloid tissue in addition to its ease in handling and low cost, probably holds an advantage over other chemotherapeutic drugs. Whether or not 6-MP will be as effective remains to be seen. The results at the present time are encouraging and like myeleran prolonged remissions may follow its use, and it seems equally specific in its action on myeloid tissue. It is easy to handle and patients on maintenance therapy are generally seen at intervals of no less than a month at the out-patient clinic and experience suggests that patients may beseen at longer intervals without giving rise to anxiety. 6-MP is also an important contribution to the therapy of chronic myeloid leukaemia in that it may be effective in the acute terminal phase (case 36) (Burchenal et al. 1953) and is probably unique in being effective in both the acute and chronic form of leukaemia.

Conclusions.

It has been demonstrated that a new compound, 6-mercaptopurine, an antimetabolite allied to the folic acid analogues, has been discovered with which

dramatic remissions may be obtained in a percentage of cases of acute leukaemia. Unlike aminopterin and amethopterin, 6-MP may be effective in adults and has a low toxicity. It can therefore be used with little danger of added discomfort to the patient - an important consideration in the management of cases of leukaemia. That the remissions are only temporary suggests the value of 6-MP as a research compound will at present outweigh its clinical usefulness. However, with the knowledge that aminopterin and amethopterin, A.C.T.H. and Cortisone, and 6-MP may all produce clinical and haematological remissions in acute leukaemia, it seems desirable to give patients the advantage of such chemotherapy. Symptomatic relief may be obtained (Fountain and Towers, 1955) and by sequential use these drugs may offer several months of useful life. A number of physicians, however, are of the opinion that in the consideration of humanity, treatment should be limited to the relief of pain and distress, and in view of the eventual fatal outcome no attempt to induce remissions and prolong life should be made. Such an attitude is not likely to lead to therapeutic advances and can only lead to a demoralised patient, relative and doctor and in a broader sphere a negative therapeutic approach to many other forms of malignant neoplasms. Each physician must consider

these factors himself. In all cases of leukaemia in children the author has discussed the treatment and its shortcomings with the parents. Suffice it to say that, without bringing any pressure to bear, on no occasion has treatment been refused and never have the parents regretted the period of remission of the disease, however brief it may have been.

Although the period of follow-up of patients with chronic myeloid leukaemia treated with 6-MP must be longer before the ultimate value of 6-MP is known, initial observations suggest it may well hold a place in the management of the disease. Like chemotherapy as a whole it is relatively cheap, easy to give and more readily available to the population as compared with radiotherapy. Moreover, it is of low toxicity and therefore easy to control. Whether it is recognised by the physician as an advance in treatment or not, the fact that chronic leukaemia in addition to the acute form, can be controlled by an antagonist of a nucleic acid precursor is of considerable fundamental interest and will surely lead to further research in this field.

SUMMARY.

1. The results of treating fifty patients suffering from leukaemia or allied disorders with a purine antagonist, 6-mercaptopurine, have been presented and discussed.

2. Of twenty-eight patients with acute leukaemia seventeen responded to treatment. Complete clinical and haematological remissions followed treatment in nine patients. The results were compared with those obtained by other workers.

3. Remissions were temporary and resistance to further therapy with 6-MP eventually resulted. The duration of remissions varied from a few weeks to over a year.

4. Children responded better to 6-MP than adults, but 6-MP seemed more effective in adults than the folic acid antagonists. The lymphoblastic type of leukaemia appeared to respond best to treatment but remissions were also observed in patients with myeloblastic and monocytic leukaemia.

5. No cross resistance between 6-MP and ACTH and cortisone was observed.

6. The survival time of patients who developed remissions on the average appeared to be longer than those who failed to respond to treatment.

7. Sixteen patients with chronic myeloid leukaemia were treated with 6-MP. The effect of maintenance therapy in the majority resulted in prolonged clinical and haematological improvement.

8. Two patients with chronic lymphatic leukaemia, two with multiple myeloma, and one with reticulosis of the skin did not respond to treatment, while one patient with mycosis fungoides obtained some improvement.

9. 6-MP is of low toxicity and no serious complications have followed its use.

CASE REPORTS

Case No. 1.

A boy, aged 3½ years, was admitted to a children's ward at the General Infirmary, Leeds, on June 20, 1953. He gave a history of pains in the legs of five months' duration. Five weeks before admission he was diagnosed as having mumps and from that time his condition rapidly deteriorated. His appetite became poor and pain in the legs prevented him from walking. Increasing pallor was noticed by the parents and the temperature at times was elevated.

On admission he was found to be severely anaemic and enlarged lymph glands, ½ to 1 cm. in diameter, were palpable in the cervical, axillary and inguinal regions. Numerous small scalp tumours up to 5 mm. in diameter were observed. The skin, throat and mouth appeared healthy and the liver and spleen were not palpable. There was no pyrexia.

Blood studies were in keeping with a diagnosis of acute lymphoblastic leukaemia. Six days after admission, on the day treatment with 6-MP was started the blood findings were as follows: haemoglobin 6 G., red cells 1,900,000 per c.mm., reticulocytes 1.9%, platelets 60,000 per c.mm., white cells 4,800 per c.mm. Differential white cell count: blast cells 17%,

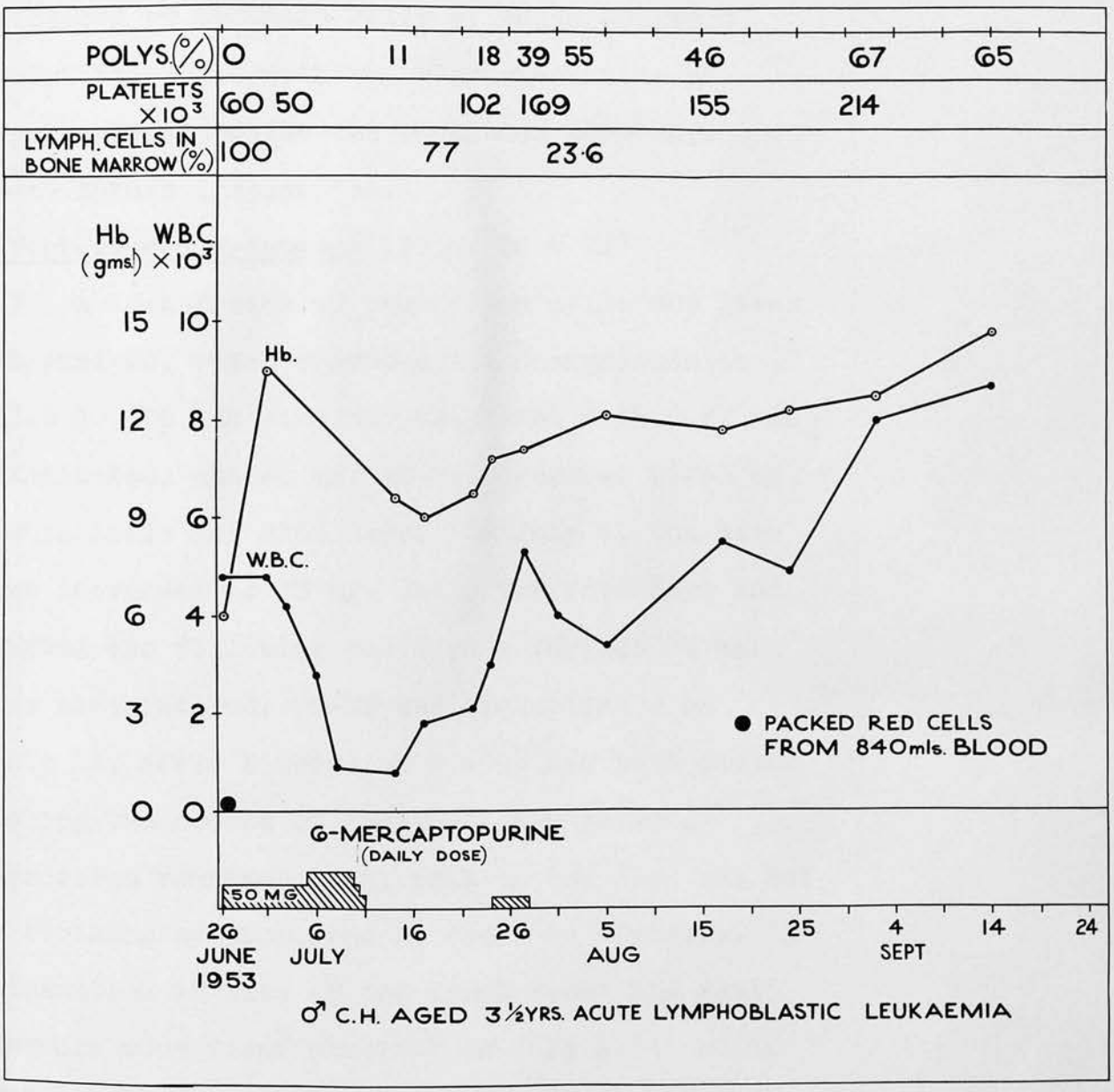

POLYS.(%) 0 11 18 39 55 46 67 65
PLATELETS ×10³ 60 50 102 169 155 214
LYMPH. CELLS IN BONE MARROW (%) 100 77 23·6
Hb (gms.) W.B.C. ×10³
15 10
12 8
9 6
6 4
3 2
0 0
Hb.
W.B.C.
PACKED RED CELLS FROM 840mls. BLOOD
6-MERCAPTOPURINE (DAILY DOSE)
50 MG
26 JUNE 1953
6 JULY
16
26
5 AUG
15
25
4 SEPT
14
24
♂ C.H. AGED 3½YRS. ACUTE LYMPHOBLASTIC LEUKAEMIA

FIG. 20.

lymphocytes 83%. On tibial puncture the bone marrow was found to be highly cellular and replaced by lymphoid cells of which 56% were primitive cells with the characteristics of lymphoblasts, while the remainder were more or less mature lymphocytes.

Progress and Treatment (Figs. 20 & 21)

A transfusion of packed red cells was given on June 26, which elevated the haemoglobin to 13.5 G. On the same day treatment with 6-MP was instituted, and 50 mg. of the drug was given by mouth daily for nine days. On July 5, the dose was increased to 75 mg. daily for five days and during the following two days a further 75 mg. was administered. 6-MP was discontinued on July 11, after a total of 0.9 G. had been given. During the course of treatment the child's condition remained good, pain in the legs was not a striking symptom, and he remained afebrile. A diminution in size of the lymph nodes and scalp tumours were first observed on July 5, at which time the leucocyte count was 2,800 per c.mm. On July 14, the glands and scalp tumours were impalpable, the white cell count having fallen to 850 per c.mm. (polymorphs 11%, lymphocytes 89%) and the haemoglobin 9.6 G. A tibial puncture on July 17 showed a hypocellular marrow with 20% blast cells, 57% lymphocytes, 18% myeloid cells

and 3% erythroid cells. Leucopenia persisted for approximately three weeks and was followed by haematological remission. 6-MP, 25 mg. daily, for four days, was given when the white cell count began to rise, but was discontinued when it became clear that haematological improvement was occurring. On July 27, the child developed a boil in the gluteal region and for eleven days Penicillin (Distaquaine) 300,000 units b.d. was given. The infection remained localised, there was no pyrexia and a satisfactory response to penicillin treatment was obtained.

Although not considered highly significant at the time, the appearance of polymorphonuclear cells in the peripheral blood on July 14, three days after treatment was withdrawn, was probably the first sign of haematological remission. Three days later bone marrow examination revealed the presence of returning granulopoiesis and erythropoiesis, which was mirrored in the peripheral blood thereafter by a rising haemoglobin and an increase in granular cells. An increase in the platelet count was also observed at this stage and by July 28 had risen to 169,000 per c.mm. On August 1, the white cell count was 4,000 per c.mm. of which 55% were polymorphs and 45% lymphocytes, and tibial puncture revealed a fall in the number of blast cells in the bone marrow to 6.4%.

Lymphocytes 17.2%, myeloid cells 46.4% and erythroid cells 30.0% were present and the marrow still showed a diminished cellularity. The presence of erythroid elements was reflected in the rising haemoglobin and red cell count, which on August 5, had reached 12.2 G. and 4,000,000 per c.mm. respectively.

The patient was discharged home in apparently normal health on August 11. Physical examination revealed no abnormality, his appetite was good and he regained full activity. His weight had increased by over four pounds. He remained in full clinical and haematological remission for nine weeks when relapse occurred. He was re-admitted to hospital on Oct. 2, 1953, and following a second course of treatment with 6-MP lasting ten weeks, he again developed a complete remission. This remission, during which a daily maintenance dose of 25 mg. 6-MP was prescribed, lasted four months. He had now become resistant to 6-MP and treatment with A.C.T.H. was instituted. No definite haematological improvement occurred, however, and his condition gradually deteriorated and death resulted on August 4, 1954.
Post-mortem examination was not performed.

Comment.

A child aged 3½ years developed a remission following 6-Mp therapy. Following relapse a

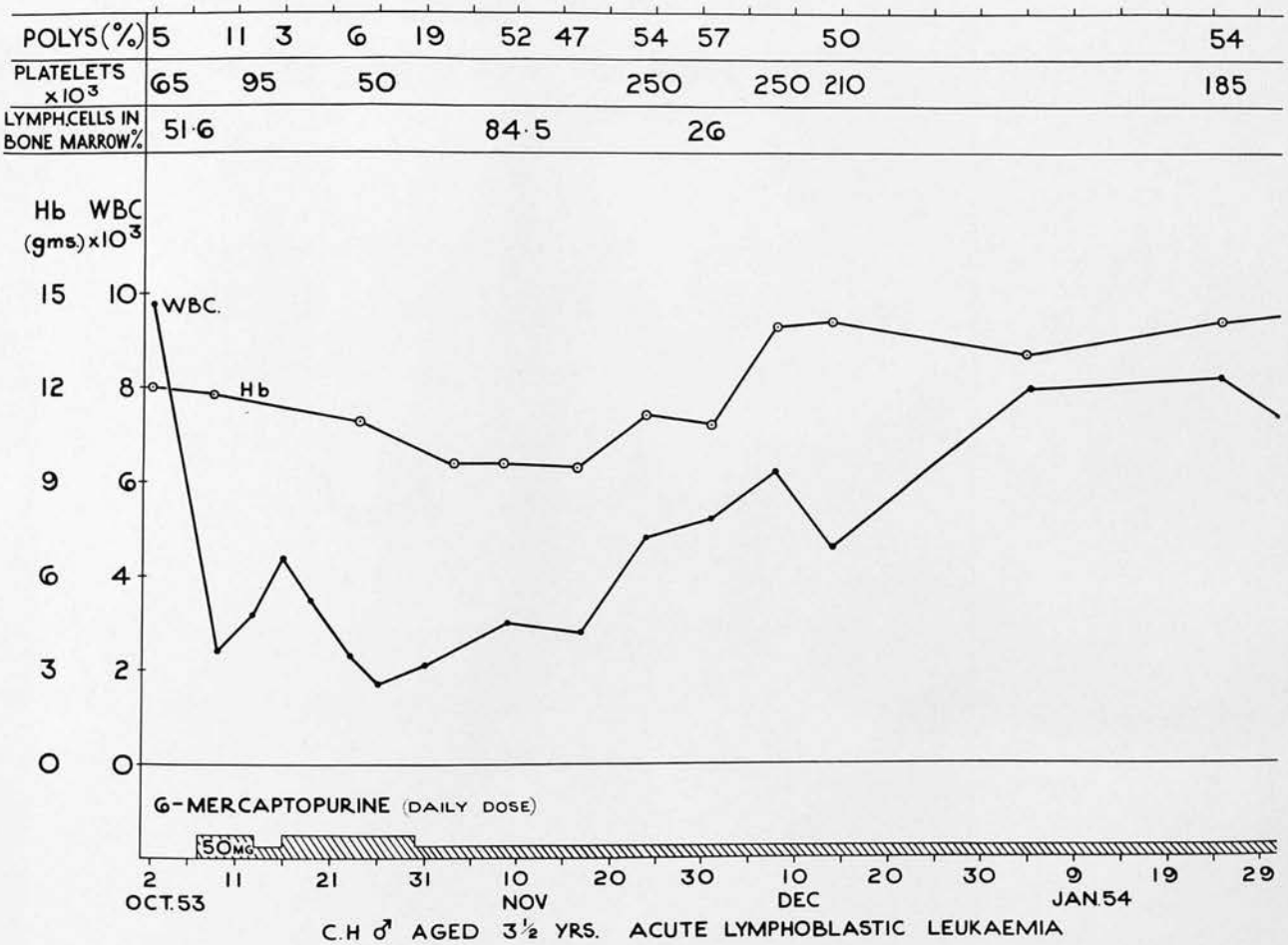

POLYS (%) 5 11 3 6 19 52 47 54 57 50 54
PLATELETS x10³ 65 95 50 250 250 210 185
LYMPH.CELLS IN BONE MARROW% 51·6 84·5 26
Hb (gms.)
WBC x10³
15 10
12 8
9 6
6 4
3 2
0 0
WBC.
Hb
6-MERCAPTOPURINE (DAILY DOSE)
50 MG.
2 11 21 31 10 20 30 10 20 30 9 19 29
OCT.53 NOV DEC JAN.54
C.H ♂ AGED 3½ YRS. ACUTE LYMPHOBLASTIC LEUKAEMIA

FIG. 21.

a second remission lasting four months followed further treatment with 6-MP. Thereafter he was resistant to treatment.

He survived 13½ months from the time of diagnosis, or 18½ months from the onset of symptoms.

Case No. 2.

A female child, aged 4½ years, was admitted to the Leeds General Infirmary on February 10, 1954, with a history of increasing pallor, pains in the lumbar region and legs, lassitude and loss of weight of six weeks' duration.

On admission she was found to be anaemic and several lymph glands about ½ cm. in diameter were palpable in the cervical, axillary and inguinal regions. The spleen and liver were not palpable and there was no bleeding. The temperature was 99°F.

Blood examination gave the following findings: haemoglobin 5.9 G., red cells 1,900,000 per c.mm., reticulocytes 3%, platelets 64,000 per c.mm., white cells 9,000 per c.mm., differential white cell count:- blast cells 19%, lymphocytes 69%, neutrophils 11%, myelocytes 1%.

Sternal bone marrow showed almost total replacement by large, deeply staining primitive cells having little cytoplasm and deeply staining nuclei. Many of the cells contained nucleoli. The differential cell count was as follows: blast cells 89.6%, lymphocytes 2.0%, myeloid cells 1.6%, erythroid cells 6.0%, other cell types 0.8%.

The peripheral blood and bone marrow cytology was in keeping with a diagnosis of acute

lymphoblastic leukaemia.

Progress and Treatment (Fig. 22)

Treatment with 6-MP, 50 mg. daily, was begun immediately on February 10. A transfusion of packed red cells was given on February 11, which raised the haemoglobin to 13.8 G. and produced considerable clinical improvement. The temperature settled within forty-eight hours and the patient's general condition remained good. She was very active and played normally. The blood picture was, however, slow in improving. A leucopenia developed two days after the start of therapy and persisted for two months. 6-MP., 25 or 50 mg. daily, was continued during this period when the leucocytes fluctuated between 2-4,000 per c.mm. By March 15, approximately five weeks after the introduction of treatment, the platelet count had risen to 150,000 per c.mm. and the bone marrow showed a definite improvement:- blast cells 31%, erythroid cells 35.2%, myeloid cells 28.8%, lymphocytes 4.8%, Leukaemic cells were still the predominant cell, however, in the peripheral blood. Two weeks later, as her general condition was so good, she was discharged home and thereafter was observed as an out-patient. Clinical examination on discharge (March 30) showed slight anaemia and a few small cervical

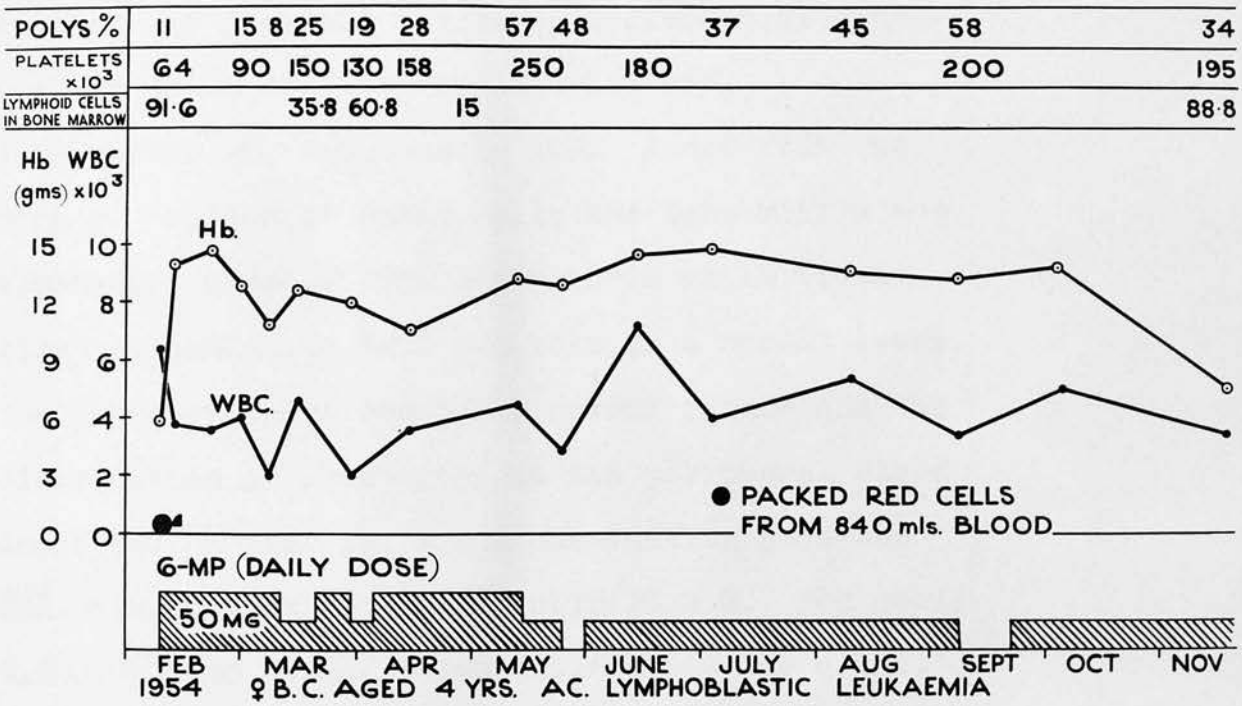

POLYS %
11
15 8 25
19
28
57 48
37
45
58
34
PLATELETS ×10³
64
90 150 130 158
250
180
200
195
LYMPHOID CELLS IN BONE MARROW
91·6
35·8 60·8
15
88·8
Hb (gms)
WBC ×10³
15 10
12 8
9 6
6 4
3 2
0 0
Hb.
WBC.
● PACKED RED CELLS FROM 840 mls. BLOOD
6-MP (DAILY DOSE)
50 MG
FEB 1954
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
♀ B.C. AGED 4 YRS. AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 22.

lymph nodes.

It was not until April 29, after eleven weeks of treatment, that the haematological evidence of a complete remission appeared. The bone marrow on that day gave the following differential count:- myeloid cells 37%, erythroid cells 48%, lymphocytes 5%, smear cells 10%. Apart from the high proportion of smear cells the bone marrow was apparently normal. The haemoglobin which to this time had tended to fall now rose to a normal level, the platelet count reached a normal figure and the distribution of leucocytes in the peripheral blood improved. On May 13, blood examination gave the following results:- haemoglobin 13.3 G., red cells 4,500,000 per c.mm., white cells 4,500 per c.mm., differential white cell count:- polymorphs 57%, lymphocytes 39%, monocytes 4%, platelets 250,000 per c.mm.

She was now given a maintenance dose of 25 mg. 6-MP daily. She started school and remained in apparently normal health until November, 1954, the only suggestion of the disease being a few persistent small cervical lymph glands and at times a slight preponderance of lymphocytes in the peripheral blood. It was not thought necessary to carry out further bone marrow examinations during this period.

On November 22, she was admitted again to hospital complaining of pains in the legs, difficulty in walking and lassitude. She was pyrexial, anaemic and enlarged lymph glands were again obvious. The blood picture was as follows:- haemoglobin 7.5 G., white cells 2,900 per c.mm. (neutrophils 34%, lymphocytes 66%), platelets 195,000 per c.mm. Bone marrow:- blast cells 39.6%, lymphocytes 49.2%, myeloid cells 6.8%, erythroid cells 4.0%, other cell types 0.4%. After an initial blood transfusion, 6-MP was increased to 50 mg. daily. Symptomatic improvement followed and the temperature settled after two weeks. She was allowed home on December 18, the blood still showing evidence of leukaemia but symptom free. In mid-January, 1955, however, she required a further blood transfusion. As no further benefit was being afforded by 6-MP, A.C.T.H. therapy was given in March. No haematological improvement resulted and in May, 1955, she was again admitted to hospital. Further courses of 6-MP and A.C.T.H. were prescribed with little effect on the blood picture and repeated transfusions have been necessary.

Comment.

A child, aged 4½ years, with acute lymphoblastic leukaemia responded slowly to

treatment with 6-MP. After almost three months continuous therapy a complete remission occurred. She was maintained in good health, returned to school and carried out normal activities for thirty-six weeks after the initial response to 6-MP. Relapse then occurred and 6-MP failed to bring about haematological improvement a second time, although she obtained temporary symptomatic relief. A.C.T.H. was later prescribed with little beneficial effect.

She has survived 19 months since the diagnosis was made, or 20¼ months since the onset of symptoms.

Case No. 3.

A 6½ year old female child was admitted to Wakefield General Hospital on November 2, 1954, with a history of increasing pallor and loss of energy of five weeks' duration, and more recent breathlessness on exertion.

On admission the temperature was 102°F. She was very anaemic, and bruises were present over the arms and back, and lymph glands were palpable in the cervical, axillary and inguinal regions. The spleen tip was just palpable.

Blood examination gave the following result:- haemoglobin 5.2 G., red cells 1,510,000 per cu.mm., white cells 12,400 per c.mm., differential white cell count:- neutrophils 5%, lymphocytes 22%, blast cells 73%. The blast cells were considered to be lymphoblasts. They measured approximately 10-12 u in diameter and were almost entirely composed of nucleus, cytoplasm being very scanty. Nucleoli were present in several. 3 normoblasts of types B and C, were seen while counting 100 white cells.

Bone marrow was easily obtained from the sternum and was found to be abundantly cellular. 95% of the cells had the characteristics of primitive blast cells, granular and red cell precursors being almost completely absent.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment (Fig. 23)

A transfusion of whole blood was given shortly after admission which raised the haemoglobin to 9.7 G., and produced symptomatic improvement. Treatment with 6-MP, 50 mg. daily, was begun on November 8. The dose was reduced to 25 mg. daily after four days due to a steadily falling leucocyte count, and on November 18, when the count was 2,800 per c.mm., to 12.5 mg. per day. Treatment was witheld for four days between November 27 and December 1, when the leucocytes fell below 2,000 per c.mm. During this period of 6-MP therapy the child was fairly well. She was out of bed and playing each day and rarely complained. The temperature, however, remained elevated between 99° - 100°F., her appetite was poor and the spleen and lymph nodes were still palpable. The haemoglobin level gradually fell and although the leucocyte count was always low no improvement in the distribution of leucocytes in the peripheral blood occurred. After three weeks treatment therefore no clinical or haematological improvement had occurred. 6-MP, 12.5 mg. daily, was re-started on December 1 and the same dosage continued until December 8, when

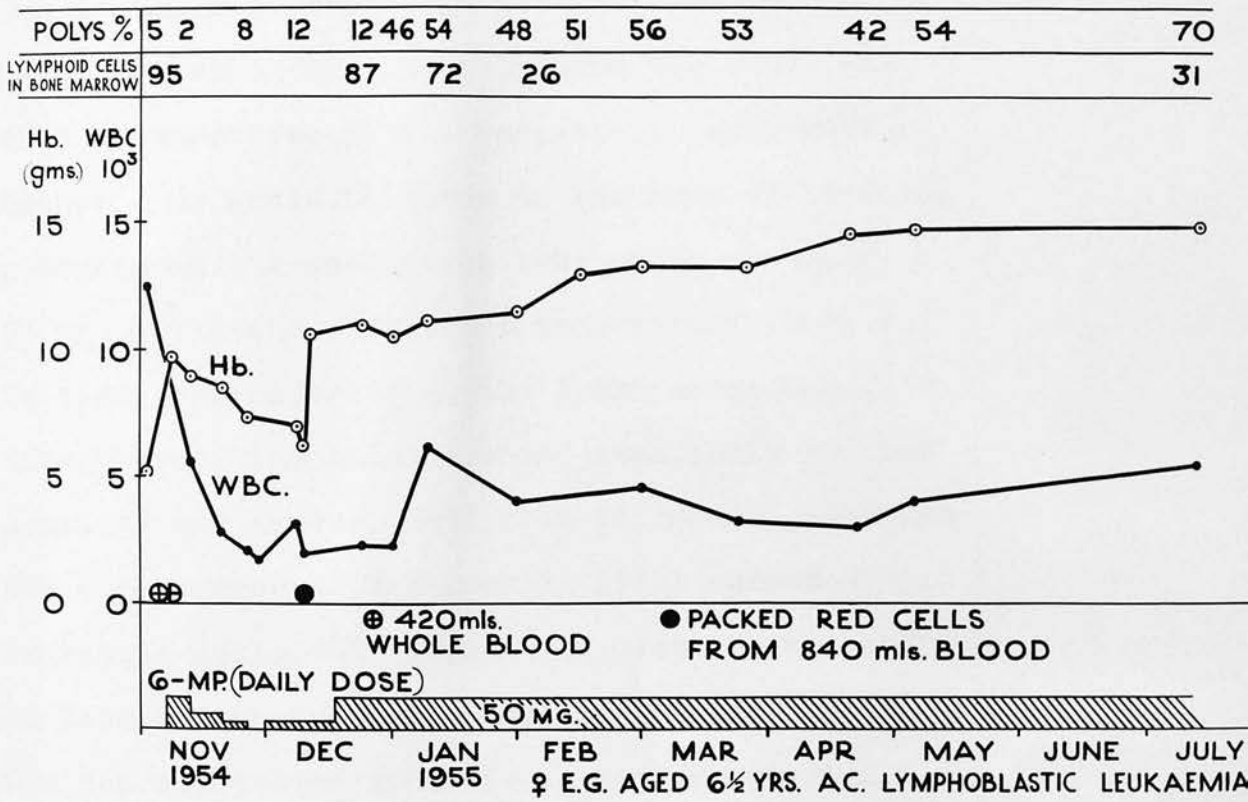

POLYS %
5 2 8 12 12 46 54 48 51 56 53 42 54 70
LYMPHOID CELLS IN BONE MARROW
95 87 72 26 31
Hb. (gms.)
WBC 10³
15 15
10 10
5 5
0 0
Hb.
WBC.
⊕ 420mls. WHOLE BLOOD
● PACKED RED CELLS FROM 840 mls. BLOOD
6-MP (DAILY DOSE)
50 MG.
NOV 1954
DEC
JAN 1955
FEB
MAR
APR
MAY
JUNE
JULY
♀ E.G. AGED 6½ YRS. AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 23.

it was increased to 50 mg. daily. Still no improvement in the blood picture had occurred and a further blood transfusion was necessary on December 9. On reviewing the case at this time the conclusion arrived at was that the dose of 6-MP prescribed had possibly been too small and that irrespective of the persistent leucopenia a higher dose would be given in the hope of inducing a remission. A week after increasing the drug to 50 mg. daily the persistent temperature finally settled, the majority of the lymph nodes had disappeared, the spleen became impalpable and her appetite and general condition improved. Although the bone marrow on December 21 still showed 87% leukaemic cells, the peripheral blood by the end of December showed 46% mature polymorphs. This was the first time since treatment was started 7½ weeks previously that the percentage of polymorphs had risen over 12%. She was discharged from hospital on January 1, 1955. Two weeks later a further bone marrow still showed a preponderance of leukaemic cells. Continued improvement in the peripheral blood - a rising haemoglobin and platelets and a return of the leucocyte picture to normal - suggested a return of the bone marrow to nearer normality. This was confirmed by further marrow examination in early February when erythroid cells 28.4%, lymphoid cells 20%, were

observed, the remainder of the cells being mature granular cells. For the following eight months she has been seen at regular intervals as an out-patient. Throughout this period she had attended school without a break. She is entirely symptom free and possesses apparently normal health. On July 12 blood examination showed:- haemoglobin 14.8 G., white cells 5,200 per c.mm. (polymorphs 70%, lymphocytes 27%, monocytes 3%). Bone marrow examination showed:- lymphocytes 31%, myeloid cells 32%, erythroid cells 35%, other cell types 2%. She continues to have 50 mg. 6-MP daily.

Comment

a 6½ year old schoolgirl with acute lymphoblastic leukaemia developed a clinical and haematological remission after seven and a half weeks 6-MP therapy. The delay in response was thought to be due to sub-optimal dosage. The remission continues thirty-eight weeks later, and she is being "maintained" on a daily dose of 50 mg. 6-MP.

She has survived 11 months since diagnosis, or 12½ months since the onset of symptoms.

Case No. 4.

A female child aged 2½ years was admitted to Wakefield General Hospital on July 31, 1954, with a 6 weeks' history of vomiting, pyrexia and "swelling of the glands". A diagnosis of glandular fever was made by her doctor but the gradual development of severe anaemia, constant tiredness, anorexia, loss in weight and finally the appearance of extensive impetigo necessitated her transfer to hospital for further investigation.

On admission she was found to be extremely anaemic. Ecchymoses were present on the arms and legs and impetigo was observed affecting the circumoral region, right temple, right pinna and external auditory meatus. The left eye was infected, swollen and closed, and numerous enlarged lymph glands were palpable in the cervical, axillary and inguinal regions. The liver and spleen were not palpable. The temperature was raised to 101°F.

Blood examination gave the following findings:- haemoglobin 2.8 G., red cells 945,000 per c.mm., white cells 3,800 per c.mm., differential white cell count:- polymorphonuclear cells 2%, lymphoid cells 98%. The majority of the latter cells were primitive blast cells.

Bone marrow obtained from the iliac crest was

found to be highly cellular and composed entirely of blast cells. No cells of the erythroid or granular series were present.

The marrow and peripheral blood picture indicated a diagnosis of acute lymphoblastic leukaemia (aleukaemic type).

Progress and Treatment (Fig. 24)

A transfusion of packed red cells was given immediately, raising the haemoglobin to 7.0 G. Penicillin 250,000 U. 6 hourly i.m., and Ung. Hydrarg. Ammon. dil. locally to the impetiginous areas on the face and head, and Guttae Penicillin to the infected eye were also prescribed.

6-MP was begun on August 1, at a dose of 100 mg. daily. It was hoped by giving this larger initial dose that improvement might be hastened. Five days later the dose was reduced to 50 mg. daily. The impetigo and infection of the left eye cleared rapidly with local treatment.

Haematological improvement was not observed, however, until almost five weeks after 6-MP therapy was started when the peripheral blood showed the presence of 30% mature polymorphs. During this period she developed chicken-pox with widespread skin sepsis and two further blood transfusions were necessary. On both occasions the transfusion wound became septic and there was,

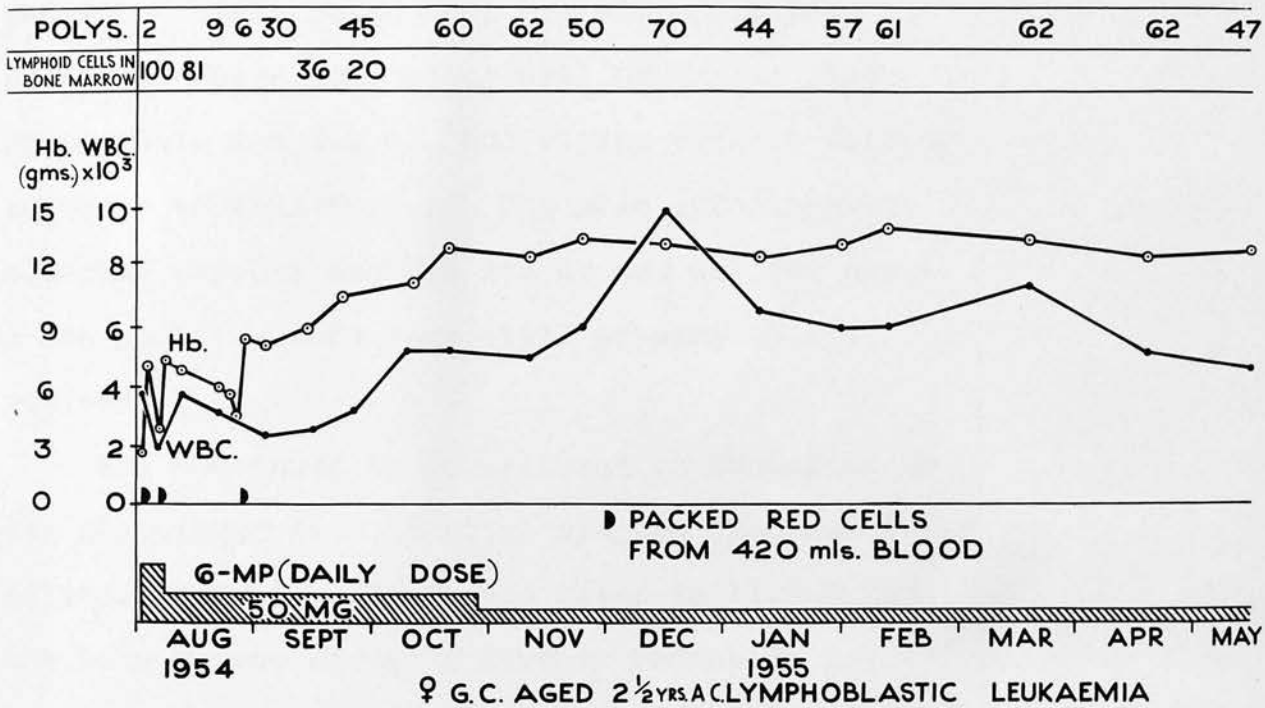

POLYS. 2 9 6 30 45 60 62 50 70 44 57 61 62 62 47
LYMPHOID CELLS IN BONE MARROW 100 81 36 20
Hb. (gms.)
WBC x10³
15 10
12 8
9 6
6 4
3 2
0 0
Hb.
WBC.
PACKED RED CELLS FROM 420 mls. BLOOD
6-MP (DAILY DOSE) 50 MG.
AUG 1954
SEPT
OCT
NOV
DEC
JAN 1955
FEB
MAR
APR
MAY
♀ G.C. AGED 2½ YRS. A.C. LYMPHOBLASTIC LEUKAEMIA

FIG. 24.

apart from a week in mid-August, continuous fever. On September 3, the temperature settled and clinical improvement coincided with haematological improvement. By September 16, the bone marrow showed:- blast cells 36%, myeloid cells 39%, erythroid cells 23%, other cell types 2%; the haemoglobin was 9.0 G., and rising without further recourse to transfusion. The skin infection was clearing rapidly and she was up and walking about. A few shotty glands were still present in all regions.

She continued to do well and on September 28 was discharged from hospital symptom free and fully active. The haemoglobin had risen to 11.0 G. and the bone marrow showed a further reduction in lymphoid cells to 20%. Erythroid cells numbered 36%, myeloid cells 43% and monocytes 1%.

A maintenance dose of 50 mg. 6-MP daily was at first prescribed but this was reduced to 25 mg. daily on October 26.

When last seen in the out-patient department, fifty-two weeks after the onset of the remission, she was still well and in apparently normal health. Since her discharge from hospital the haemoglobin has remained over 13.0 G. and apart from an occasional slight lymphocytosis the blood film has shown no suggestion of leukaemia. Further bone

marrow studies have not been carried out.

Comment

A 2½ year old female child with acute lymphoblastic leukaemia developed a complete clinical and haematological remission under treatment with 6-MP. Five weeks elapsed before treatment became effective and she continues in normal health fifty-two weeks after the onset of the remission, and 13½ months after the diagnosis was made. She is taking a maintenance dose of 25 mg. 6-MP daily.

She has survived 13½ months since diagnosis, or 15 months since the onset of symptoms.

Case No. 5.

A male child, aged 2 years, was admitted to St. James's Hospital, Leeds, on January 25, 1955. A history was obtained of 'influenza' a month previously following which he remained pale and debilitated. The parents noticed the pallor increasing and on this account sought the advice of their doctor.

On admission he was found to be extremely anaemic, slight enlargement of the inguinal lymph glands were detected and the spleen was palpable 2 cms. below the costal margin. The liver edge could just be felt. Systematic examination was otherwise negative and the temperature was normal.

Blood examination gave the following results:- haemoglobin 4.48 G., red cells 1,400,000 per c.mm., reticulocytes 0.6%, white cells 3,200 per c.mm., differential white cell count:- blast cells and lymphocytes 70%, polymorphonuclear cells 24%, myelocytes 1%, monocytes 4%, Tinck cells 1%. 1 nucleated red cell per 100 white cells was present, and many of the lymphocytes were seen to be immature. A platelet count carried out shortly after treatment was started was 90,000 per c.mm.

Bone marrow obtained from the iliac crest was found to be extremely cellular and the differential cell count was as follows:- blast

cells 87.0%, myeloid cells 11%, erythroid cells 0.5%, lymphocytes 1.5%. A diagnosis of acute leukaemia probably of lymphoblastic type was made.

Progress and Treatment (Fig. 25)

On January 28, a transfusion of packed red cells was given which raised the haemoglobin to normal and produced considerable symptomatic benefit. Treatment with 6-MP 50 mg. daily was started on February 1, and a few days later in view of his excellent clinical condition he was allowed home. When seen as an out-patient on February 23, he was found to be symptom free and fully active. Clinical examination was negative, the liver and spleen being now impalpable. The blood picture, however, was still typical of leukaemia:- haemoglobin 12.4 G., platelets 35,000 per c.mm., white cells 2,700 per c.mm., differential count:- neutrophils 7%, monocytes 3%, lymphocytes 90%. In view of the low leucocyte count 6-MP was reduced to 12.5 mg. daily. Two weeks later, the haemoglobin had fallen to 7.1 G. Other feature of the blood picture, however, suggested improvement, the platelet count now being normal at 350,000 per c.mm. and the bone marrow findings:- blast cells 7.5%, myeloid cells 51.0%, erythroid cells 5.5%, lymphocytes 26%, monocytes 10%.

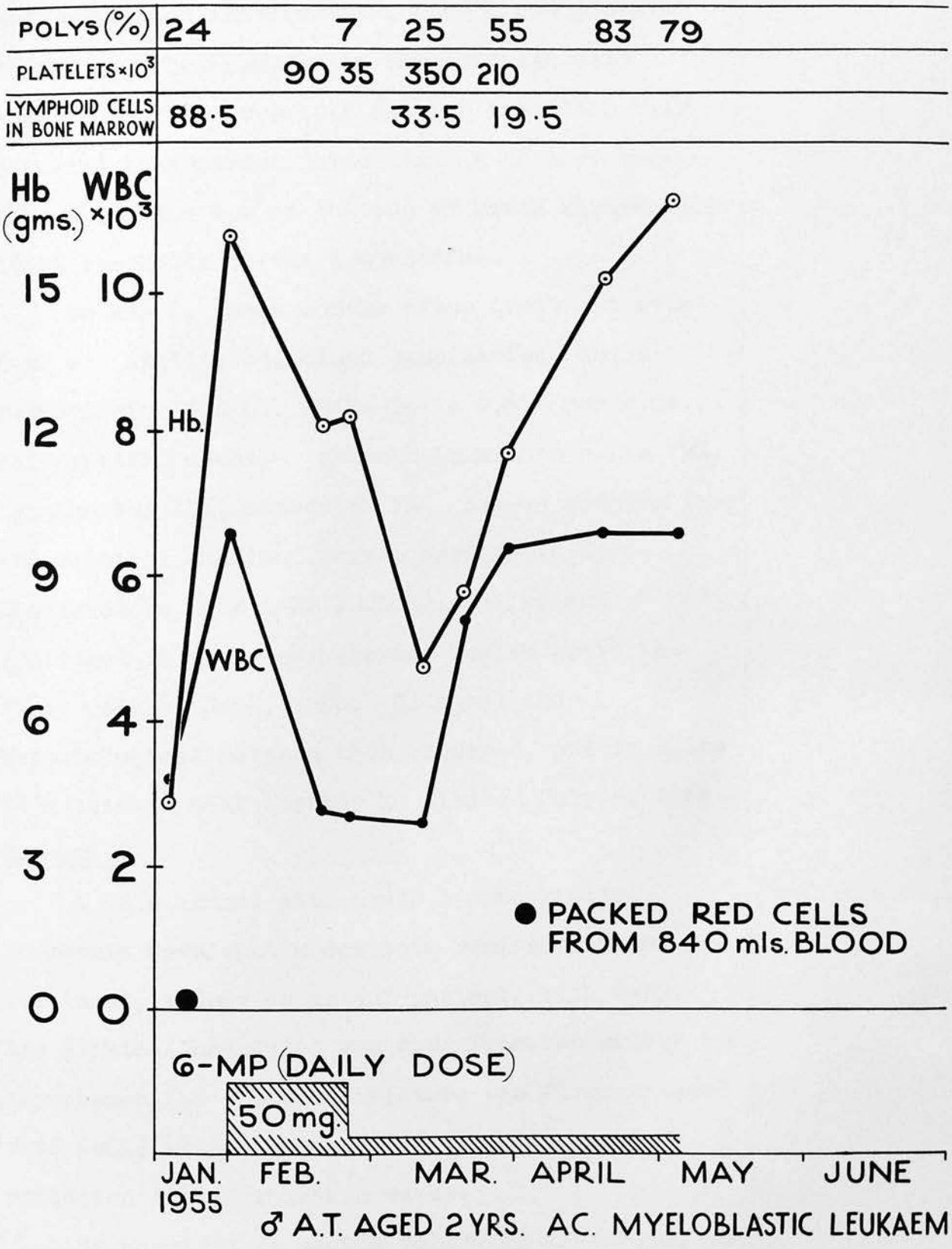

POLYS (%) 24 7 25 55 83 79
PLATELETS ×10³ 90 35 350 210
LYMPHOID CELLS IN BONE MARROW 88·5 33·5 19·5
Hb (gms.)
WBC ×10³
15 10
12 8
9 6
6 4
3 2
0 0
Hb.
WBC
● PACKED RED CELLS FROM 840 mls. BLOOD
6-MP (DAILY DOSE)
50 mg.
JAN. 1955
FEB.
MAR.
APRIL
MAY
JUNE
♂ A.T. AGED 2 YRS. AC. MYELOBLASTIC LEUKAEMIA

FIG. 25.

Continued improvement in the blood picture followed. The haemoglobin rose without blood transfusions to over 14.8 G., and the blood film returned to apparent normality. A further bone marrow examination at the end of March showed only 19.5% lymphoblasts and lymphocytes.

On May 2, three months after treatment with 6-MP was instituted, blood examination showed haemoglobin 16.8 G., white cells 9,600 per c.mm., differential count:- polymorphonuclear cells 79%, lymphocytes 17%, monocytes 4%. He was symptom free, and clinical examination was negative. He continued to take 6-MP 12.5 mg. daily, and continued in apparently normal health until the first week of June, 1955. Clinical and haematological relapse then occurred, and in spite of continued 6-MP therapy he died on July 8, 1955.

Comment.

A male infant with acute lymphoblastic leukaemia developed a complete remission under treatment, mainly as an out-patient, with 6-MP. His clinical condition was good from the outset and improvement of the blood picture was first noticed five weeks after the start of treatment The remission lasted thirteen weeks.

He survived 5½ months following diagnosis, or 6½ months since the onset of symptoms.

Case No. 6.

A girl aged 10½ years was admitted to Seacroft Hospital, Leeds, on April, 3, 1955, with an illness which started on Christmas Day, 1954, while en route by ship to Canada with her parents. Her initial complaint was low back pain which persisted for three days. On landing at Halifax, Nova Scotia, she felt improved and early in January she started to attend school. A recurrence of the low back pain occurred and she was noticed to be becoming progressively paler. She lacked energy, lost her appetite and became pyrexial. A diagnosis of rheumatic fever was made and as a result of failure to respond to salicylate therapy was admitted to Hamilton General Hospital, Ontario, on February 24, 1955. Further investigations, including two sternal marrow examinations revealed her to have leukaemia. The parents were informed of the hopeless prognosis of the disease and returned to England by air on March 31, 1955.

On admission she was found to be severely anaemic and complained of severe pain in the lumbar region on movement. The temperature was 99°F. A few small lymph glands were palpable in the cervical region and in both groins. The spleen tip was just palpable. There was no purpura or

other evidence of haemorrhage.

Blood examination on admission was as follows:- haemoglobin 8.7 G., red cells 2,900,000 per c.mm., platelets 89,000 per c.mm., white cells 2,700 per c.mm. Differential white cell count:- lymphoid cells 79%, polymorphonuclear cells 17%, monocytes 4%. Many of the lymphoid cells were immature and showed the characteristics of blast cells. A few normoblasts were identified.

Bone marrow aspirated from the left iliac crest was found to be highly cellular, a differential cell count showing 94.6% blast cells, 2.2% myeloid cells, 2.6% erythroid cells and 0.6% other cell types.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment (see Fig. 26)

Treatment with 6-MP was begun on April 6, 1955, a dose of 50 mg. b.d. being prescribed. During the following three weeks no improvement occurred, she had recurrent attacks of pain in the back and joints and on April 18, the haemoglobin had fallen to 5.2 G A transfusion of concentrated red cells was given which raised the haemoglobin to 9.1 G.

On April 27, the temperature returned to normal and the spleen was impalpable, the pain in the joint and lumbar region cleared, her appetite improved and she was able to get up. The peripheral blood, however, still showed a preponderance of leukaemic

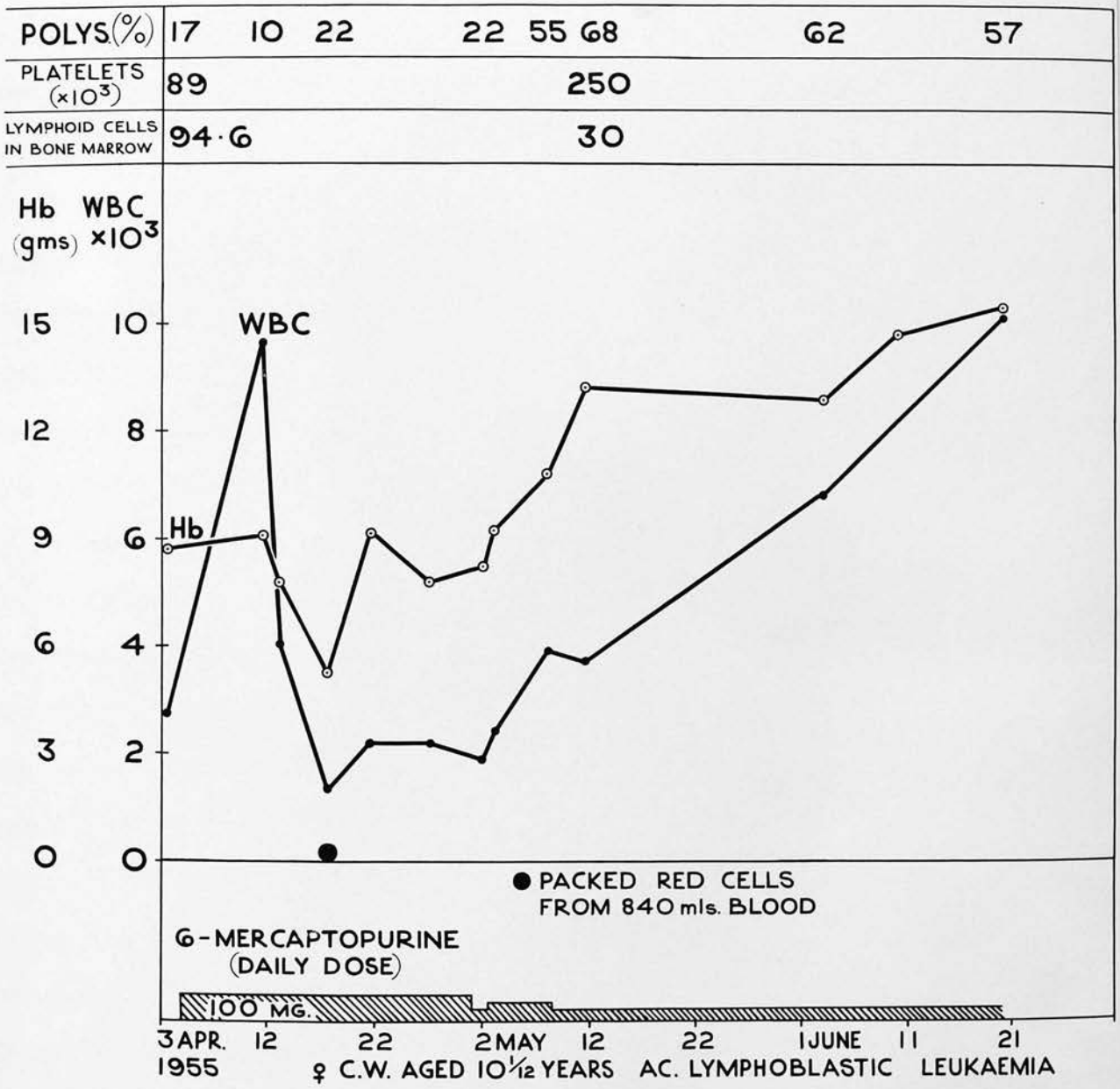

POLYS (%) 17 10 22 22 55 68 62 57
PLATELETS (×10³) 89 250
LYMPHOID CELLS IN BONE MARROW 94·6 30
Hb (gms) WBC ×10³
15 10
12 8
9 6
6 4
3 2
0 0
WBC
Hb
● PACKED RED CELLS FROM 840 mls. BLOOD
6-MERCAPTOPURINE (DAILY DOSE)
100 MG.
3 APR. 12 22 2 MAY 12 22 1 JUNE 11 21
1955
♀ C.W. AGED 10½ YEARS AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 26.

cells. The dose of 6-MP was reduced to 25 mg. b.d. on April 30 and apart from a period of a week when it was increased to 25 mg. t.d.s. this dose was continued as maintenance therapy. On May 11 the haemoglobin had risen to 13.2 G., the white cell count was 4,700 per c.mm. and the differential count showed polymorphs 68%, lymphocytes 28%, monocytes 4%. The platelet count was normal at 250,000 per c.mm. A further bone marrow examination showed a reduction in cellularity and the following cell picture: blast cells 9.6%, myeloid cells 23.8%, lymphocytes 20.4%, erythroid cells 44.2%, other cell types 2%. When seen a month later she was fully active and symptom free. The haemoglobin had risen to over 14.8 G., the peripheral blood picture appeared normal and there was no clinical evidence of leukaemia. She continued in full remission for ten weeks. On July 8, she complained, however, of pains in the limbs and the blood picture revealed evidence of relapse. She was re-admitted to hospital and found to be resistant to further 6-MP therapy. A downhill course followed and she died on August 7, 1955.

Comment

A 10½ year old girl with acute lymphoblastic leukaemia developed a complete remission following treatment with 6-MP. The remission, during which

the child was in normal health with no apparent clinical or haematological evidence of leukaemia, lasted for ten weeks. She failed to respond a second time to treatment.

She survived for 5½ months after the diagnosis was made, or 7½ months after the onset of symptoms.

Case No. 7.

A 44 year old baker was admitted to St. James's Hospital, Leeds, on June 9, 1953, with a four weeks history of pain in the lumbar region, sweating and dyspnoea on exertion. On admission widespread ecchymoses were found and two small skin tumours, the size of a farthing, were observed in the region of the xiphisternum. Sacral oedema was present and the liver and spleen were palpable 6 cms. and 5 cms. respectively below the costal margins. The temperature was normal and no other abnormalities were detected on physical examination. A radiograph of the chest was normal and a biopsy of one of the skin tumours showed it to be due to leukaemic infiltration.

His blood count on June 11, was as follows:- haemoglobin 8.5 G., red cells 2,800,000 per c.mm., reticulocytes 1%, platelets 66,000 per c.mm., white cells 6,800 per c.mm. Differential white cell count: blast cells 11%, myelocytes 11%, neutrophils 30%, eosinophils 11%, basophils 1%, lymphocytes 22%, monocytes 14%. On sternal puncture the bone marrow showed: blast cells 59%, myeloid cells 12%, erythroid cells 8.5%, lymphocytes 15%, other cell types 5.5%. Megakaryocytes were scanty. Many of the primitive blast cells were of a monocytoid type, the appearance suggesting a

Naegali type monocytic leukaemia.

Progress and Treatment (Figs. 27 & 28)

Treatment with 6-MP was begun on June 15, 100 mg. being given daily by mouth for seven days. On June 22, the patient became severely dyspnoeic due to a large left sided pleural effusion. Two pints of haemorrhagic fluid were removed and the dose of the drug increased to 200 mg. daily. A transfusion of whole blood was given on the fifth day of treatment elevating the haemoglobin to 10.5 G. No change in the clinical or haematological state occurred during the first three weeks of treatment. On July 8, the patient stated he was feeling much better, the pain in the lumbar region had disappeared and his appetite improved.

Physical examination showed no change, however, in the size of the liver and spleen and a large pleural effusion was still present. Blood examination showed the haemoglobin to have fallen and a further transfusion of whole blood was performed. The leucocyte count was 4,200 per c.mm. and the platelets 85,000 per c.mm. Thereafter the white cell count continued to fall and treatment was discontinued on July 14, when the white cells reached 1,200 per c.mm.; a total dose of 5.3 G of 6-MP had been given. The patient was now

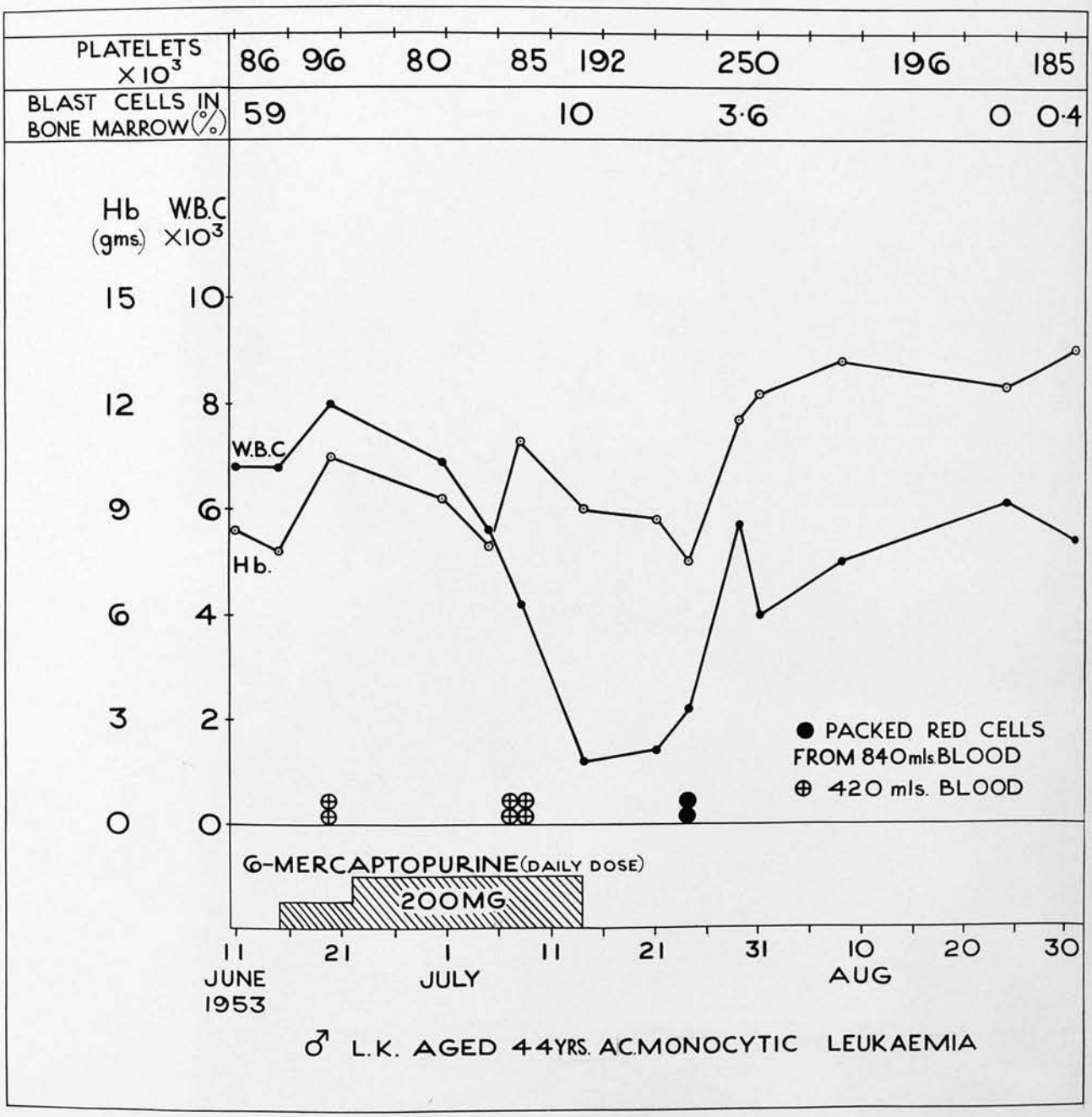
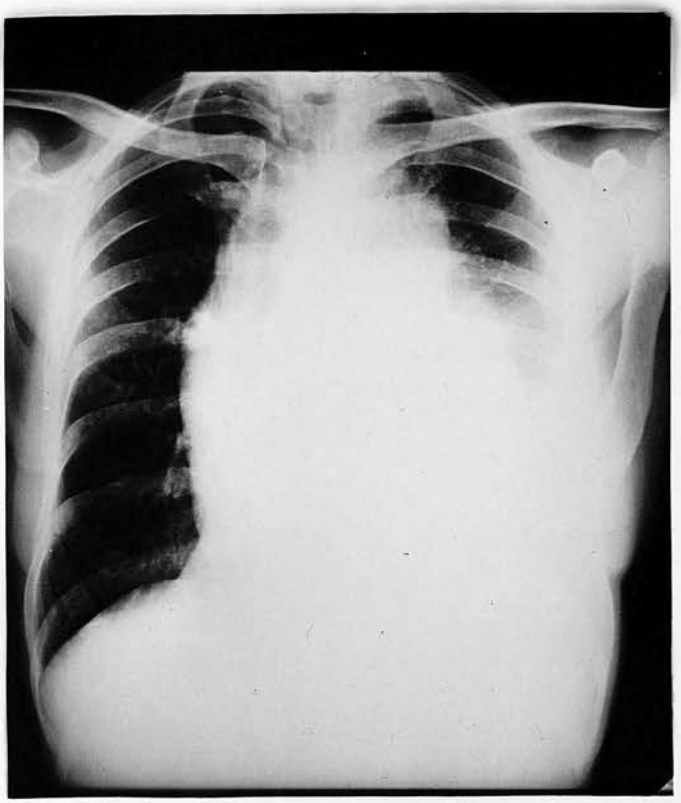
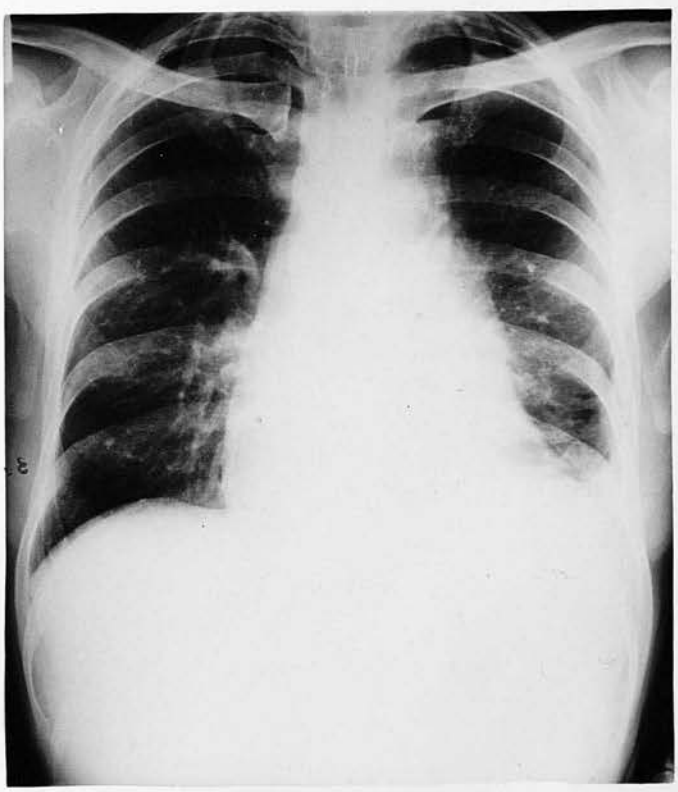

PLATELETS ×10³
86 96 80 85 192 250 196 185
BLAST CELLS IN BONE MARROW (%)
59 10 3·6 0 0·4
Hb (gms.)
W.B.C ×10³
15 10
12 8
9 6
6 4
3 2
0 0
W.B.C.
Hb.
● PACKED RED CELLS FROM 840mls BLOOD
⊕ 420 mls. BLOOD
6-MERCAPTOPURINE (DAILY DOSE)
200MG
11 21 1 11 21 31 10 20 30
JUNE 1953
JULY
AUG
♂ L.K. AGED 44YRS. AC.MONOCYTIC LEUKAEMIA

FIG. 27.



(a)



(b)

FIG. 28. Case No. 7. Radiograph of chest (a) before treatment, and (b) after treatment with 6-MP, showing diminution in size of pleural effusion and mediastinal lymph nodes.

symptom free, the liver had become impalpable and the spleen had regressed so that the lower pole could only be felt on deep infiltration. The remaining skin tumour over the sternum had disappeared. Fluid was still present in the left pleural cavity and a further two pints were removed on July 13. A sternal puncture on July 14 revealed a hypocellular bone marrow and a diminution in blast cells to 10%. Erythropoiesis was depressed but normoblastic in type and there was a relative increase in lymphocytes:- myeloid cells 38%, erythroid cells 3.5%, lymphocytes 44%, other cell types 4.5%.

The platelet count three days after treatment was stopped rose to 192,000 per c.mm. and thereafter remained within normal limits. Leucopenia persisted for two weeks and the leucocyte count then rising to a normal level. A fall in haemoglobin to 7.5 G on July 23 necessitated a transfusion of packed red cells, and thereafter a satisfactory haemoglobin level was maintained without further recourse to blood transfusions. On July 29, physical examination revealed the tip of the spleen to be palpable, otherwise no abnormality could be detected. Blood examination gave the following results: haemoglobin 11.5 G., red cells 3,800,000 per c.mm.,

reticulocytes 1%, platelets 250,000 per c.mm., white cells 5,700 per c.mm. Differential white cell count: neutrophils 78%, eosinophils 2%, lymphocytes 16%, monocytes 5%. The sternal bone marrow was still hypocellular but the differential cell count showed considerable improvement, blast cells being 3.6%, myeloid cells 47.6%, erythroid cells 16.4%, lymphocytes 13.6%, and degenerate cells 18.8%.

The patient was discharged home on August 1, with instruction to attend for follow-up examination as an out-patient. A maintenance dose of 6-MP was not prescribed. For seven weeks after therapy was discontinued he was symptom free and carried out normal activities. There was no clinical evidence of leukaemia. A radiograph of the chest one week after discharge was normal. The haemoglobin remained above 12.0 G. and the leucocyte count, differential and platelet counts were normal. A sternal puncture carried out on August 25 showed a relatively normal bone marrow appearance. In early September he began to complain of a cough and dyspnoea on exertion, and examination revealed a reaccumulation of fluid in the left pleural cavity. In addition the skin tumour re-appeared over the lower sternum, the liver and spleen became palpable and the blood

again showed the characteristic changes of acute leukaemia. Treatment with 6-MP was again instituted but the disease was now found to be completely refractory and the patient died on October 8, 1953.

Post-mortem. The significant findings were extensive glandular infiltration in the thorax and abdomen, blood-stained effusions in both pleural cavities and early broncho-pneumonia. In addition a large mass of leukaemic tissue was observed on the front of the sternum and small plaques were present on the parietal pleura. The liver showed discrete leukaemic infiltrations around bile ducts in both lobes, some attaining the size of one inch in diameter. There was no diffuse infiltration of the liver. The spleen showed no definite macroscopic evidence of leukaemia. The kidneys presented an unusual appearance in that a rim of leukaemic tissue about $\frac{1}{8}$" wide was present beneath the capsule into which haemorrhage had occurred. At certain points the tissue dipped into the renal cortex forming small diffuse masses about $\frac{1}{2}$" in diameter. In addition very large infiltrations in several of the medullary papillae were observed. Leukaemic infiltration was also observed in the wall of the bladder and in the myocardium. Microscopically

the visceral infiltrations were found to be composed of very primitive cells in keeping with the ante-mortem diagnosis. Histological evidence of leukaemia was also observed in the spleen.

Comment

A 44 year old male with acute monocytic leukaemia developed a dramatic clinical and haematological remission following treatment with 6-MP. Evidence of the disease, including a pleural effusion, skin tumours and hepato-splenomegaly completely disappeared for seven weeks when relapse occurred.

He survived 4 months after diagnosis, or 5 months after the onset of symptoms.

Case No. 8.

A 62 year old housewife was admitted to the General Infirmary, Leeds, on July 23, 1953, with a ten weeks history of increasing pallor, lassitude, loss of appetite and dyspnoea on exertion. During this period she noticed some distension of the abdomen developing, together with flatulence and tenderness in the left hypochondrium, but no abdominal pain. For three weeks she had been troubled with night sweats which were severe and soaked her bed clothes.

On admission the temperature was 100.4°F. She was found to be anaemic and lymph glands up to 1½ cms. in diameter were palpable in the axillae and groins. The liver was palpable 5 cms. and the spleen 12 cms. below the costal margin. Slight sacral oedema and crepitations at both lung bases were also detected. There were no other abnormalities on physical examination. A radiograph of the chest suggested that the right half of the diaphragm was raised and associated with slightly increased pulmonary markings in the right lower lobe.

Blood findings were as follows:- haemoglobin 7.8 G., red cells 2,800,000 per c.mm., platelets 119,000 per c.mm., white cells 1,900 per c.mm. Differential white cell count: polymorphs 36%,

monocytes 2%, lymphocytes 62%. Nucleoli were observed in a percentage of the lymphocytes.

On sternal puncture the bone marrow was found to be moderately cellular and 66.4% of the cells were of the lymphoid series, about half being lymphoblasts. The remaining cells were equally divided between the myeloid and erythroid series. The picture was consistant with a diagnosis of aleukaemic lymphatic leukaemia.

Progress and Treatment. (Fig.29)

The patient received a transfusion of packed red cells three days after admission and 6-MP in doses of 200 mg. daily, was begun on August 7. No change in her condition occurred during the following nine days, her main complaints being abdominal distension, flatulence, anorexia, sweating and general weakness. A painful sore on her nose also appeared. On August 17 she stated that she felt better and that her appetite was improving. Physical examination at that time revealed no change, but four days later the spleen had diminished in size, and the feeling of abdominal distension had lessened. The sacral oedema had disappeared and the infection of the nose was healing. The dose of 6-MP was reduced to 100 mg. daily on August 24, and finally discontinued seven days later; a total of 4.2 G. had been given. At this stage the spleen was

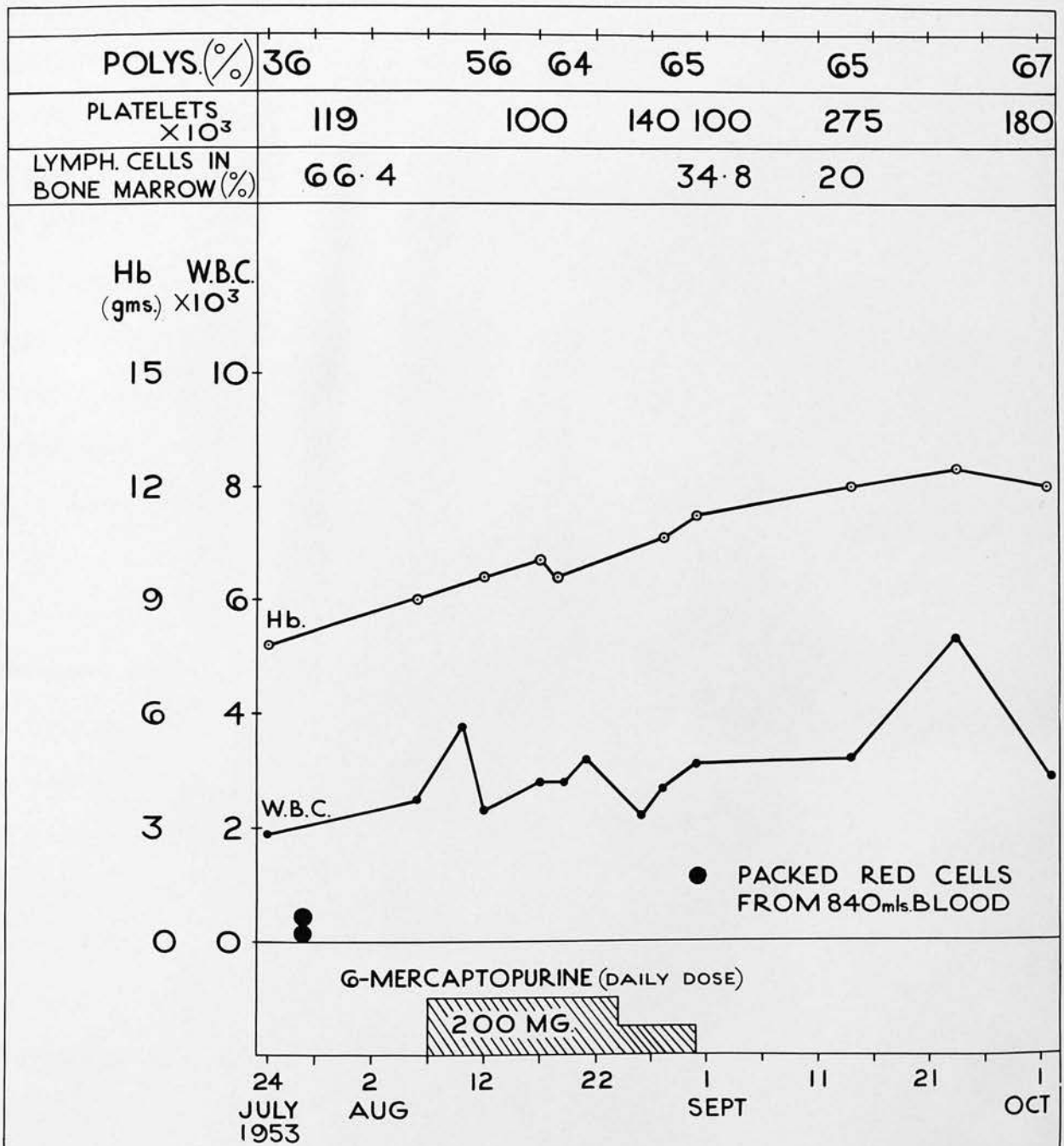

POLYS. (%) 36 56 64 65 65 67
PLATELETS ×10³ 119 100 140 100 275 180
LYMPH. CELLS IN BONE MARROW (%) 66·4 34·8 20
Hb (gms.)
W.B.C. ×10³
15 10
12 8
9 6
6 4
3 2
0 0
Hb.
W.B.C.
PACKED RED CELLS FROM 840mls. BLOOD
6-MERCAPTOPURINE (DAILY DOSE)
200 MG.
24 JULY 1953
2 AUG
12
22
1 SEPT
11
21
1 OCT
♀ L.F. AGED 62 YRS. ALEUKAEMIC LYMPHATIC LEUKAEMIA

FIG. 29.

impalpable and liver edge could be felt only on deep inspiration. A few small, fleshy glands remained in the axillae. Physical examination was otherwise negative, the temperature was normal and the patient symptom free.

Unlike the patients described previously no significant reduction in white cells followed the administration of the drug, and at no stage did the count fall below the initial figure. An improvement in the distribution of leucocytes in the peripheral blood was, however, observed within ten days of the start of therapy. A steady rise in haemoglobin occurred, but there was no significant increase in platelets at this stage. On completion of treatment the blood findings were as follows: haemoglobin 11.2 G., red cells 3,700,000 per c.mm., platelets 100,000 per c.mm., white cells 3,100 per c.mm. of which 65% were polymorphs. The bone marrow showed a diminished cellularity. Very few lymphoblasts comprising only 1.2% of the marrow cells were seen; 33.6% of the cells were lymphocytes. Erythropoiesis was active and normoblastic, 34.0% of cells being of this series. The remainder were developing granulocytes.

The patient was discharged on September 5 feeling well. She was seen again as an out-patient ten days later, improvement having been maintained. The haemoglobin had risen to 12 G. and the platelet

count to 275,000 per c.mm. A further bone marrow examination revealed only 20% blast cells, and lymphocytes, 34.8% being red cell precursors and the remainder granular cells. Thereafter she remained in good health with an apparently normal blood picture for a period of 7 months. During this time she was fully active, carrying out her household duties and living a normal life. Maintenance therapy was not given during the period of remission and following relapse in April 1954 the disease was refractory to further treatment with 6-MP. A gradual downhill course occurred and she died on August 23, 1954.

She survived a period of 13 months following diagnosis, or 15½ months after the onset of symptoms.

Case No. 9.

An adult male aged 41 years was admitted to the Leeds General Infirmary on April 7, 1954. His history was of increasing dyspnoea, lassitude and loss in weight of six weeks' duration. For four days he had repeated epistaxes and noticed a rash on the legs.

On admission he was found to be pale and showed signs of weight loss. Enlarged lymph glands were present in the cervical, axillary and inguinal regions and a purpuric eruption was present over both lower limbs. The trachea was displaced to the right and there were signs of an extensive pleural effusion on the left side. There was no splenomegaly.

A chest x-ray confirmed the presence of fluid in the left pleural sac and also showed a large mediastinal mass.

Blood examination on April 8 was as follows:- haemoglobin 11.8 G., red cells 3,800,000 per c.mm., platelets 35,000 per c.mm., white cells 129,000 per c.mm. A blood film showed only 2% polymorphonuclear cells. A few typical myelocytes were present but the majority were leukaemic blast cells which showed very fine granules on peroxide staining. On sternal puncture the bone marrow showed: blast cells 85.2%, myeloid cells 0.4%,

erythroid cells 0.4%, lymphocytes 13.2%.

A diagnosis of acute myeloblastic leukaemia was made.

Progress and Treatment (Fig. 30)

In view of the patient's extremely critical condition an attempt was made to induce an early response to 6-MP by giving a large initial dose. 1 G. of 6-MP was given over the 24 hour period on April 8, followed by 200 mgm. daily for five days. The haemoglobin fell rapidly after admission and a transfusion of whole blood was necessary on April 12, raising the haemoglobin to 10 G. The white cells fell to 4,800 per c.mm. in five days with considerable clinical improvement. The leucocyte count having fallen to 2,000 per c.mm. on April 14 the dose of 6-MP was reduced to 100 mg. daily and eventually discontinued ten days later when the count was 600 per c.mm. During this period clinical improvement continued, the enlarged lymph glands disappeared, the dyspnoea was relieved and the mediastinal mass and pleural effusion diminished in size as shown radiologically. Paracentesis had not been carried out. On May 1, blood examination gave the following results: haemoglobin 12.2 G., platelets 450,000 per c.mm., white cells 5,400 per c.mm., differential white cell count: polymorphs 65%, lymphocytes 34%,

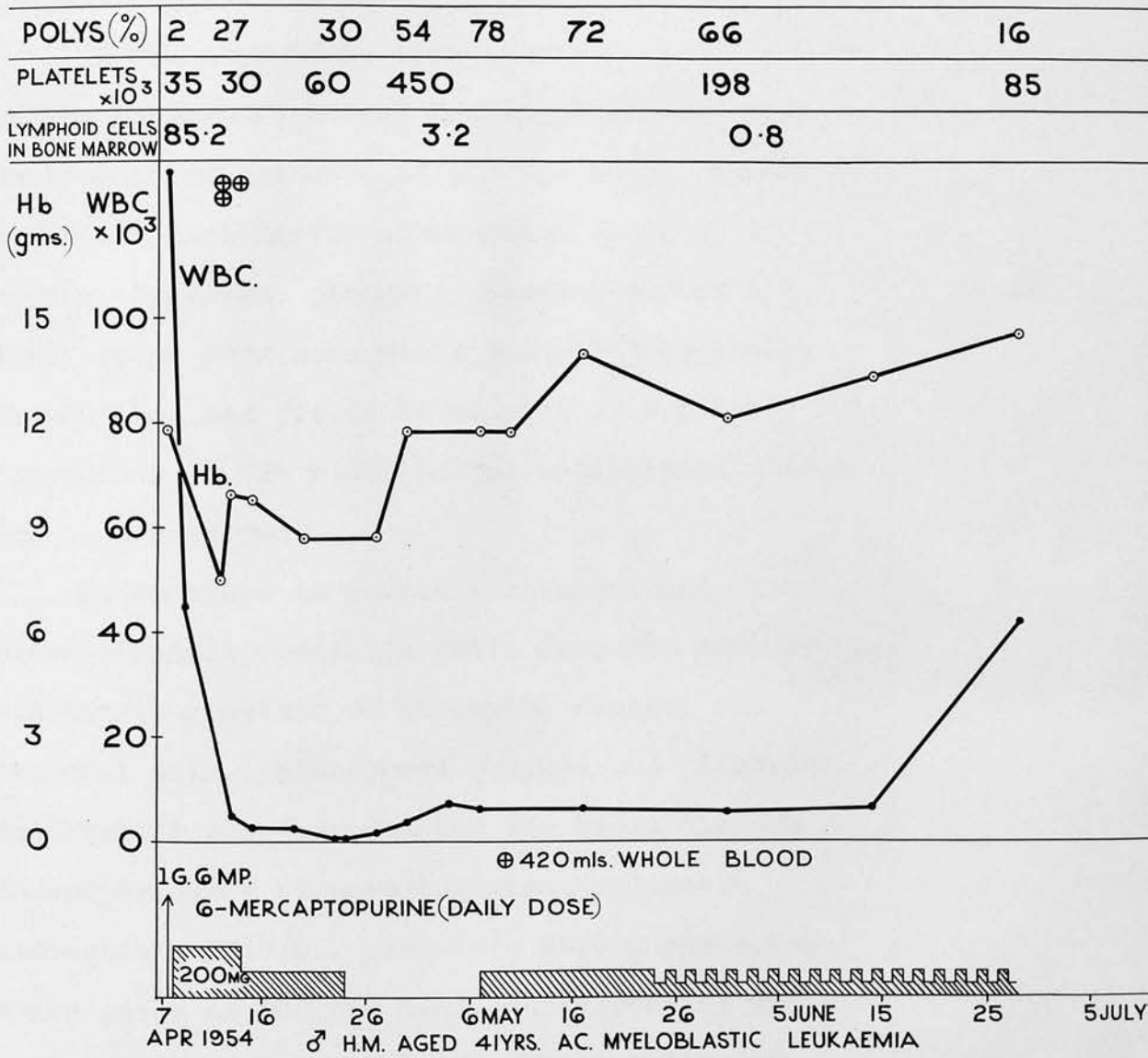

POLYS (%) 2 27 30 54 78 72 66 16
PLATELETS ×10³ 35 30 60 450 198 85
LYMPHOID CELLS IN BONE MARROW 85·2 3·2 0·8
Hb (gms.)
WBC ×10³
WBC.
Hb.
15 100
12 80
9 60
6 40
3 20
0 0
⊕ 420 mls. WHOLE BLOOD
1G. 6MP.
6-MERCAPTOPURINE (DAILY DOSE)
200MG
7 16 26 6MAY 16 26 5JUNE 15 25 5JULY
APR 1954
♂ H.M. AGED 41YRS. AC. MYELOBLASTIC LEUKAEMIA

FIG. 30.

monocytes 1%. Sternal bone marrow: blast cells 3.2%, myeloid cells 44.4%, lymphocytes 12%, erythroid cells 39.2%, other cell types 1.2%.

He was discharged from hospital fully active and in apparently normal health on May 8, on a daily maintenance dose of 100 mg. 6-MP. On May 31, a further bone marrow examination revealed an apparently normal picture. Chest x-ray at this time, apart from some minor pleural thickening, showed the lung fields to be clear. Further diminution in the width of the mediastinal shadow had also occurred.

He remained in complete clinical and haematological remission until June 28, when he started to complain of anorexia, nausea, and skeletal pain. Widespread purpura and glandular enlargement was observed and the blood picture showed evidence of haematological relapse:- haemoglobin 14.8 G., platelets 85,000 per c.mm., white cells 41,500 per c.mm., differential white cell count:- polymorphs 16%, blast cells 84%. He was re-admitted to hospital but in spite of intensive therapy with 6-MP went downhill rapidly and died on July 17, 1954. A post-mortem was not performed.

Comment.

A case of acute myeloblastic leukaemia

showing a dramatic response to 6-MP therapy. A complete remission resulted - the duration from the onset of clinical and haematological improvement being nine and a half weeks. On relapse he was completely resistant to further treatment with 6-MP.

He survived 3½ months following diagnosis, or 5 months after the onset of symptoms.

Case No. 10.

Miss P.P., aged 9 years, was transferred from another hospital to Leeds General Infirmary on July, 12, 1954. Four days previously a diagnosis of acute lymphoblastic leukaemia had been made and a blood transfusion was given.

She gave a history of pains in the back and legs with difficulty in walking for three months. She had been noticed to be becoming increasingly pale and during the week prior to admission had been troubled with epistaxis.

On admission she was found to be slightly anaemic and several lymph glands were palpable in the cervical, axillary and inguinal regions. There was no evidence of bleeding, purpura, or splenomegaly or other abnormality on physical examination. The temperature was normal.

Blood examination gave the following findings: haemoglobin 11.9 G., red cells 4,000,000 per c.mm., platelets 35,000 per c.mm., white cells 3,400 per c.mm. Differential white cell count:- lymphoid cells 96%, neutrophils 4%. Many of the lymphoid cells were immature blast cells. Sternal bone marrow examination was in keeping with the diagnosis of acute lymphoblastic leukaemia, 96% being lymphoid cells, the majority having the characteristics of blast cells. 1.2% myeloid cells and 2.8% erythroid cells were also present.

Progress and Treatment

Treatment with 6-MP, 100 mg. daily, was begun on the day of admission. A rise in temperature three days later, associated with a discharging right ear, necessitated giving penicillin. She, however, did not complain and her general condition was good, the only clinical indication of disease being anaemia and enlarged cervical lymph nodes. No improvement in the blood picture occurred for several weeks. Two weeks after admission the haemoglobin had fallen to 6.5 G. and the peripheral blood showed 92% leukaemic cells. The leucocyte count had been low from the outset and on July 29, when 1,700 per c.mm., the dose of 6-MP was reduced to 25 mg. b.d. The count thereafter fluctuated between 1-2,000 per c.mm. for a further three weeks. An improvement in the peripheral blood was observed on August 13, almost five weeks after the start of treatment. At this time the presence of polymorphonuclear cells in substantial numbers (34%) was noticed for the first time. The temperature, which had persisted for four weeks, had settled and she was symptom free. On August 19, blood examination was as follows: haemoglobin 7.5 G., red cells 2,500,000 per c.mm., platelets 274,000, white cells 3,200 per c.mm. Differential white cell count: polymorphs 44%,

monocytes 2%, lymphocytes 54%. A further bone marrow examination showed:- lymphoid cells 44%, myeloid cells 26%, erythroid cells 30%, a percentage of the lymphoid series being blast cells.

In view of the clinical and haematological improvement she was discharged home on August 21, 1954, on a maintenance dose of 25 mg. 6-MP daily. She was seen on August 30, when the haemoglobin had risen to 11.8 G. without further transfusion. Thereafter she was maintained symptom free for the following thirty-six weeks and she was able to attend school and carry out full activities. During this time the haemoglobin level was consistantly normal. The differential leucocyte count, however, frequently showeda slight preponderance of lymphocytes and the platelet count tended to be subnormal. A few lymph glands persisted also in the neck.

On April 18, 1955, she began to complain of pains in the legs and abdomen and loss of energy. The blood picture showed haemoglobin 11.8 G., white cells 5,700 per c.mm. of which 85% were lympho-blasts. The dose of 6-MP was thereafter increased to 75 mg. daily but no improvement occurred in the following three weeks. She was re-admitted on May 14, 1955, to hospital. On admission a blood transfusion was required which raised the

haemoglobin to 10.5 G. Platelets were 12,000 per c.mm. and there was epistaxis. Over 90% of the white cells in the peripheral blood were lymphoblasts and the spleen was enlarged. 6-MP was stopped on admission and A.C.T.H., 25 I.U. i.m. 8 hourly prescribed. A marked drop in leucocytes occurred followed by clinical improvement, a rise in the platelet count and mature leucocytes in the peripheral blood. Further transfusions were not required. On June 16, the blood showed: haemoglobin 12.6 G., white cells 2,700 (polymorphs 72%, lymphocytes 28%), platelets 150,000 per c.mm. The bone marrow still showed a marked preponderance of leukaemic cells but she was discharged home the next day. A.C.T.H. was stopped and 6-MP 20 mgs. daily given as maintenance therapy. When seen at the medical out-patient department on June 27, further improvement had occurred and the haemoglobin had risen to 14.8 G. The differential count was normal but platelets remained low at 130,000 per c.mm. Improvement was, however, short lived, and she proved completely refractory to further treatment and died in late July, 1955.

Comment

A case of acute lymphoblastic leukaemia in a 9 year old girl who responded satisfactorily and

almost completely to 6-MP therapy. For thirty-six weeks following treatment she lived a normal life. There was no anaemia during that time and the only evidence of the underlying disease was a slight preponderance of lymphocytes in the peripheral blood, a generally rather low normal platelet count and a few enlarged cervical lymph nodes.

After relapse temporary improvement, both clinical and haematological, followed treatment with A.C.T.H.

She survived a period of 12½ months after the diagnosis of acute leukaemia was made, or 15½ months after the onset of symptoms.

Case No. 11.

A male child aged 3 years was admitted to Pontefract General Infirmary on March 2, 1955, with a history of swelling of the parotid glands and increasing pallor of two weeks' duration.

On admission the temperature was raised to 100°F. He was found to be anaemic and numerous bruises and petichiae were present over the lower limbs. There was bilateral parotid enlargement and a right sided facial palsy. The spleen and liver were enlarged and lymph glands were palpable in the cervical, axillary and inguinal regions. Ulceration of the gums was also observed.

Blood examination gave the following results:- haemoglobin 7.6 G., white cells 320,000 per c.mm. A blood film showed practically all the leucocytes to be blast cells. Platelets were absent.

A diagnosis of acute leukaemia was made.

Progress and Treatment

6-MP, 50 mg. daily, was started on March 3, and increased to 100 mg. daily on March 8. On March 11 a blood transfusion was given which raised the haemoglobin from 5.2 G. to 8.1 G. A rapid fall in the leucocyte count occurred and on March 11 had reached 3,000 per c.mm. 6-MP was reduced at this stage to 50 mg. daily. Two weeks after the start of treatment he was feeling greatly

improved, the facial swelling had subsided and the facial movements improved. The spleen and liver were impalpable and the lymph nodes smaller. The haemoglobin was rising and the peripheral blood contained 32% mature polymorphs. The temperature, however, persisted. By March 29 he was symptom free and the blood picture was: haemoglobin 11.1 G., white cells 6,500, differential count: neutrophils 65%, lymphocytes 35%. Thereafter he remained well with no clinical evidence of disease apart from a persistent slight anaemia. He was maintained on 25 mg. 6-MP daily and the remission lasted twenty-two weeks. He was then found to be refractory to treatment with 6-MP and also A.C.T.H. and he died on September 26, 1955, just less than seven months after the diagnosis was made.

Comment

A child aged 3 years responded dramatically to treatment with 6-MP. Considerable clinical and haematological improvement occurred but the haemoglobin level never completely reached normal. He continued in remission twenty-two weeks after a satisfactory response to 6-MP therapy was first noted.

He survived a period of $6\frac{3}{4}$ months following diagnosis, or $7\frac{1}{4}$ months after the onset of symptoms.

Case No. 12.

A male child aged 3 years 9 months was admitted to the Leeds General Infirmary on October 2, 1954, with a history of loss of energy and increasing pallor of 2 months' duration and night sweats and distension of the abdomen for 2 weeks.

On admission his temperature was 100°F, and he was sweating profusely. Anaemia was evident and there was bruising of the right forearm. Lymph gland enlargement was present in the cervical, axillary and inguinal regions and there was hepato-splenomegaly. The liver extended 3-4 cms. and the spleen 8 cms. below the costal margins respectively. There were no other abnormal findings.

Blood examination gave the following findings: haemoglobin 6.2 G., red cells 2,100,000 per c.mm., platelets 95,000 per c.mm., white cells 137,600 per c.mm., differential white cell count: polymorphonuclear cells 5%, lymphocytes 15%, blast cells 80%. Three nucleated red cells per 100 WBC's were observed in the blood film.

Sternal bone marrow was found to be highly cellular, the differential cell count being as follows: blast cells 84.8%, lymphocytes 6.4%, myeloid cells 7.6%, erythroid cells 0.8%.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment (Fig. 31)

A blood transfusion was given on October 7 which raised the haemoglobin to 12.2 G. and treatment with 6-MP, 50 mg. daily, was begun on the following day. Four days later the leucocyte count had fallen to 40,800 per c.mm. but the clinical picture was unchanged. By October 19, after eleven days of 6-MP therapy, the white cells were 1,700 per c.mm., 23% of which were mature polymorphs, the remainder being leukaemic cells. The child was now lively and active, the sweating had cleared and the temperature had almost settled. The liver and spleen were reduced in size. Improvement continued and after three weeks treatment with 6-MP he had regained normal activity, there was no fever, the enlarged liver and lymph glands had disappeared and the only clinical evidence of the disease was the spleen which was just palpable. Blood examination was as follows: haemoglobin 13.0 G., platelets 140,000 per c.mm., white cells 4,900 per c.mm., differential leucocyte count: polymorphs 49%, lymphocytes 48%, monocytes 3%. A sternal bone marrow showed only 20% blast cells, the remainder of the cells being approximately equally divided between myeloid and erythroid precursors.

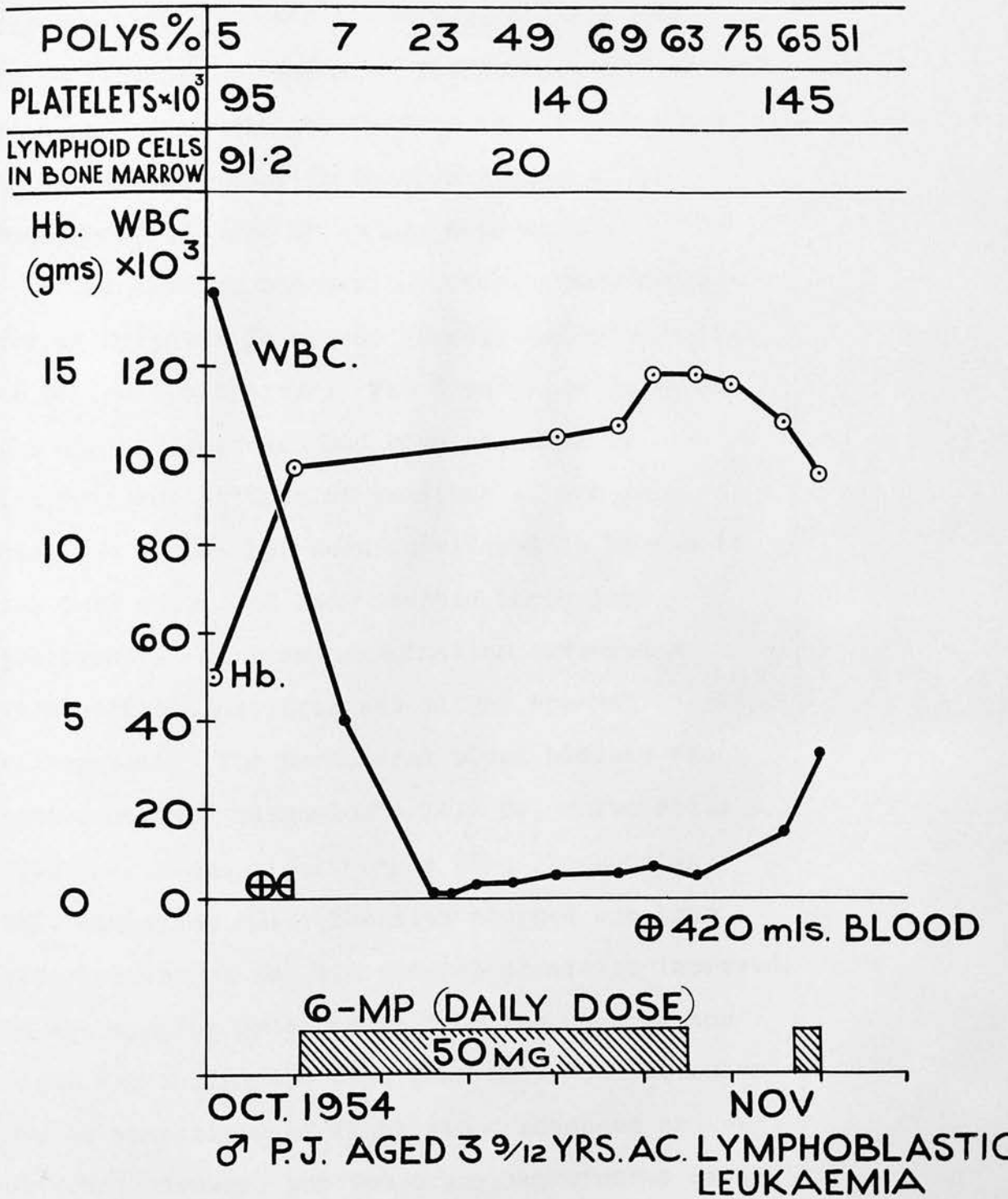

POLYS % 5 7 23 49 69 63 75 65 51
PLATELETS ×10³ 95 140 145
LYMPHOID CELLS IN BONE MARROW 91·2 20
Hb. (gms)
WBC ×10³
15 120
100
10 80
60
5 40
20
0 0
WBC.
Hb.
⊕420 mls. BLOOD
6-MP (DAILY DOSE)
50 MG.
OCT. 1954
NOV
♂ P.J. AGED 3 9/12 YRS. AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 31.

The copenia had persisted for a week when irrespective of continued treatment the leucocyte count returned to normal. The haemoglobin level rose to normal without further transfusions and he was allowed home on October 30, on a "maintenance" dose of 50 mg. 6-MP daily.

The improvement was, however, short lived for on November 15 he was re-admitted to hospital in status epilepticus. The fits which began on the day of admission had been preceded by intermittent attacks of vomiting of six days duration. This had been considered to be due to the 6-MP which had been stopped three days previously. Clinical examination revealed a right sided hemiplegia and slight splenic enlargement. The peripheral blood picture was satisfactory; haemoglobin 14.5 G., white cells 6,400 per c.mm. (polymorphs 75%, lymphocytes 24%, monocytes 1%). The fits stopped one hour after admission and his general condition improved. He was symptom free, apart from weakness of the right leg during the next four days. On November 20, he complained of right sided headache and was obviously drowsy, but temporary improvement again occurred. However, on November 22, he developed persistent vomiting, gradually became comatose and died on November 24.

The cerebro-spinal fluid was examined on two

occasions and showed:

November 15, 1954: cells 1,800 per c.mm. (polys 80%, lymphs 20%), protein 198 mg.%, chloride 720 mg.%, sugar 70 mg.%, culture sterile.

November 22, 1954: cells 3,720 per c.mm. (lymphs 100%), protein 290 mg.%, chloride 710 mg.%, sugar 70 mg.%.

From November 15-24 the clinical picture other than the haematological complications showed no apparent change. The blood showed a gradually developing anaemia, the leucocyte count rose with the re-appearance of 8% leukaemic cells indicating early relapse. 6-MP was re-started on November 20 with no effect. Further bone marrow studies were not performed.

The nature of the neurological manifestations and its relationship to the underlying leukaemic process was obscure. Leukaemic deposits in the meninges or localised bleeding was a considered possible explanation.

The relevant post-mortem findings are briefly mentioned below.

1. Slight enlargement of the paratracheal, retropancreatic and mesenteric lymph nodes.

2. Enlargement and leukaemic infiltration of spleen and kidneys.

3. Petechial haemorrhages and a little fresh haemorrhage in the epidural space overlying the upper cervical segment. No evidence of leukaemic infiltration in the meninges.

4. On sectioning the brain intense generalised capillary congestion was observed. The petichial haemorrhages were confined to the meninges and the underlying grey matter was normal. No visible leukaemic infiltration was present. The spinal cord was normal.

Histology. 1. Infiltration by lymphoid cells of liver, kidney, spleen, pancreas, thyroid and lymph nodes.

2. Considerable meningeal infiltration by lymphocytic cells, mainly perivascular and infiltrating adventia. The cortex showed similar infiltrates around vessels in grey, but more especially around veins in white matter. Similar infiltrates throughout basal ganglia. Section from spinal cord also showed similar changes.

Comment

A 3¾ year old boy with acute lymphoblastic leukaemia responded dramatically to 6-MP therapy, with a return of the peripheral blood picture to apparent normality. The remission was not complete as the spleen, although markedly reduced in size,

remained palpable. Approximately two weeks after discharge from hospital he was re-admitted with bizzare neurological complications and died in coma nine days later. The peripheral blood showed early indications of relapse prior to death. Post-mortem revealed widespread infiltration of viscera by leukaemic cells. Deposits in the brain and leptomeninges suggested that that was the cause of the neurological features.

He survived 1¾ months following diagnosis, or 3¾ months after the onset of symptoms.

Case No. 13.

A 3½ year old female child was admitted to Pontefract General Infirmary on November 29, 1954, with a history of mumps, diagnosed a month earlier, listlessness, increasing pallor, epistaxis, anorexia and recent vomiting.

On admission she was found to be anaemic and the temperature was elevated to 99.8°F. Widespread ecchymoses and petichiae were present mainly on the limbs and lymph node enlargement could be felt in the cervical, axillary and inguinal regions. The spleen was palpable 4 cms. below the costal margin and the liver edge could just be felt.

Blood examination gave the following findings: haemoglobin 5.8 G., red cells 1,900,000 per c.mm., white cells 250,000 per c.mm. Platelets 15,000 per c.mm. The blood film showed less than 1% mature polymorphonuclear cells, virtually all the leucocytes being primitive blast cells.

Bone marrow obtained from the iliac crest showed 99% lymphoid cells, the majority of which were blast forms.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment (Fig. 32)

A blood transfusion was given on December 2, which raised the haemoglobin to 7.8 G. 6-MP,

50 mg. daily, was begun on December 4. Five days later the leucocyte count had fallen from 250,000 to 30,000, the child was feeling happier and there was a reduction in size of the spleen and lymph glands. On December 14, after ten days treatment, the white cells were 2,000 per c.mm. and the dose of 6-MP was reduced to 25 mg. daily. A further transfusion was given on December 17 and thereafter a sustained rise in the haemoglobin level occurred without further recourse to blood transfusion. The temperature had settled by December 21, the purpuric skin eruption had faded, the spleen tip was just below the costal margin and the liver could not be palpated. The peripheral blood picture was also greatly improved - 43% of leucocytes being mature polymorphs. The child was very lively, running about and playing normally and on January 4 she was well enough to be discharged home. Clinical examination now failed to reveal any evidence of leukaemia. Blood examination was as follows:- haemoglobin 11.8 G., platelets 110,000 per c.mm., white cells 5,500 per c.mm. (polymorphs 79%, lymphocytes 17%, monocytes 4%). A maintenance dose of 50 mg. 6-MP daily was prescribed and she was instructed to attend the out-patient clinic at regular intervals. She remained in apparently

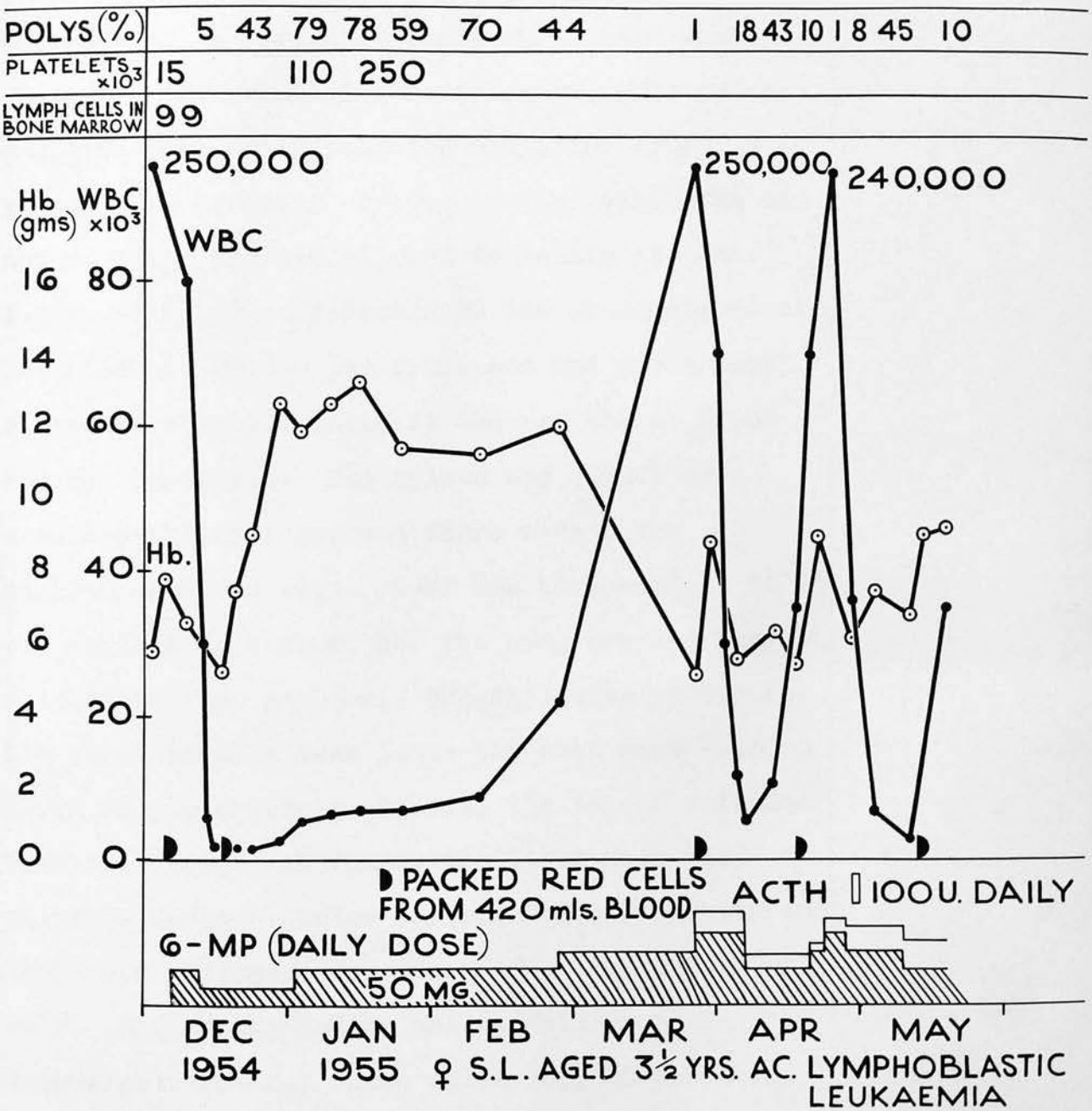

POLYS (%) 5 43 79 78 59 70 44 1 18 43 10 18 45 10
PLATELETS ×10³ 15 110 250
LYMPH CELLS IN BONE MARROW 99
250,000
WBC
250,000
240,000
Hb WBC (gms) ×10³
16 80
14
12 60
10
8 40
6
4 20
2
0 0
Hb.
PACKED RED CELLS FROM 420 mls. BLOOD
ACTH 100U. DAILY
6-MP (DAILY DOSE)
50 MG
DEC 1954
JAN 1955
FEB
MAR
APR
MAY
♀ S.L. AGED 3½ YRS. AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 32.

normal health without any indication of the underlying disease apart from an occasional mild anaemia (haemoglobin range 11.2 to 13.2 G.) until February 11, a period of eight and a half weeks since the beginning of the remission. The spleen could then be again palpated and a few lymph glands were becoming obvious in the neck. She did not complain and was allowed to remain at home. Two weeks later on February 25 the leucocyte count had risen to 22,000 per c.mm. and the percentage of mature granular cells in the peripheral blood had fallen to 44%. The spleen and glands were considerably enlarged and there were a few ecchymoses to be seen. 6-MP was increased to 75 mg. per day and in view of her few symptoms she was allowed to stay at home. She failed to return for her appointment a week later and when next seen on March 25 was severely anaemic, the spleen extended into the left iliac fossa, the liver edge was palpable 2-3 cms. below the right costal margin and there was widespread purpura. The temperature was 99°F. Blood examination was as follows: haemoglobin 5.2 G., white cells 250,000 per c.mm. (polymorphonuclear cells 1%, blast cells 99%). The dose of 6-MP was increased to 100 mg. daily and A.C.T.H. 75 I.U. daily in 8 hourly doses of 25 I.U. i.m., was prescribed in addition. After

twelve days (Apr.6) the leucocytes had fallen to 5,000, and the clinical evidence of leukaemia was much reduced. The dose of 6-MP and A.C.T.H. was now reduced to 50 mg. and 50 U. daily respectively and an improvement in the distribution of leucocytes in the peripheral blood followed, 43% polymorphs being present by April 13. The haemoglobin, however, failed to rise and further transfusions were necessary.

Although clinical and haematological improvement occurred it was only short lived. 6-MP and A.C.T.H. were continued but irrespective of this relapse occurred, the leucocyte count on April 23 rose to 240,000 per c.mm. By increasing the dose of 6-MP and A.C.T.H. it was again possible to temporarily reduce the total white cells and for two weeks improve the differential leucocyte count. The anaemia was persistent, however, and transfusions were again necessary. The fall in the white cell count was not associated as previously with such a marked reduction in the evidence of leukaemia elsewhere, i.e. lymph glands, spleen, etc. and the child's condition gradually deteriorated with bleeding from the mucous membranes and renal tract, and she died on June 16.

Comment

A 3½ year old female child with acute

lymphoblastic leukaemia developed a clinical and haematological remission lasting eight and a half weeks while under treatment with 6-MP. Relapse then occurred and temporary improvement of two weeks duration followed combined therapy with 6-MP and A.C.T.H. Further treatment with increased doses of the two drugs again reduced the leucocyte count and improved briefly the differential cell count but failed to produce any clinical improvement.

She survived 6½ months following diagnosis, or 7½ months after the onset of symptoms.

Case No. 14.

Master R.W., aged 14 years, was admitted to the Leeds General Infirmary on February 7, 1955. His main complaints were cough and progressive dyspnoea of three weeks' duration. For two weeks he had noticed swellings, first in the left axilla and then in the right cervical region. His appetite had deteriorated and on one occasion he had vomited.

On admission he was found to have a good colour. Numerous enlarged cervical and axillary lymph glands about ½ cm. in diameter were palpable. The spleen was enlarged 4 cms. below the left costal margin and the liver edge extended 3 cms. below the right costal margin. Signs suggestive of pleural effusion were present at the left base. The temperature was normal. X-ray of the chest confirmed the presence of a pleural effusion and in addition showed massive enlargement of the mediastinal lymph glands. (Fig. 33a)

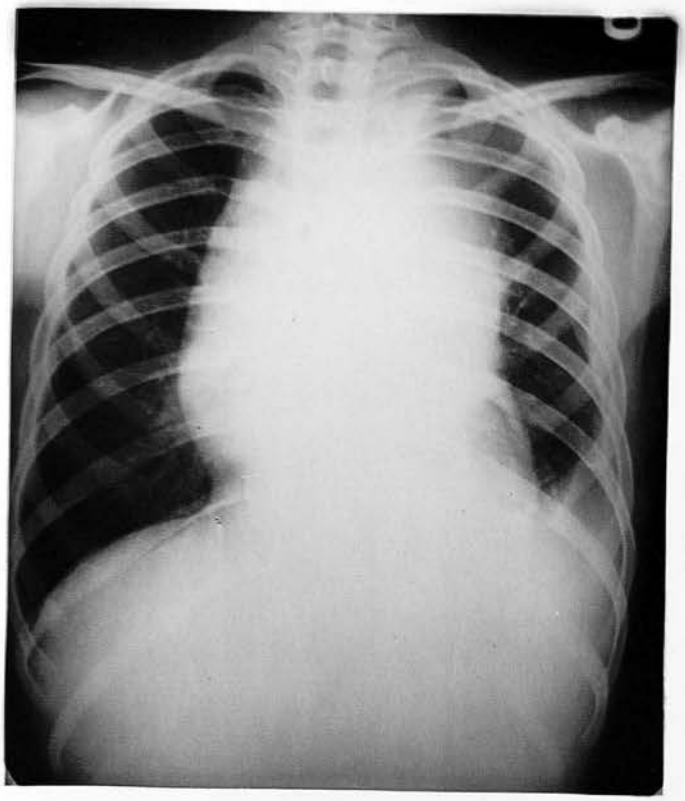
Blood examination on admission gave the following findings: haemoglobin 14.7 G., red cells 5,000,000 per c.mm., platelets 210,000 per c.mm., white cells 24,600 per c.mm. Differential white cell count:- neutrophils 51%, lymphocytes 49%. The blood film showed that many of the lymphocytes were immature having the appearance of lymphoblasts.

A sternal bone marrow examination confirmed the diagnosis of acute lymphoblastic leukaemia, 78.8% of the cells being lymphoid cells the majority primitive blast cells. Myeloid cells 11.2% and erythroid cells 10.0% were also observed.

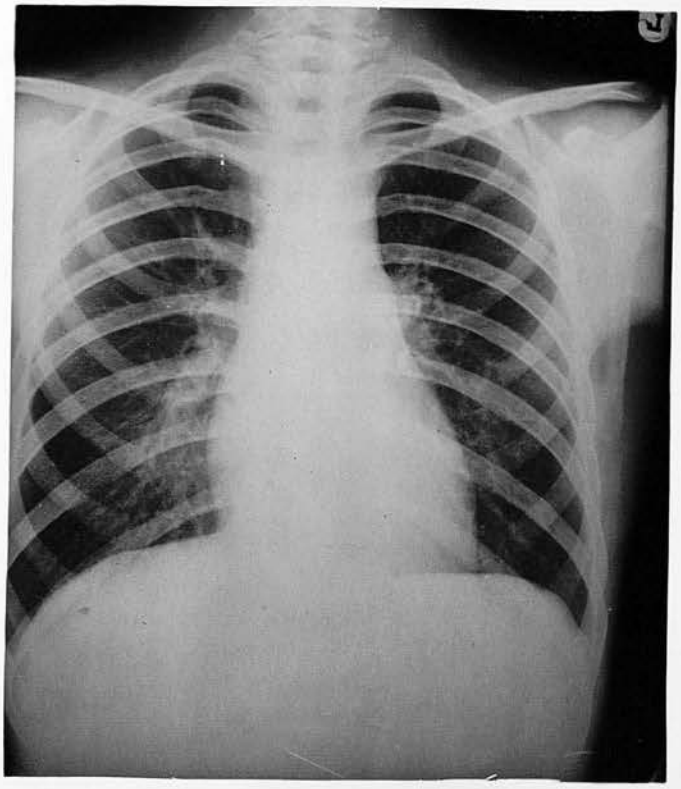
Progress and Treatment (Figs. 33 & 34)

6-MP was begun on February 11, a dose of 150 mg. daily being prescribed. No change in his clinical condition occurred during the next thirteen days. During this time he complained of severe stabbing pain in the left chest and dyspnoea on exertion and the physical findings remained unaltered. On February 24, the lymph glands, liver, and spleen were reduced in size and he began to feel better. The chest pain cleared and the breathlessness became less. On February 28, a further chest x-ray showed considerable reduction in size of the mediastinal glands and the effusion was much less.

After an initial rise in the leucocyte count to 67,000 per c.mm. on February 15 a gradual fall occurred and on February 25 it was 4,200. The haemoglobin level had also fallen at that time to 10.4 G. The dose of 6-MP was reduced to 50 mg. daily on February 28 and after a week's convalescence he was discharged home on March 7, symptom free and fully active. On March 4 (fig.33b)



(a)



(b)

Fig. 33. Case No. 14. Radiograph of chest (a) before treatment and (b) after treatment with 6-MP, showing clearance of pleural effusion and almost complete disappearance of mediastinal lymph nodes.

a further x-ray revealed clearing of the pleural effusion and a return of the mediastinum to almost normality. Blood examination on March 5 was as follows: haemoglobin 10.5 G., platelets 300,000 per c.mm., white cells 4,000 per c.mm., differential white cell count:- neutrophils 68%, lymphocytes 31%, monocytes 1%. A further bone marrow examination showed a less cellular picture with the following differential cell count: lymphocytes and blast cells 21%, erythroid cells 32.5%, myeloid cells 45.5%, other cell types 1.0%. A maintenance dose of 50 mg. 6-MP daily was given and he remained in good health for a period of six weeks. The differential leucocyte count remained normal and a gradual rise in the haemoglobin occurred. Some evidence of the disease persisted, however, for a chest x-ray on March 28 still showed some enlargement of mediastinal glands, and the spleen remained palpable. On April 23, he was re-admitted to hospital in relapse. He was extremely dyspnoeic with marked enlargement of the mediastinal and cervical lymph glands. Splenomegaly was also prominent. The blood picture showed haemoglobin 14.6 G., platelets 235,000 per c.mm., white cells 22,400 per c.mm., differential white cell count: lymphoid cells 69%, neutrophils 31%. Many of the lymphoid cells were

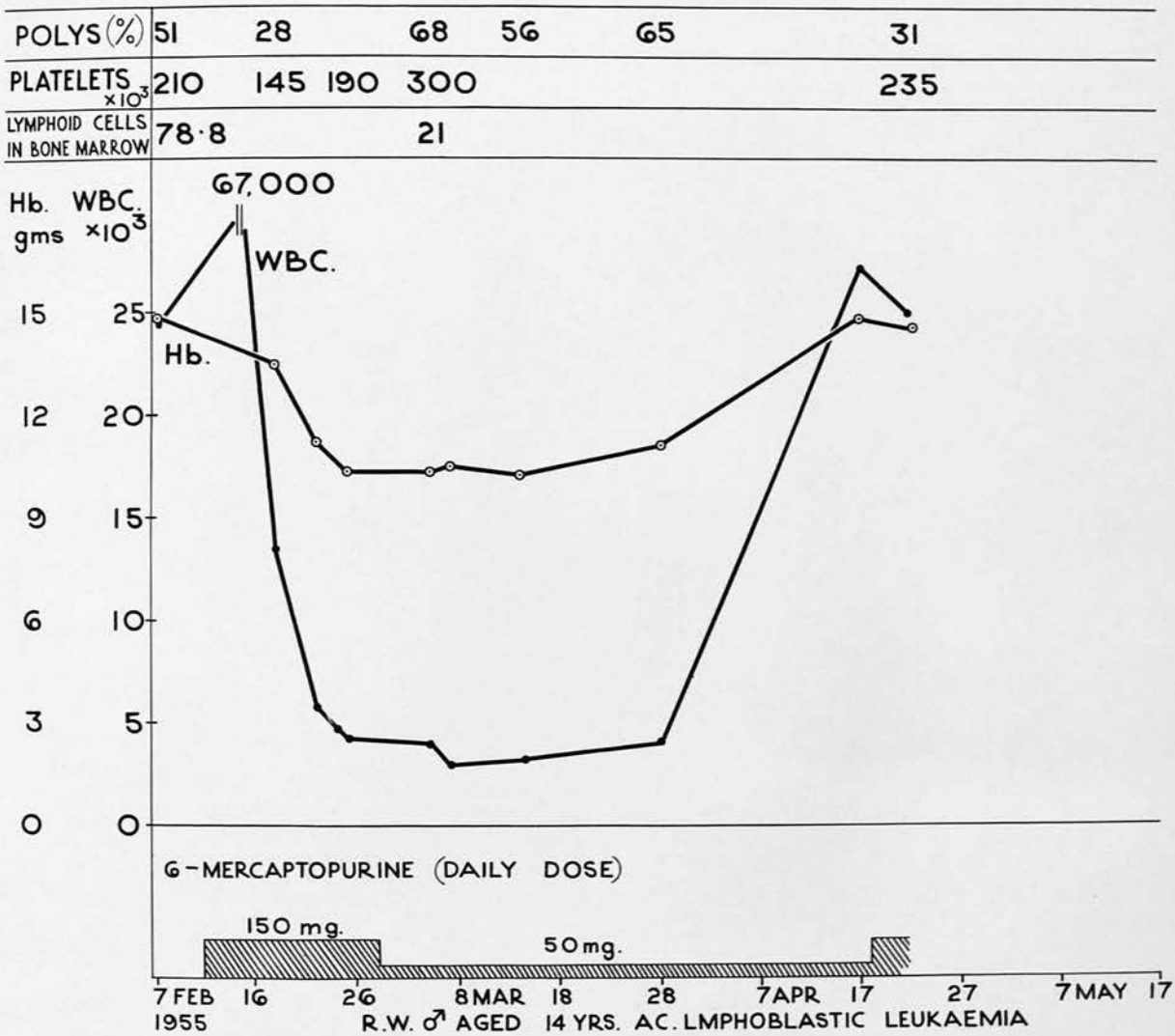

POLYS (%) 51 28 68 56 65 31
PLATELETS ×10³ 210 145 190 300 235
LYMPHOID CELLS IN BONE MARROW 78·8 21
Hb. gms
WBC. ×10³
67,000
WBC.
Hb.
15 25
12 20
9 15
6 10
3 5
0 0
6-MERCAPTOPURINE (DAILY DOSE)
150 mg.
50 mg.
7 FEB 1955
16
26
8 MAR
18
28
7 APR
17
27
7 MAY
17
R.W. ♂ AGED 14 YRS. AC. LMPHOBLASTIC LEUKAEMIA

FIG. 34.

primitive types. 6-MP, 150 mg. daily, and A.C.T.H. 25 mg., 6 hourly, was prescribed during the next fourteen days with marked symptomatic benefit. The dyspnoea was relieved and the enlarged mediastinal lymph nodes and splenomegaly again regressed. The peripheral blood picture when discharged on May 12 showed:haemoglobin 9.3 G., red cells 3,180,000 per c.mm., platelets 270,000, white cells 3,600 per c.mm. Differential white cell count:- neutrophils 57%, lymphocytes 43%. A bone marrow examination revealed the following cellular distribution:- lymphoid cells 57%, myeloid cells 18%, erythroid cells 25%.

Treatment with 6-MP daily and A.C.T.H. gel 25 mgs. twice weekly, was continued at home. The haemoglobin rose to 11.5 G. in the following two weeks but relapse soon followed. He was again admitted to hospital on May 29, and found to be completely refractory to further therapy. Irrespective of treatment the leucocytes rose to 288,000 per c.mm., and he died on June 23.

Comment

A youth aged 14 years with acute lymphoblastic leukaemia responded dramatically to 6-MP therapy initially. Respiratory distress, a consequence of superior mediastinal obstruction from glandular enlargement was relieved, and both clinical and

haematological improvement occurred. Relapse followed some six weeks later. A combination of 6-MP and A.C.T.H. produced temporary benefit but a rapid deterioration followed and death ultimately occurred.

He survived 4½ months following diagnosis, or 5¼ months after the onset of symptoms.

Case No. 15.

Miss A.B., aged 5 years, was transferred from another hospital to Leeds General Infirmary on March 30, 1954. Her main complaints were loss of appetite, lassitude and weight loss for three months. For a month prior to admission her parents had noticed she had "swollen glands" in her neck.

On admission the temperature was found to be normal. There was slight anaemia and numerous hard, cervical lymph glands ½-1 cm. in diameter were palpable. The tip of the spleen was just palpable. No other abnormal findings were detected.

Blood examination gave the following results: haemoglobin 11.9 G., red cells 3,800,000 per c.mm., platelets 275,000 per c.mm., white cells 2,400. Differential white cell count:- polymorphonuclear cells 39%, lymphoid cells 61%. The blood film showed a high percentage of the lymphoid cells to be immature blast cells.

Sternal bone marrow examination gave the following differential cell count: blast cells 96.8%, myeloid cells 2.0%, erythroid cells 2.0%.

A diagnosis of acute lymphoblastic leukaemia (aleukaemic type) was made.

Progress and Treatment (Fig. 35)

6-MP, 50 mg. daily, in divided doses of 25 mg.

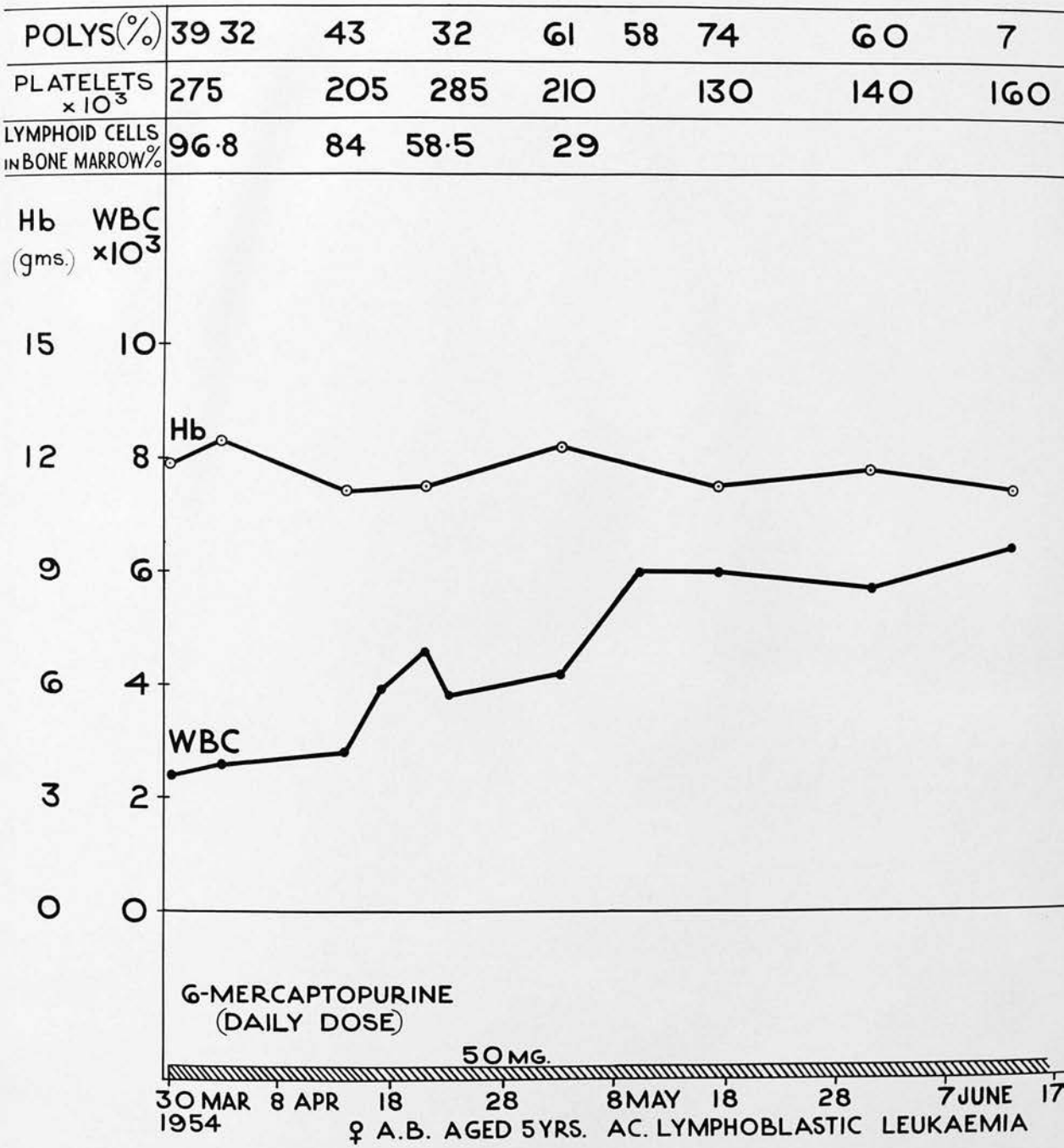

POLYS(%) 39 32 43 32 61 58 74 60 7
PLATELETS ×10³ 275 205 285 210 130 140 160
LYMPHOID CELLS IN BONE MARROW% 96·8 84 58·5 29
Hb (gms.)
WBC ×10³
15 10
12 8
9 6
6 4
3 2
0 0
Hb
WBC
6-MERCAPTOPURINE (DAILY DOSE)
50 MG.
30 MAR 1954
8 APR
18
28
8 MAY
18
28
7 JUNE
17
♀ A.B. AGED 5 YRS. AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 35.

each, was started on March 30. No change in her clinical condition occurred during the following two weeks. On April 14, a further bone marrow examination showed no significant change in the haematological picture, 84% of the cells being of the lymphoid series. A week later, on April 21, definite improvement was observed. She was feeling well and was fully active. The spleen was impalpable and the cervical lymph nodes reduced in size. The bone marrow picture was improving:- lymphoid cells 58.5%, myeloid cells 24.5% and erythroid cells 17%.

She was discharged home on April 23, 1954, on 50 mg. 6-MP daily and thereafter her progress was followed as an out-patient. Ten days later the peripheral blood picture showed haemoglobin 12.2 G., red cells 4,200,000 per c.mm., differential count:- polymorphs 61%, lymphocytes 38%. The bone marrow now showed 29% lymphoid cells all of which appeared mature. Myeloid cells constituted 66% of all marrow cells, red cells being scanty at 5%.

She remained in apparently normal health during the next six weeks. The blood picture never completely returned to normal. There was a persistent anaemia and the platelet count fell after three weeks. The differential leucocyte count remained normal until clinical relapse was observed on June 14. She failed to respond to

further treatment and died on July 13, 1954. Post-mortem examination was not carried out.

Comment

A child aged 5 years with acute lymphoblastic leukaemia developed a partial remission following 6-MP therapy lasting seven and a half weeks.

She survived 3½ months after the diagnosis of acute leukaemia was made, or 6½ months following the onset of symptoms.

Case No. 16.

A female aged 30 years of Belgian nationality was admitted to St. James's Hospital on December 19, 1953. Her main complaints were of increasing weakness and lassitude of 2 months' duration, nausea and occasional vomiting for 1 month, and dizziness and dyspnoea on exertion for 3 weeks. For two weeks prior to admission she noticed that she bruised very easily.

On admission she was found to be very anaemic, ecchymoses were present over the arms and lymph glands were palpable in the cervical and axillary regions. The liver extended 2 cms. and the spleen 3 cms. below the costal margins. The right tonsil was inflamed but the temperature was normal at 97.8°F.

Blood examination gave the following findings: haemoglobin 6.9 G., red cells 2,260,000 per c.mm., platelets 101,000 per c.mm., white cells 69,000 per c.mm., differential white cell count:- blast cells and primitive monocytoid cells 58%, polymorphonuclear cells 21%, lymphocytes 18%, monocytes 3%. There were 2 nucleated red cells per 100 white cells.

Sternal bone marrow was found to be highly cellular, in which the predominant cells were blasts and primitive monocytoid cells, peroxidase negative. The differential cell count was as follows: blasts

and primitive monocytoid cells 84%, myeloid cells 9.6%, erythroid cells 2.4%, lymphocytes 2.4%, other cell types 1.6%.

A diagnosis of monocytic leukaemia, probably Naegali type, was made.

Progress and Treatment

No change had taken place in her condition when treatment with 6-MP, 200 mg. daily, was begun on December 26, a week after admission, and the blood picture was essentially unaltered, the haemoglobin being 6.1 G., and leucocytes 60,000 per c.mm. of which the majority were primitive cells. In the following week approximately 2.5 litres of blood were transfused but the haemoglobin level rapidly fell and by January 5 was 7.4 G. The leucocytes had fallen to 6,800 per c.mm. on January 5, the spleen was reduced in size and the liver impalpable. Her clinical condition, however, had deteriorated. She was febrile and troubled with diarrhoea and nausea. Penicillin was prescribed and the dose of 6-MP reduced to 100 mg. daily. A further transfusion of approximately 2.5 litres of blood was given which only raised the haemoglobin to 9.1 G. 6-MP was stopped on January 7, after a total of 2.3 G. had been given, in view of the leucocytes having reached 2,500 per c.mm. The diarrhoea rapidly cleared and by

January 12 definite clinical and haematological improvement was observed, the bone marrow showing considerable progress in maturation. Blast cells were 25%, myeloid cells 48.5%, erythroid cells 14.5%, lymphocytes 4.0%, monocytes 3.5%, and other cell types 4.5%, 6-MP, 50 mg. daily, was re-started on January 12, in order to try and further improve the clinical and haematological state. The temperature settled, the spleen and lymph glands regressed, the platelet count rose to 180,000 per c.mm. and the haemoglobin, without further transfusion, to 11.1 G. The distribution of leucocytes in the peripheral blood also improved with an increase in percentage of mature cells. A few leukaemic cells remained however and in spite of continued 6-MP relapse soon occurred. The leucocyte count rose to 41,000 per c.mm. on January 25, and leukaemic cells in the marrow and peripheral blood increased. The temperature rose, and a widespread purpuric eruption appeared together with severe menorrhagia. She developed a dental abscess and irrespective of increased doses of 6-MP she rapidly deteriorated and died following a cerebral haemorrhage on February 16, 1954. Autopsy was not performed.

Comment

A 30 year old woman with monocytic leukaemia

improved both clinically and haematologically following a course of 6-MP therapy. Improvement was short-lived lasting no more than two weeks and further treatment proved of no value.

She survived 2 months following diagnosis, or 4 months after the onset of symptoms.

Case No. 17.

A boy aged 14 years was admitted to St. James's Hospital, Leeds, on February 5, 1954, with a two weeks' history of lassitude, anorexia, attacks of dizziness and vomiting. On three occasions during the week prior to admission he had coughed up blood.

On admission the temperature was 100.4°F. He was very anaemic, lymph glands were palpable in the cervical, axillary and inguinal regions and the spleen was palpable 11 cms. below the left costal margin. A purpuric eruption was present over both lower limbs and the left chest.

Blood examination gave the following results: haemoglobin 4.1 G., red cells 1,410,000 per c.mm., platelets 79,000 per c.mm., white cells 29,000 per c.mm. Differential white cell count:- blast cells 92%, lymphocytes 6%, neutrophils 1%, metamyelocytes 1%.

Bone marrow aspirated from the left iliac crest was found to be extremely cellular. 96% of the cells were myeloblasts, the remainder being polymorphs and normoblasts.

Progress and Treatment (Fig. 36)

Transfusions of whole blood were given on February 8, and again on February 12, which raised the haemoglobin to 12.8 G. 6-MP, 100 mg. daily,

was started on February 9. By February 26 no clinical or haematological improvement had occurred and the dose (of 6-MP) was increased to 150 mgs. daily. The leucocyte count had fallen to 4,000 per c.mm. on March 2, three weeks after the start of treatment and by March 9 was 1,000 per c.mm. 6-MP was then stopped. The spleen was reduced in size being 8 cms. below the costal margin. The bone marrow however still showed 95.5% of blast cells. Although two further blood transfusions were given during the following three weeks, the spleen became impalpable and the lymph nodes reduced in size. By March 29, approximately three weeks after discontinuing treatment, the proportion of mature polymorphonuclear cells had risen to 44%. Platelets, which previously had been almost non-existent, rose to 69,000 per c.mm. The patient was feeling much improved, was eating better and the temperature was normal. Bone marrow examination, however, again showed a marked preponderance of leukaemic cells. It was therefore decided to begin treatment with A.C.T.H., 25 units 6 hourly, on April 2. Three days later the blood count was as follows: haemoglobin 13.3 G., red cells 4,600,000 per c.mm., platelets 123,000 per c.mm., white cells 3,000 per c.mm., differential count:- polymorphs 72%, lymphocytes 28%. No abnormal white cells were

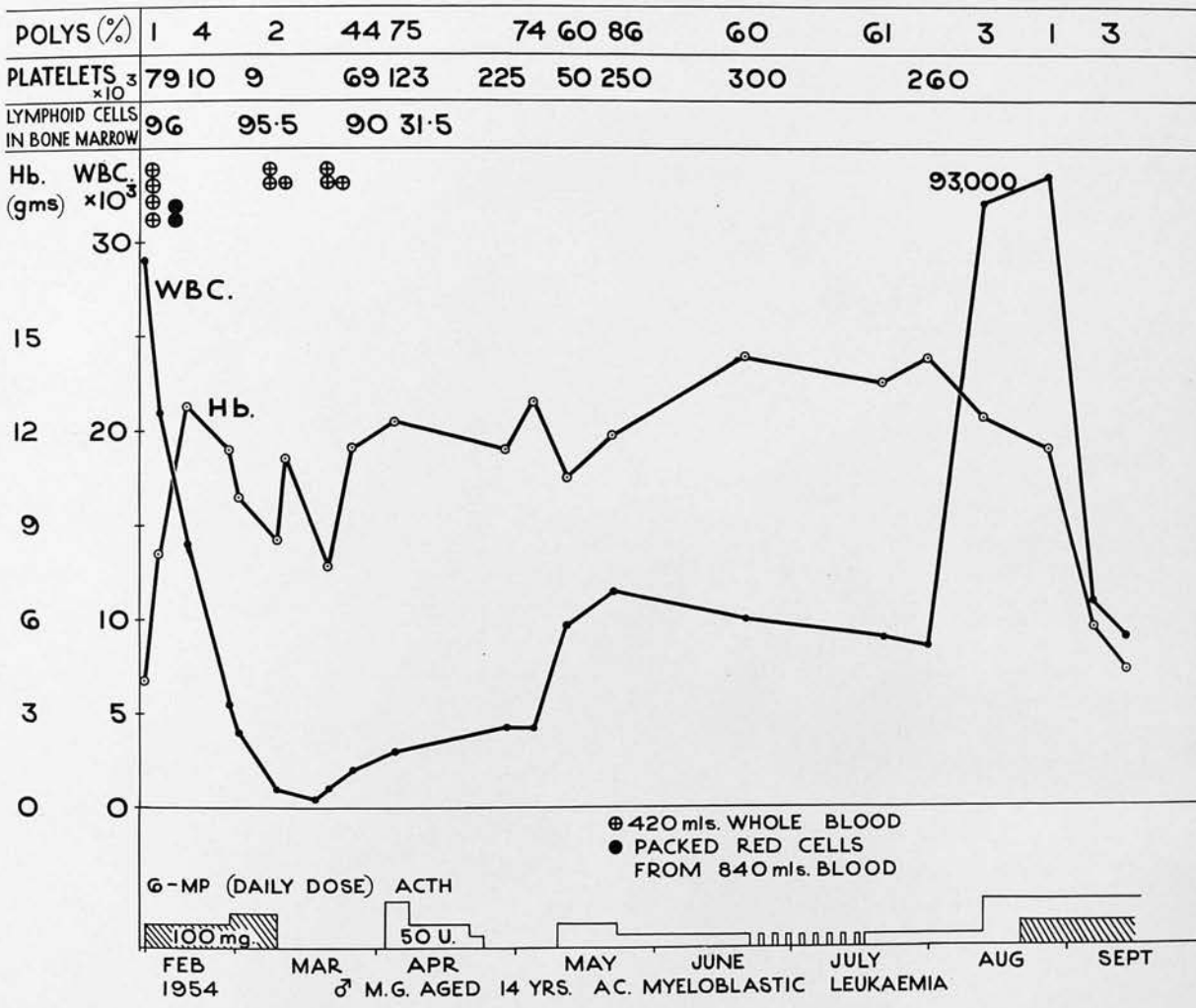

POLYS (%) 1 4 2 44 75 74 60 86 60 61 3 1 3
PLATELETS ×10³ 79 10 9 69 123 225 50 250 300 260
LYMPHOID CELLS IN BONE MARROW 96 95·5 90 31·5
Hb. (gms)
WBC. ×10³
30
15
12
9
6
3
0
20
10
5
0
WBC.
Hb.
93,000
⊕ 420 mls. WHOLE BLOOD
● PACKED RED CELLS FROM 840 mls. BLOOD
6-MP (DAILY DOSE)
100 mg.
ACTH
50 U.
FEB 1954
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
♂ M.G. AGED 14 YRS. A.C. MYELOBLASTIC LEUKAEMIA

FIG. 36.

seen. Another bone marrow examination on April 8 showed a less cellular picture and the following differential count: blast cells 51%, myeloid cells 31.5%, erythroid cells 20.0%, lymphoid cells 17.5%. A.C.T.H. was continued at a reduced dose until April 25. Clinical examination was now negative.

Two weeks later he began to complain of pains in the hip, the spleen was just palpable and the platelet count fell to 50,000 per c.mm. A.C.T.H. was again given at an initial dose of 20 units b.d. and later 20 units daily. He was finally put on a maintenance dose of 20 units every third day. On this regime he remained in apparently normal health during the next three months. An occasional immature leucocyte was observed in the peripheral blood but on most occasions the blood picture appeared normal.

In mid-August, a rapid enlargement of the spleen occurred, numerous blast cells reappeared in the blood and irrespective of treatment with A.C.T.H. and 6-MP he died on September 16, 1954. The only effect treatment in this terminal stage had was to produce a fall in the leucocyte count.

Comment

A boy with acute myeloblastic leukaemia had an initial course of 6-MP lasting four weeks. A fall in the leucocyte count and disappearance of

the splenomegaly with reduction in size of lymph glands and clinical improvement followed. Although an increase in polymorphonuclear cells and platelets in the peripheral blood occurred the bone marrow showed little change. Three days after treatment with A.C.T.H. was started considerable clinical and haematological improvement was observed. Whether this was due to A.C.T.H. or to a continuation of the improvement following 6-MP therapy is difficult to ascertain. Thereafter after a temporary set-back the patient remained in apparently normal health for a period of four and a half months after A.C.T.H. therapy was instituted.

He survived 7½ months after the diagnosis was made, or 8 months following the onset of symptoms.

Case No. 18.

A female child aged 2½ years was admitted to the Leeds General Infirmary on October 14, 1953. She was well until seven weeks previously when she developed diarrhoea which persisted for three weeks. She never picked up, however, and was listless, her appetite was poor and she became increasingly pale. Repeated epistaxis occurred during the two weeks prior to admission and for five days slept almost continuously.

On admission the temperature was 101°F. She was found to be well nourished but extremely anaemic. Bruising was marked over the right iliac crest and both calves, and numerous petichiae were observed in the mouth and lips. Lymph glands were palpable in the cervical, axillary and inguinal regions and the liver and spleen were enlarged. Fine crepitations were audible over both lung bases.

Blood examination gave the following results: haemoglobin 5.3 G., platelets 33,000 per c.mm., white cells 65,000 per c.mm. A differential white cell count showed that 100% of cells were of the lymphoid series and over half were blast forms.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment

A blood transfusion was given immediately following admission which raised the haemoglobin to 14.7 G. Bleeding from the nose continued and she remained drowsy. On October 15 the leucocytes had fallen to 2,900 per c.mm., with no change in the differential cell count. 6-MP, 50 mg. daily, was started on October 16, but no clinical or haematological improvement occurred and she died in coma five days later. It was thought probable that she had intra-cranial bleeding, but autopsy was not performed.

Comment

A female child of 2½ years with acute lymphoblastic leukaemia died seven days after admission and five days after starting 6-MP therapy. A fall in the leucocyte count from 65,000 to a leucopenic level occurred within twenty-four hours of admission and prior to beginning treatment with 6-MP. A blood transfusion was considered a possible cause of this fall in the white cells.

She survived one week after diagnosis, or 2 months following the onset of symptoms.

Case No. 19.

A female infant was born on May 1, 1954, weighing 6 lbs. 13 ozs. The baby was rather shocked at birth and was given Coramine and Lobeline with gradual improvement. On the eighth day the baby developed a 'sticky eye' and was put on Penicillin. On the following day the eye was better but the umbilicus was noticed to be rather inflamed. The cord was still attached and the base was about 1" in diameter. The baby was given Chloromycetin, in addition to Penicillin, with improvement. Chemotherapy was discontinued on the eighteenth day. On the twenty-sixth day the cord had still not separated and there was still some inflammation. The temperature rose again and the abdomen became distended and tympanitic. The possibility of intestinal obstruction was suggested and a laparotomy was performed. At operation no obstruction or inflammation was seen. Free fluid was present in the abdomen and the spleen was greatly enlarged. The abdomen was closed and in view of the splenomegaly blood examination was performed.

Blood examination (May 28, 1954) gave the following results: haemoglobin 7.6 G., white cells 290,000 per c.mm. The blood film showed almost all the leucocytes to be primitive blast

cells. The impression obtained was that this was acute myeloblastic leukaemia.

In view of the history probably extending from birth it was considered that this was a case of congenital leukaemia. The mother's blood revealed no abnormality apart from a mild anaemia (haemoglobin 12.2 G.).

Progress and Treatment

6-MP, 50 mg. daily, in divided doses was begun immediately. The dose, based on the child's weight, was high but this seemed warranted in view of the acuteness of the disease. Treatment, however, was ineffective and the child died twenty-four hours later.

Comment

A case of congenital acute leukaemia, probably of myeloblastic type, died twenty-four hours after treatment with 6-MP was begun. The duration of treatment was too short to produce any effect.

She survived only one day following diagnosis and approximately one month after the onset of the disease.

Case No. 20.

A male child aged 2 years was admitted to Pontefract General Infirmary on December 20, 1954, with a history of lassitude, fever, difficulty in walking and progressive enlargement of the abdomen of two weeks' duration.

On admission the temperature was 102°F., he was very anaemic and breathless and numerous petichiae were present over the limbs and trunk. Lymph glands were enlarged in the cervical, axillary and inguinal regions and the spleen was palpable.

Blood examination was as follows: haemoglobin 6.7 G., red cells 2,200,000 per c.mm., platelets 50,000 per c.mm., white cells 13,600 per c.mm., differential white cell count: lymphoid cells 90%, polymorphonuclear cells 5%, metamyelocytes 4%, monocytes 1%.

Bone marrow obtained from the left iliac crest was found to be almost entirely replaced by leukaemic blast cells. Granular and red cell precursors were virtually absent.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment

An immediate transfusion of packed red cells was given which raised the haemoglobin to 11.4 G.

6-MP, 50 mg. daily, was started on December 22. The leucocyte count fell to 2,000 per c.mm. after a week and thereafter remained low. No improvement in the distribution of leucocytes in the peripheral blood, however, occurred, the haemoglobin fell and further transfusions were necessary on December 30 and January 6. No clinical improvement resulted from treatment with 6-MP, haemorrhages into the skin, eyes, mouth and urinary tract developed and on January 6, after fifteen days therapy, 6-MP was stopped and A.C.T.H. gel 10 units i.m. 12 hourly was given. This also was ineffective, the temperature rose to over 104°F. and the patient died on January 11, 1955. An autopsy was not performed.

Comment

A child aged 2 years with extremely acute leukaemia failed to respond to treatment with 6-MP and later A.C.T.H. The acuteness and rapid downhill course of the disease, and the shortness of the course of treatment were possible reasons for the failure of treatment.

The survival time was 3 weeks following diagnosis, or 1¼ months after the onset of symptoms.

Case No. 21.

A 6¾ year old girl was admitted to the Leeds General Infirmary on August 13, 1954, with a history of attacks of nausea and pain in the left gluteal region for five weeks. For one week she had complained of severe pain in the legs with difficulty in walking and tiredness.

On admission she was found to be pale, but quite happy and co-operative and did not complain of pain. She was afebrile. A few bruises were evident on the legs but the spleen could not be palpated. Small glands were present in the groins but none in the cervical or axillary regions.

Blood examination gave the following findings: haemoglobin 10.7 G., red cells 3,500,000 per c.mm., white cells 8,200 per c.mm. Differential white cell count:- neutrophils 7%, lymphoid cells 95%. Many of the latter cells were immature. A platelet count two weeks after admission was 200,000 per c.mm. Tibial bone marrow on August 23 was of normal cellularity and 99% of cells were lymphoid cells, many of which had the characteristics of lymphoblasts.

A diagnosis of acute lymphoblastic leukaemia was made.

Progress and Treatment (Fig. 37)

When first seen, over two weeks after admission, her condition was unchanged and it was evident that the leukaemic process was running a clinically subacute course. Occasional attacks of pain and lassitude were her only symptoms and clinical examination, apart from anaemia, revealed no definite evidence of the underlying disease. 6-MP, 50 mg. daily, was begun on August 31. A month later no haematological improvement had been observed. The leucocytes had fallen to a leucopenic level, and a gradual fall in haemoglobin had also occurred. Her condition, however, remained fairly good, apart from occasional leg pains, slight bruising and an occasional rise in temperature. 6-MP was increased to 100 mg. daily on October 1, as a result of (a) a failure to respond on the lower dose after thirty days treatment and (b) the leucocyte count beginning to rise irrespective of therapy. No clinical or haematological improvement, however, occurred. A blood transfusion was given on October 20 when the haemoglobin had fallen to 8.3 G. and in view of her satisfactory general condition she was allowed home on October 21. Thereafter she attended as an out-patient and 6-MP therapy was continued. The leukaemic process remained resistant to treatment,

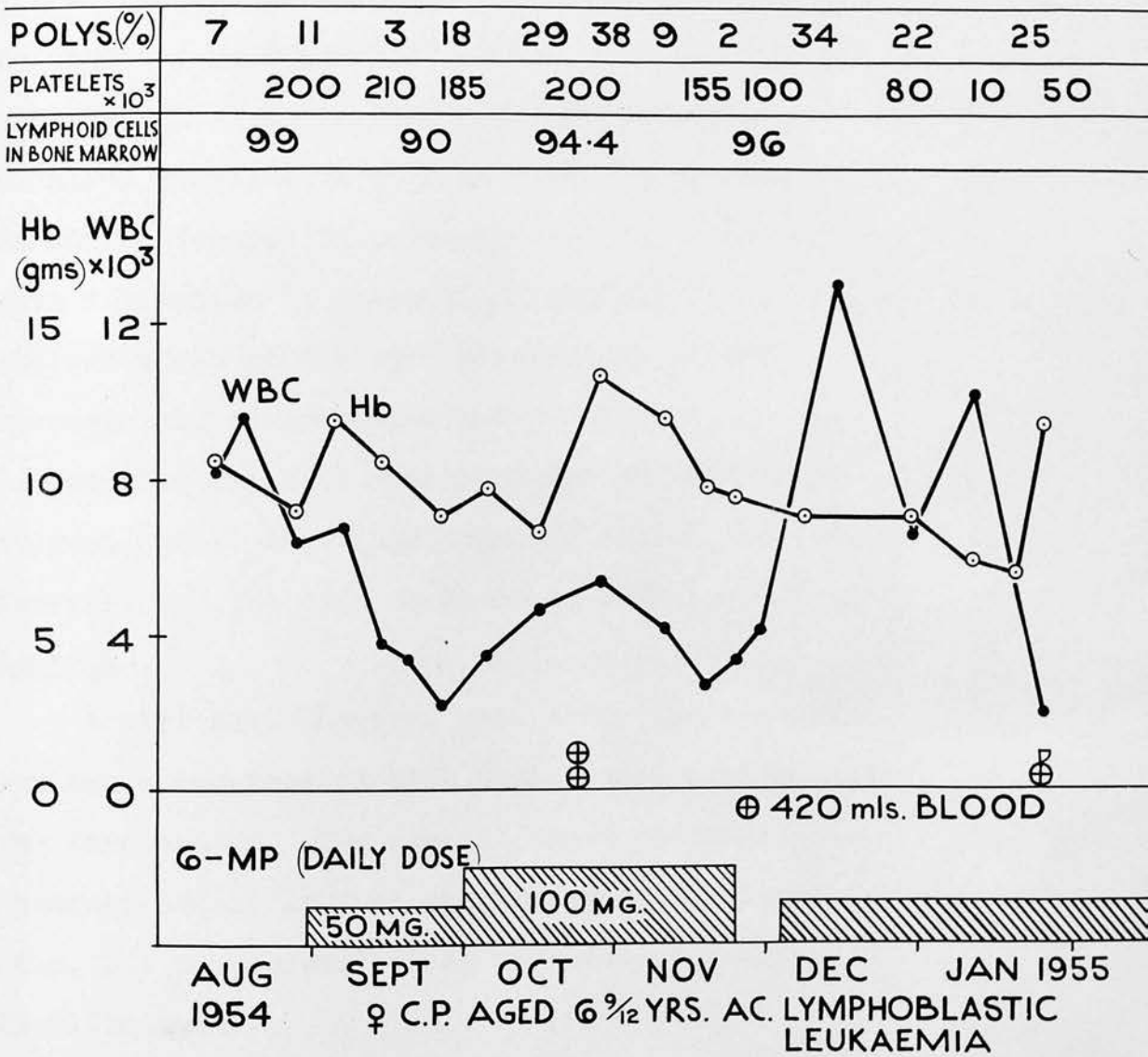

POLYS.(%) 7 11 3 18 29 38 9 2 34 22 25
PLATELETS ×10³ 200 210 185 200 155 100 80 10 50
LYMPHOID CELLS IN BONE MARROW 99 90 94·4 96
Hb (gms) WBC ×10³
15 12
10 8
5 4
0 0
WBC
Hb
⊕ 420 mls. BLOOD
6-MP (DAILY DOSE)
50 MG.
100 MG.
AUG 1954
SEPT
OCT
NOV
DEC
JAN 1955
♀ C.P. AGED 6 9/12 YRS. AC. LYMPHOBLASTIC LEUKAEMIA

FIG. 37.

the haemoglobin level fell slowly and platelets also became less numerous. She had few complaints apart from occasional pain. She was re-admitted to hospital on January 12, 1955, because of severe anaemia and given another blood transfusion. Clinical examination at this time showed anaemia, marked tenderness on pressure over the right femur with limitation of movement of the hip joint, and a shallow ulcer on the soft palate. There was no splenomegaly or lymphadenopathy. Following transfusion she felt much improved and was again allowed home. A gradual downhill course followed, however, and she died on March 4, 1955.

Comment

A girl aged $6\frac{3}{4}$ years, with acute lymphoblastic leukaemia was treated with 6-MP almost continuously for five months. The disease ran a clinically subacute course in that symptoms were relatively mild, but no haematological improvement resulted from treatment.

She survived approximately seven months following diagnosis, or $8\frac{1}{2}$ months after the onset of symptoms.

Case No. 22.

A female aged 53 years was admitted to St. James's Hospital on November 1, 1954, with a history of recurrent haematuria and bleeding from the bowel with increasing anaemia for one month.

On admission she was found to be severely anaemic, the liver was enlarged 4 cms. and the spleen 3 cms. below the respective costal margins, and a few enlarged lymph glands were present in the cervical region. Prolapsed haemorrhoids were observed. There were no skin haemorrhages and the temperature was normal.

Blood examination gave the following findings: haemoglobin 4.5 G., red cells 1,300,000 per c.mm., platelets 45,000 per c.mm., white cells 11,400 per c.mm. Differential white cell count:- blast cells 90%, promyelocytes 1%, myelocytes 1%, lymphocytes 7%, monocytes 1%. Sternal bone marrow was hypercellular and the differential cell count showed 46.5% blast cells, myelocytes and polymorphonuclear cells 9%, lymphocytes 4%, erythroid cells 40.5%.

The blast cells had the staining characteristics of myeloblasts and a diagnosis of acute myeloblastic leukaemia was made.

Progress and Treatment

An immediate blood transfusion was given which

raised the haemoglobin to 9.0 G. and produced symptomatic improvement. 6-MP, 150 mg. daily, was started on November 9 and continued until December 1, when the leucocytes had fallen to 2,000 per c.mm. On a reduced dose of 50 mg. 6-MP leucopenia persisted for 3 weeks, the liver and spleen became smaller but no improvement in the distribution of leucocytes in the peripheral blood occurred, the platelets remained low and repeated bleeding from the lower bowel necessitated further blood transfusion to maintain the haemoglobin at a satisfactory level. A.C.T.H. 40 I.U. intravenously in 500 mls. of saline given over a period of 8 hours was given on three consecutive days, and followed by A.C.T.H. gel 40 U. intramuscularly twice daily for 7 days. There was no effect on the clinical or haematological picture. On December 30, the dose of 6-MP was raised again to 150 mg. daily but apart from again producing a fall in the leucocyte count, it was ineffectual. After repeated haemorrhages, she passed into coma on January 22, and died the same day. Post-mortem examination was not performed.

Comment

A 53 year old female with acute myeloblastic leukaemia failed to obtain benefit from treatment with 6-MP over a period often weeks. The only

effect of the drug was a fall in the leucocyte count and reduction in size of the liver and spleen. Treatment with A.C.T.H. also had no effect on the leukaemic process.

She survived 2¾ months after diagnosis, or 3¾ months following the onset of symptoms.

Case No. 23.

A female aged 47 years was admitted to St. James's Hospital, Leeds, on January 6, 1955, with a two months' history of tiredness, lassitude, nervousness and "thumping noises" in the head. Over the same period she had become progressively paler and had complained of dyspnoea on exertion, flatulence and heartburn. Menstruation had latterly become scanty.

On admission the temperature was 98.0°F. She was severely anaemic and a few small lymph glands were present in the right posterior triangle of the neck. There was no enlargement of the liver or spleen or evidence of bleeding. The right breast was the seat of chronic mastitis.

Blood examination gave the following findings: haemoglobin 3.16 G., red cells 1,020,000 per c.mm., reticulocytes 1%, P.C.V. 10%, M.C.V. 103 cu., M.C.H.C. 26%, platelets 50,000 per c.mm., white cells 9,500 per c.mm. Differential white cell count:- blast cells 24%, myelocytes 3%, polymorphonuclear cells 44%, lymphocytes 21%, monocytes 7%, Tunck cells 1%. 4 nucleated red cells per 100 white cells were observed.

The sternal bone marrow was moderately cellular and obtained with difficulty. The differential cell count was as follows:- blast cells 42.4%, myeloid

cells 33.6%, lymphocytes 5.6%, plasma cells 2.8%, erythroid cells 14.4%, other cells 1.2%. Erythropoiesis was mainly megaloblastic.

Other investigations included a Rehfuse Test Meal which showed the presence of free HCl., serum bilirubin, direct 0 mgm.%, total 0.365 mg.%, plasma proteins 5.75 G.%, A/G ratio 1.4:1, Alkaline phosphatase 10.4 units. Bence-Jones Protein - absent. Blood urea 41 mgms.%. X-ray chest and pelvis - normal. Faecal urobilinogen 233 mg.%.

Progress and Treatment

The probable diagnosis was considered to be acute leukaemia with myeloblastic erythropoiesis (leukanaemia) but in view of the possibility of the blast cells in the bone marrow being atypical megaloblasts it was decided to try the effect of Vitamin B_{12} and folic acid. Vitamin B_{12} (Cytamen) 100 mgms. was given on alternate days for a fortnight with no effect on the clinical or blood picture. Folic acid 20 mgm. daily was then prescribed and continued for several weeks. It failed, however, to have any effect on erythropoiesis, no reticulocyte response occurred and repeated blood transfusions were necessary. Further bone marrow studies after both Vitamin B_{12} and folic acid showed no alteration in the cell

picture - erythropoiesis remaining essentially megaloblastic.

It was decided to start 6-MP, 150 mg. daily, on April 29. Blood examination at that time showed: haemoglobin 7.4 G., white cells 50,000 per c.mm., platelets 30,000 per c.mm. The peripheral blood contained 89% blast cells and the bone marrow 86.4%. Clinical examination was unaltered, the white cells had fallen to 10,500 after ten days and after one month's treatment were 4,200 per c.mm. There was still a preponderance of leukaemic cells, however, and the platelet count remained low. Blood transfusions were also still required. At her request she was transferred to a convalescent home on May 24 and advised to continue to take 6-MP, 100 mg. daily. When seen two weeks later her condition was unaltered and no haematological evidence of a response to treatment was observed.

6-MP had no effect on the leukaemic process in this patient, who died on July 12, 1955.

Comment

A patient with leukanaemia showed no response initially to Vitamin B_{12} and Folic Acid. Thereafter 6-MP therapy was instituted but no clinical or haematological improvement occurred following a two months course.

She survived a period of 6 months following diagnosis, or 8 months after the onset of symptoms.

Case No. 24.

A male patient aged 78 years was admitted to St. James's Hospital on November 9, 1953, with a history of generalised pruritus of one year's duration. More recently he had been troubled with increasing dyspnoea on exertion and had noticed himself becoming paler.

On admission he was afebrile and the only significant finding was severe anaemia. There was no lymphadenopathy, hepatomegaly or splenomegaly. The skin was dry but there was no eruption.

Blood examination gave the following findings: haemoglobin 7.0 G., red cells 2,000,000 per c.mm., platelets 58,000 per c.mm., white cells 4,900 per c.mm. Differential white cell count:- blast cells 11%, neutrophils 29%, eosinophils 10%, monocytes 30%, lymphocytes 18%, Tinck cells 2%.

Sternal bone marrow was poorly cellular and showed: blast cells 27%, myeloid cells 31,5%, erythroid cells 25%, monocytes 4%, other cell types 2.5%. Many of the white cell precursors had a normocytic appearance and a diagnosis of monocytic leukaemia of Naegali type was made.

Progress and Treatment

A blood transfusion was given on the day of admission which raised the haemoglobin to 9.3 G. 6-MP, 200 mg. daily, was begun on November 28,

after a further transfusion owing to a fall in haemoglobin to 7.1 G. Treatment was continued until December 17 when the leucocytes reached a level of 1,400 per c.mm., haemoglobin 7.5 G., platelets 68,000 per c.mm. The peripheral blood still showed 30% blast cells and immature monocytoid cells and blast cells were the predominant cell in the hypoplastic bone marrow. The leucocytes continued to fall to 650 per c.mm. on December 21. At this time he developed a severe soreness of the throat and was given a further transfusion which initially gave rise to rigors and shock with a pronounced fall in blood pressure. The temperature rose, he developed diarrhoea, pain in the legs, breathlessness, dryness and cracking of the lips, and a monilia infection of the mouth, and eventually died from bronchopneumonia on January 5. The white cell count remained severely depressed being 1,200 per c.mm. the day prior to death and no improvement in the differential leucocyte picture occurred. A rise in platelets to 225,000 per c.mm. on December 30 was the only improvement observed in the blood. Post-mortem showed a right lower lobe bronchopneumonia, cystitis and bilateral pyonephrosis, cor pulmonale and advanced emphysema. There was no naked eye evidence suggestive of

leukaemia in the liver, spleen or other viscera. None of the lymph glands were enlarged and the bone marrow was pale and slightly gelatinous but not characteristic of leukaemia.

Histology of lymph glands from the mediastinum and lesser curvature of stomach showed extensive but patchy reticulo-endothelial reaction, with bizarre multinucleated giant cells. The architecture of the glands was not completely destroyed and there was some trabecular fibrosis. No fixation of eosinophils in the glands was noted. The liver showed mild portal cirrhosis but no leukaemic infiltration. Sections from other sites were not unfortunately taken.

Comment

An unusual and difficult case of an elderly man with a peripheral and bone marrow picture of monocytic leukaemia who failed to benefit from treatment with 6-MP. The only effect of treatment was a lowering of the leucocyte count, although latterly the platelets did rise to a normal level. Post-mortem revealed, together with other pathologies, Hodgkin's disease.

It is suggested that the essential lesion was Hodgkin's disease with subsequent monocytic leukaemia. The absence of evidence of the latter at autopsy is difficult to explain. Histological examination was

not complete, however, and the possibility that the absence of leukaemic cells may have been a consequence of 6-MP therapy suggests itself in view of the profound terminal depression of the leucocytes in the peripheral blood and bone marrow.

He survived 1¾ months after the diagnosis was made. The survival time from the onset of symptoms was difficult to ascertain in view of the difficulty in obtaining a satisfactory history.

Case No. 25

A 50 year old female was admitted to the Brotherton Wing of the Leeds General Infirmary on October 26, 1953, with a history of night sweats, dyspnoea, recurrent purpura and increasing pallor of six months' duration. A week before admission she noticed haematuria.

On admission she was anaemic and purpura was present over the face, neck, limbs and trunk. Ulceration of the lips and a large swelling in the right submandibular region was present. Lymph glands were palpable in all areas and the spleen was enlarged. The temperature was 100.4°.

Blood examination revealed: haemoglobin 9.9 G., red cells 3,600,000 per c.mm., platelets 33,000 per c.mm., white cells 44,800 per c.mm. Differential white cell count:- polymorphonuclear cells 3%, blast cells 96%. The blast cells were peroxidase positive and many had a monocytoid appearance. The sternal marrow was hypercellular, 75% of cells being primitive blasts and 25% of the myeloid series.

A diagnosis of monocytic leukaemia of Naegali type was made.

Progress and Treatment

A transfusion of concentrated red cells was given on November 2, and 6-MP, 200 mg. daily, was

begun on the following day. The leucocyte count fell from 88,000 to 5,000 per c.mm. in the following seven days, the spleen became impalpable and the mass in the neck smaller. Her clinical condition, however, remained unchanged, the temperature persisted and further transfusions were necessary. She finally succumbed from a right lower lobe pneumonia on November 11.

Comment

Acute leukaemia affecting an adult female who died of intercurrent infection a week after the start of treatment with 6-MP. A fall in the leucocyte count and regression of leukaemic tissue was observed to follow therapy.

She survived 2 weeks following diagnosis, or 6½ months after the onset of symptoms.

Case No. 26.

A female patient aged 53 years was admitted to the Leeds General Infirmary on May 31, 1954, with a ten days history of epistaxis, purpura, haematuria, fever and pains in the back and limbs.

On examination the temperature was 102.6°F., she was severely anaemic and there was marked ulceration of the mouth and throat, widespread purpura was evident affecting the limbs, trunk and buccal cavity. Small cervical, axillary and inguinal lymph glands were palpated, the abdomen was distended but no enlargement of the liver or spleen could be detected.

Blood examination gave the following result: haemoglobin 8.7 G., red cells 3,500,000 per c.mm., platelets 71,000 per c.mm., white cells 358,600 per c.mm. The majority of the leucocytes were primitive blast cells, but a percentage were promyelocytes and myelocytes.

Sternal bone marrow was hypercellular, the predominant cells being poorly differentiated leukaemic cells of the myeloid series. Less than 1% of mature polymorphonuclear cells were seen and no red cell precursors could be found.

A diagnosis of acute myeloblastic leukaemia was made.

Progress and Treatment

In view of the extremely acute nature of the disease 6-MP, 1 Gm., was given in the 24 hour period following admission, together with a transfusion of concentrated red cells. 6-MP, 200 mg., daily, was thereafter prescribed. No beneficial effect was observed, however, she had repeated convulsions and death occurred on June 3.

Post-mortem examination revealed the characteristic findings of acute leukaemia, with massive haemorrhage in the left temporal lobe and smaller haemorrhages in the corona radiata, pons and cerebellum.

Comment

A 53 year old woman with acute myeloblastic leukaemia was given 1 G. 6-MP in 24 hours. This failed to control the leukaemic process and she died three days after admission.

She survived 3 days following diagnosis, or 14 days after the onset of symptoms.

Case No. 27

A male patient aged 42 years was admitted to St. Luke's Hospital, Bradford, on December 21, 1954, with a history of attacks of shivering and aching in the limbs and failing health of one month's duration. He had an attack of tonsillitis in early December followed by 'gastritis' which was associated with severe vomiting and lasted five days.

On admission the temperature was raised to 101.6°F. He was found to be pale, a few palpable lymph glands were present in the cervical and inguinal regions and the spleen tip could just be palpated. There was no evidence of bleeding.

Blood examination gave the following findings: haemoglobin 6.4 G., red cells 2,000,000 per c.mm., white cells 46,000 per c.mm., differential white cell count:- blast cells 56%, promyelocytes 16%, myelocytes 9%, polymorphonuclear cells 3%, lymphocytes 13%, monocytes 3%.

Bone marrow examination was not performed.

A diagnosis of acute myeloblastic leukaemia was made.

Progress and Treatment

6-MP, 200 mg. daily, was begun on December 24. Penicillin was also prescribed and a transfusion of 840 mls. whole blood was given on

admission. A week after starting treatment the leucocytes fell to 10,300 per c.mm. A further transfusion was given at this time which raised the haemoglobin to 11.1 G. By January 3, the white cells were 1,400 per c.mm., the differential being unchanged. 6-MP was stopped but within three days a rising leucocyte count necessitated re-starting treatment at a dose of 150 mg. daily. He was, however, feeling much better, the temperature had settled, the spleen was impalpable and he was symptom free and he was allowed up. Clinical improvement was short-lived, however, the anaemia was persistent and the blood continued to show evidence of leukaemia. Towards the end of January he started to complain of dysphagia and the temperature and leucocyte count rose irrespective of continued 6-MP therapy. A.C.T.H., 50 units 6 hourly, was given for one week in addition to 6-MP without any effect on the leukaemic process although the dysphagia did clear. Cortisone, 100-200 mgm. daily, was given for a month. The temperature settled for a week and the leucocytes fell to 800 per c.mm., but no clinical or haematological improvement followed. Repeated blood transfusions were given, but failed to prevent a downhill course, with sloughing necrotic lesions in the throat and mouth, gluteal abscesses,

cellularity of the right arm, and death occurred on April 8, 1955.

Post-mortem revealed a right upper lobe pneumonia and widespread infiltration of all tissues with leukaemic cells.

Comment

A 42 year old male with acute myeloblastic leukaemia failed to respond to 6-MP. A fall in the leucocyte count with regression of the enlarged spleen was noted initially together with clinical improvement of short duration. No response to A.C.T.H. or Cortisone was observed.

He survived 3½ months after diagnosis, or 4½ months following the onset of symptoms.

Case No. 28.

A female aged 34 years was admitted to St. Luke's Hospital, Bradford, on February 26, 1955, complaining of tiredness, general debility and dyspnoea on exertion of three weeks' duration.

On admission she was very anaemic and the temperature was 100.6°F. Palpable lymph glands were present in the neck and groin and the spleen extended to below the umbilicus.

Blood examination gave the following findings: haemoglobin 5.1 G., white cells 40,000 per c.mm., platelets 30,000 per c.mm. Differential leucocyte count:- blast cells 48%, promyelocytes 13%, myelocytes 3%, metamyelocytes 6%, polymorphonuclear cells 11%, lymphocytes 19%. 2 nucleated red cells per 100 white cells were observed.

A diagnosis of acute myeloblastic leukaemia was made. Bone marrow examination was not performed at this stage.

Progress and Treatment

Initial blood transfusions resulted in a temporary rise in haemoglobin. 6-MP, 200 mg. daily, was begnn on March 11, when the haemoglobin was 6.9 G., white cells 24,000, platelets 31,000 per c.mm. The differential white cell count still showed a preponderance of leukaemic cells. No change was observed during the following two weeks.

On March 24, the leucocytes fell to 3,000 and six days later were 2,000 per c.mm. The differential count was, however, unchanged and bone marrow showed 95% myeloblasts. Her clinical condition showed a regression in size of the spleen and lymph glands but the temperature remained elevated. 6-MP was stopped on March 30 and the white cells continued to fall. On April 5, they were 640 per c.mm. The count remained low for several days and on April 15 it was 600 per c.mm. The spleen was now impalpable and the lymph glands much less obvious. The haemoglobin, however, remained low at 5.7 G., and she developed an ulcerated throat for which penicillin was prescribed.

She was transferred to another hospital on April 19, 1955. The sore throat improved and the temperature gradually settled. Definite improvement in the differential leucocyte count occurred with an increase in the percentage of mature polymorphonuclear cells. The haemoglobin level had risen without further transfusion and the enlargement of the lymph glands and spleen had subsided completely. She felt well in herself and was discharged home on the 7th May. The bone marrow showed a reduction in blast cells to 34%. 6-MP was witheld and when seen at the out-patient department on May 19 the haemoglobin was 10.4 G. The differential

leucocyte count still showed 50% polymorphonuclear cells but myelocytes and a few blast cells could be observed. She was feeling well, and refused to be admitted to hospital for further treatment at that time. On June 22, she was, however, re-admitted in pronounced clinical and haematological relapse and she died following what appeared to be a cerebral haemorrhage on June 24.

Comment

A 34 year old woman with acute myeloblastic leukaemia developed both clinical and haematological improvement after treatment with 6-MP. The beneficial effects of therapy lasted, however, only seven weeks.

The survival time following diagnosis was 4 months, or $4\frac{3}{4}$ months after the onset of symptoms.

Case No. 29.

A 49 year old hospital porter had been in good health up to the day prior to admission to the Leeds General Infirmary on September 25, 1953. At that time he experienced a sudden attack of pain in the left side of the abdomen associated with vomiting. A past history of peptic ulceration with perforation in 1945, and partial gastrectomy in 1949, led to his immediate admission to hospital.

On admission he was found to be a pale, thin man, in obvious pain and who vomited repeatedly during the examination. A large rubbery, mobile lymph gland the size of a walnut was present in the left axilla and the spleen was palpable 8 cms. below the left costal margin. No other abnormalities were found on clinical examination.

Blood examination was in keeping with a diagnosis of chronic myeloid leukaemia and was as follows: haemoglobin 9.0 G., red cells 3,300,000 per c.mm., reticulocytes 2%, platelets 300,000 per c.mm., white cells 128,000 per c.mm. Differential white cell count: myelocytes 2%, metamyelocytes 17%, neutrophils 79%, eosinophils 1%, lymphocytes 1%. An occasional nucleated red cell was observed on examination of the peripheral blood film. Sternal puncture revealed a hypercellular bone marrow, with a marked

preponderance of granular cells, 93.6% being of this series.

Progress and Treatment (Fig. 38)

Treatment with 6-MP was begun on September 28 and continued until October 23, a total dose of 4.4 G. being prescribed. Within 48 hours of admission the abdominal pain and vomiting ceased but some tenderness over the left upper abdomen persisted for about one week. The blood picture remained unaltered for just over 3 weeks, when on October 21, the white cell count fell to 60,000 per c.mm. Two days later it was 44,000 per c.mm. at which time treatment was stopped. Coinciding with the fall in the leucocyte count, the spleen, which during the first three weeks of treatment had increased in size, became smaller. He was discharged home on October 28 and thereafter his progress was followed, at regular intervals, at the out-patient department. His condition remained satisfactory, the haemoglobin level rose to 11.2 G. and he returned to work during the following month. On November 30 the spleen was much larger and the white cell count had risen to 56,000 per c.mm. He was feeling well, however, and he continued working. 6-MP was re-started at a dose of 100 mg. daily and after a period of three weeks clinical and haematological improvement was again observed. On December 30, the haemoglobin

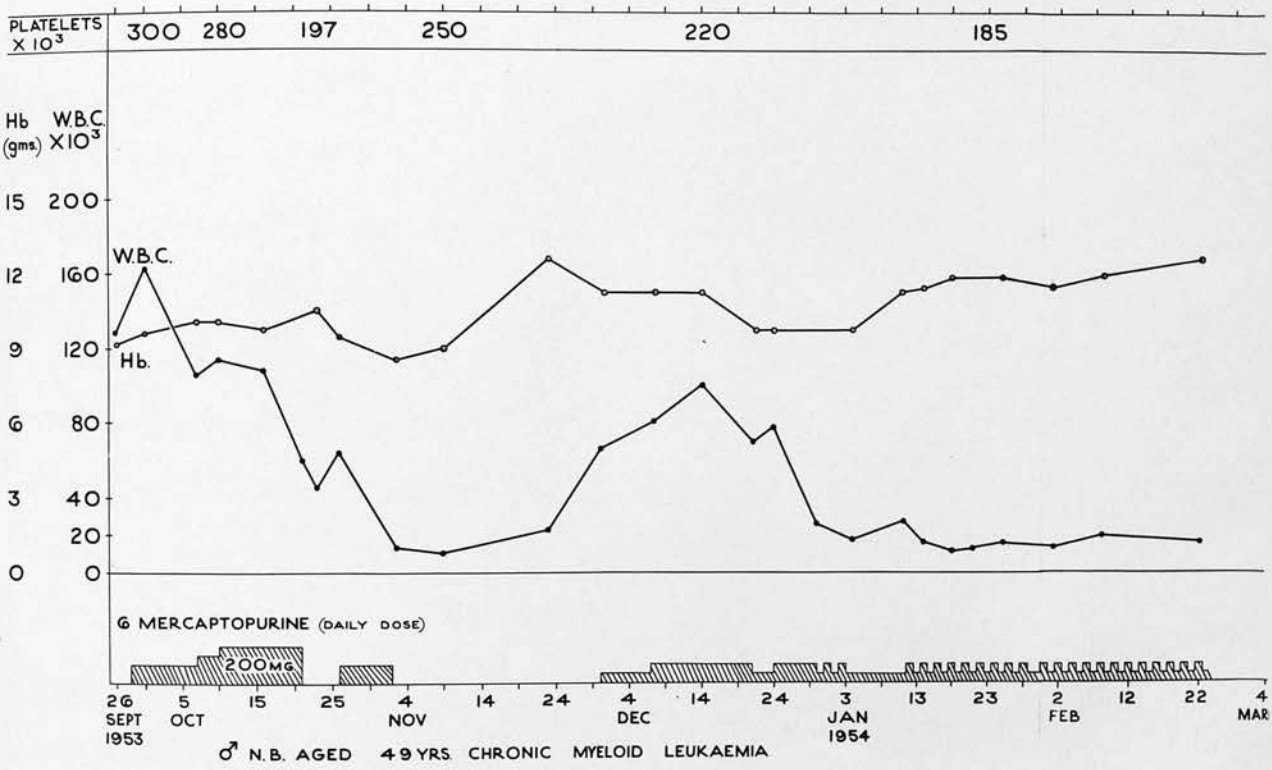

PLATELETS X 10³
300 280 197 250 220 185
Hb (gms.)
W.B.C. X10³
15 200
12 160
9 120
6 80
3 40
0 20 0
W.B.C.
Hb.
6 MERCAPTOPURINE (DAILY DOSE)
200MG.
26 SEPT 1953
5 OCT
15
25
4 NOV
14
24
4 DEC
14
24
3 JAN 1954
13
23
2 FEB
12
22
4 MAR
♂ N.B. AGED 49 YRS. CHRONIC MYELOID LEUKAEMIA

FIG. 38.

was 9.9 G. and the leucocyte count 26,400 per c.mm.

In view of the previous experience and that of others it was obvious that relapse would follow within a month or six weeks of stopping treatment and a daily maintenance dose of 6-MP seemed necessary if a satisfactory clinical and haematological state was to be continued for any length of time.

A dose of 100 mg. or 50 mg. 6-MP daily was thereafter prescribed as maintenance therapy. On this basic dosage scheme the patient has remained in good health. He has not lost a day's work for over two years. The spleen has been almost invariably palpable 1-2 cm. below the costal margin, the leucocyte count satisfactory, and the haemoglobin level after a slow initial rise has remained at a normal level.

Comment

A male aged 49 years with chronic myeloid leukaemia responded well to 6-MP. The evidence of disease regressed and the blood picture returned to normal. By the use of maintenance therapy he has continued in good health with a normal blood picture for over two years.

Case No. 30.

A male aged 25 years was referred to the medical out-patient department of the Leeds General Infirmary on October 7, 1953. He had been discharged from Her Majesty's Forces three weeks previously, suffering from chronic myeloid leukaemia and had received a course of deep x-ray therapy immediately prior to discharge. He was symptom free and was about to begin work.

On examination his colour was good, there was no lymphadenopathy and the only abnormal finding was a palpable spleen about 1-2 cms. below the left costal margin.

Blood examination gave the following findings: haemoglobin 13.3 G., red cells 4,600,000 per c.mm., platelets 210,000 per c.mm., white cells 23,700. Differential white cell count: myelocytes 2%, metamyelocytes 8%, polymorphonuclear cells 72%, lymphocytes 15%, monocytes 3%.

Progress and Treatment (Fig. 39)

In view of his satisfactory clinical and haematological state, and recent radiotherapy, no further treatment was indicated. He was seen at monthly intervals and for the following eleven months he remained in good health, and carried out his job without interruption. The haemoglobin level was always above normal and the spleen was

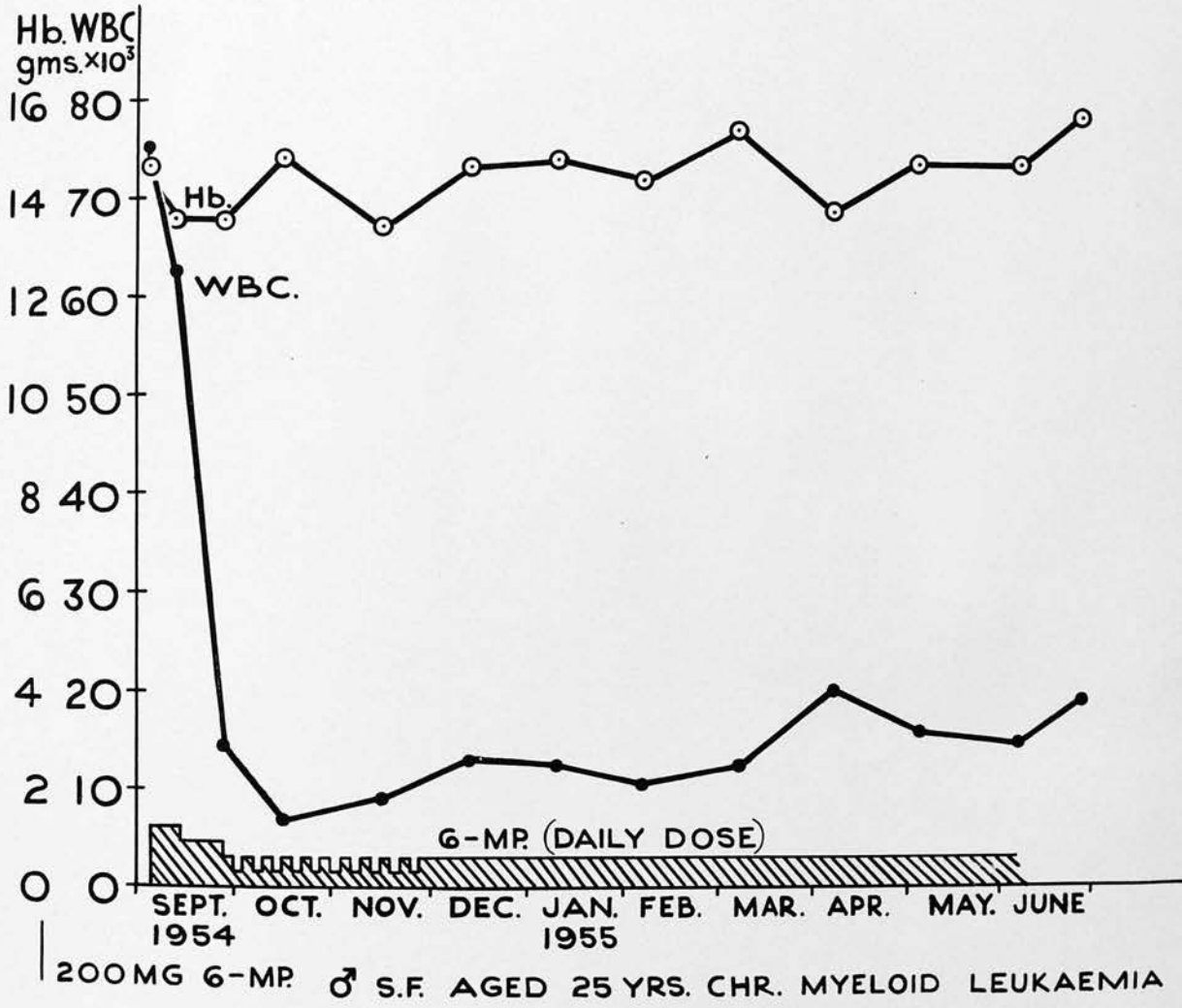

Hb. WBC
gms. x10³
16 80
14 70
12 60
10 50
8 40
6 30
4 20
2 10
0 0
Hb.
WBC.
6-MP. (DAILY DOSE)
SEPT. 1954
OCT.
NOV.
DEC.
JAN. 1955
FEB.
MAR.
APR.
MAY.
JUNE
200 MG 6-MP.
♂ S.F. AGED 25 YRS. CHR. MYELOID LEUKAEMIA

FIG. 39.

just palpable. A gradual slow rise in the leucocyte count occurred. On September 1, 1954, the haemoglobin was 14.5 G., and the white cells 75,200 per c.mm., the spleen had also begun to increase in size at this time and although he was still symptom free it was decided to attempt to improve the blood picture by chemotherapy. 6-MP, 200 mg. daily, as an out-patient, was started on September 6. Seven days later the dose was reduced to 150 mg. daily. Three weeks after the start of 6-MP therapy, on September 27, the leucocyte count was 14,600 per c.mm. and the tip of the spleen was just palpable. On a maintenance dose of 50 or 100 mg. 6-MP daily he continued in good health a year later with a normal haemoglobin and platelet count. The leucocytes have been maintained at a satisfactory level throughout. On September 5, 1955, clinical examination showed the spleen to be palpable 1 cm. below the costal margin and was otherwise negative. Examination of the blood on the same day gave the following findings:- haemoglobin 14.6 G., white cells 11,200, platelets 190,000 per c.mm.

Comment

A male aged 25 years with chronic myeloid leukaemia responded satisfactorily to deep x-ray treatment. Eleven months later evidence of

haematological relapse was appearing and treatment with 6-MP started. The leucocyte count returned to normal after three weeks and thereafter his clinical and haematological state has been consistently normal over a period of twelve months.

Case No. 31.

A male child aged 9 years was admitted to St. James's Hospital, Leeds, on July 31, 1954, with a 5 months' history of loss in weight, progressive dyspnoea on exertion and lassitude.

On admission he was found to be extremely pale and thin. Many bruises were present on the lower limbs. Several small lymph glands were palpable in the groins and neck. The spleen was enormously enlarged, the lower pole reaching almost to the symphysis pubis. The liver was palpable 4 cms. below the right costal margin.

Blood examination gave the following results: haemoglobin 6.9 G., red cells 2,000,000 per c.mm., reticulocytes 1%, platelets 25,000 per c.mm., white cells 300,000 per c.mm. Differential white cell count:- myeloblasts 4%, promyelocytes 6%, myelocytes 27%, metamyelocytes 30%, polymorphonuclear cells 31%, lymphocytes 1%, monocytes 1%. Sternal bone marrow was hypercellular, the myeloid-erythroid ratio 26 to 1 and blast cells numbering only 1.6%.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment (Fig. 40)

A transfusion of packed red cells was given which raised the haemoglobin to 9.6 G. and treatment with 6-MP, 100 mg. daily, was started on August 7.

No clinical or haematological change occurred for a period of two weeks. On August 21, the spleen had regressed and the leucocyte count fallen to 125,000 per c.mm. Treatment was continued and ten days later the leucocyte count was normal. He was feeling well, fully active and clinical evidence of disease, although still present, showed a marked regression. By September 7, the liver edge was almost impalpable, the spleen half way between the costal margin and the umbilicus and a few glands in the groins just palpable. The haemoglobin level was rising without further transfusion and the leucocyte count was at a satisfactory level. Platelets, however, were still scanty but apart from a few bruises no bleeding occurred. The dose of 6-MP was reduced to 50 mg. daily on September 7, and quite unexpectedly, oneweek later, a relapse occurred, the leucocyte count rose to 300,000 per c.mm., the haemoglobin started to fall and the spleen and liver increased in size. He had no complaints, however, apart from a septic thumb. Penicillin intramuscularly was prescribed and much bruising resulted. The dose of 6-MP was increased to 150 mg. daily on September 21. Clinical and haematological improvement was observed on September 28, two weeks after the relapse began and one week after raising the dose of 6-MP. The dose of 6-MP was thereafter

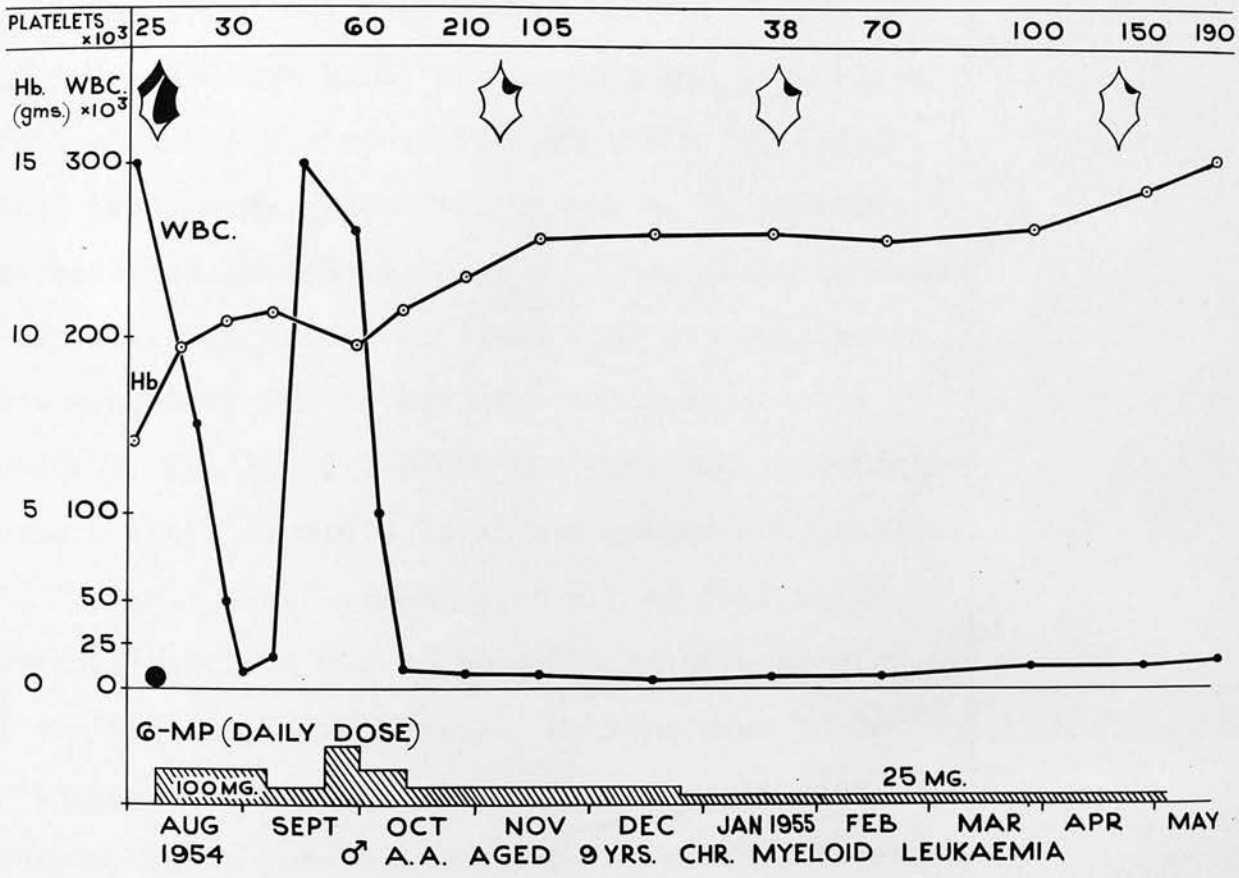

PLATELETS ×10³
25 30 60 210 105 38 70 100 150 190
Hb. (gms.)
WBC. ×10³
15 300
10 200
5 100
50
25
0 0
WBC.
Hb
6-MP (DAILY DOSE)
100 MG.
25 MG.
AUG 1954
SEPT
OCT
NOV
DEC
JAN 1955
FEB
MAR
APR
MAY
♂ A.A. AGED 9 YRS. CHR. MYELOID LEUKAEMIA

FIG. 40.

reduced to 100 mg. daily on September 30, and on October 12 to 50 mg. daily. He was now well and returned home, the white cells numbering 10,600 per c.mm., and the haemoglobin having climbed to 10.8 G. A month later the haemoglobin had reached 13 G. and the platelets 210,000 per c.mm. Since that time he has been followed as an out-patient. He returned to school shortly after discharge from hospital, the spleen at times has been impalpable, but generally felt a few cms. below the costal margin. The blood picture has remained satisfactory except for a fluctuation in the number of platelets. On April 27 blood examination was as follows:- haemoglobin 14.4 G., white cells 14,000 per c.mm., platelets 150,000 per c.mm. He continues to be maintained on 50 mg. 6-MP daily and is in good health, fully active and attending school, thirteen months after 6-MP therapy was instituted The blood picture on August 17, 1955 was: haemoglobin 13.1 G., white cells 13,500 and platelets 185,000 per c.mm.

Comment

A boy of nine years with chronic myeloid leukaemia responded successfully to treatment with 6-MP. Clinical and haematological improvement resulted and thirteen months after 6-MP therapy was instituted he continues in good health and at school.

Regression of the liver and spleen and lymph nodes with a fall in the leucocyte count, and spontaneous rise in the haemoglobin, followed initial therapy. After a temporary relapse he has been maintained satisfactorily on 50 mg. 6-MP daily. The blood picture has been consistently good and the platelet count, at the outset 25,000 per c.mm., rose to normal levels following treatment.

Case No. 32.

A male aged 18 years was admitted to the Leeds General Infirmary on July 30, 1954, with a history of general debility of two years' duration and recurrent attacks of pain in the left hypochondrium for six months.

On admission lymph glands were palpable in the right side of neck and both axillae. The spleen was greatly enlarged, the lower pole being palpable in the right iliac fossa. The liver was palpable 3 cms. below the right costal margin. No other abnormality was found.

Blood examination on August 6, 1954, gave the following results: haemoglobin 10.4 G., red cells 3,500,000 per c.mm., platelets 175,000 per c.mm., white cells 259,200 per c.mm. Differential white cell count:- blast cells 2%, myelocytes 35%, metamyelocytes 7%, neutrophils 51%, lymphocytes 5%.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment (Fig. 41)

6-MP, 200 mg. daily, was started on August 6, and continued until August 25, when the dose was reduced to 100mg./50 mg. on alternate days. The leucocyte count was now 78,400 per c.mm., the spleen considerably reduced in size, the lower pole reaching to the umbilicus. Platelets were

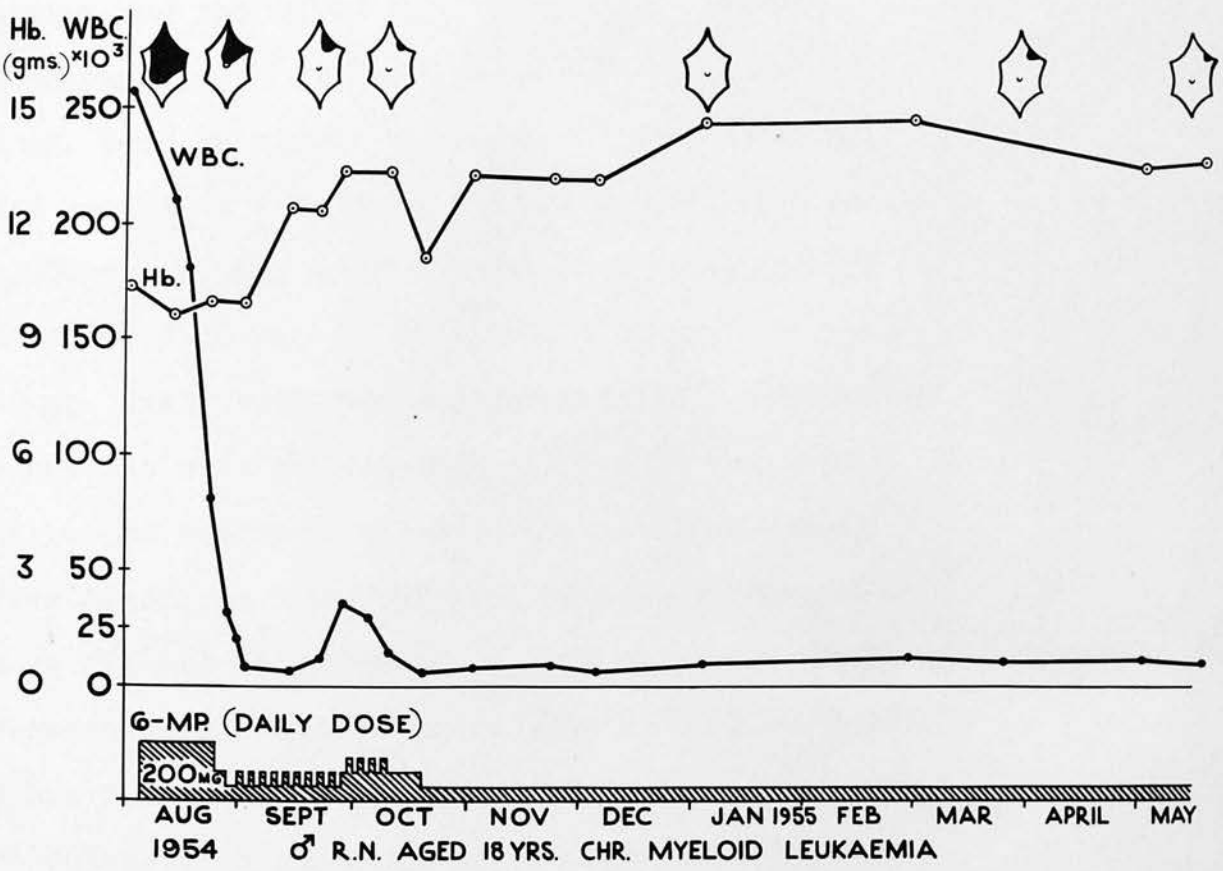

Hb. WBC
(gms.) ×10³
15 250
12 200
9 150
6 100
3 50
25
0 0
WBC.
Hb.
6-MP. (DAILY DOSE)
200MG
AUG
SEPT
OCT
NOV
DEC
JAN 1955
FEB
MAR
APRIL
MAY
1954
♂ R.N. AGED 18 YRS. CHR. MYELOID LEUKAEMIA

FIG. 41.

normal at 300,000 per c.mm. The leucocyte count continued to fall and by September 2 had reached 8,000 per c.mm. By this time the patient was symptom free and the spleen had further regressed in size and the liver was impalpable. He was discharged on September 8 on a dose of 100 mg./ 50 mg. 6-MP on alternate days. On September 27, as a result of a rise in the leucocyte count to 36,800 per c.mm., 6-MP was increased temporarily to 100 mg./150 mg. on alternate days and later to 100 mg. daily with the desired results. Thereafter he was put on a maintenance dose of 50 mg. 6-MP daily. He returned to work almost immediately after discharge from hospital and has continued to do so without interruption. Over one year after treatment was instituted, he remains in good health. He has been seen on the average once per month, at the out-patient department, and the blood picture on each occasion has been satisfactory. On September 5, 1955, the haemoglobin was 13.9 G. and white cells 10,100 per c.mm., and the spleen was impalpable. Since October, 1954, he has continually received 50 mg. 6-MP daily as maintenance therapy.

Comment

A male aged 18 years with chronic myeloid leukaemia responded satisfactorily to treatment

with 6-MP, and on maintenance therapy remains well and doing a full day's work thirteen months after treatment was instituted. The blood picture is normal, and there is no clinical evidence of disease.

Case No. 33.

A female aged 42 years was admitted to the Leeds General Infirmary on January 25, 1954, with severe bronchitis of four months' duration and for which she had been in hospital in November, 1953. She was known to have had chronic myeloid leukaemia since November, 1952, and had since been treated with urethane. She had remained fairly well during the following year, the haemoglobin remained at a satisfactory level and the white cells ranging from 35-75,000 per c.mm. The lower pole of the spleen, however, was always 2-6 cms. below the umbilicus and had at times given rise to discomfort.

On admission she was found to be slightly anaemic and the spleen was palpable 2 cms. below the umbilicus. There was no lymphadenopathy. Rales were audible over both lung fields and air entry was diminished at the bases. She was extremely breathless, febrile and troubled with a severe cough. Much purulent sputum wasbeing expectorated.

Progress and Treatment

Urethane therapy was continued for the next six weeks, during which period she had recurrent attacks of intense dyspnoea responding only to oxygen therapy and she was maintained in a tent almost permanently for five weeks. A variety of

antibiotics were given in an effort to suppress the respiratory infection.

It was decided to treat the leukaemia with 6-MP in March, 1954, and treatment was begun on March 6, at a dose of 200 mg. daily. The blood picture at that time was:- haemoglobin 11.8 G., red cells 4,200,000 per c.mm., white cells 96,000 per c.mm., differential white cell count:- myelocytes 5%, metamyelocytes 26%, polymorphonuclear cells 68%, lymphocytes 1%. The spleen was palpable 12 cms. and the liver 6 cms. below the costal margins. Two days after 6-MP was started the breathing became easier and the respiratory infection began to clear.

The leucocyte count fell gradually and after reducing the dose to 150 mg. daily on March 11, 6-MP was stopped on March 17, after a total dose of 3.05 G. had been given. The leucocyte count on March 29 was normal at 6,000 per c.mm. and a daily maintenance dose of 50 mg. 6-MP was prescribed. The liver and spleen were considerably reduced in size and on April 2 the liver was impalpable and the spleen extended 5 cms. below the left costal margin.

Her chest condition recurred in a lesser degree and persisted for fourteen days, up to April 12. She was discharged home a week later

and in view of a rising white count advised to take 6-MP, 100 mg./50 mg. on alternate days.

During the next six months, apart from occasional pain in the chest, she remained well, the blood picture was satisfactory, and the only clinical evidence of leukaemia was the spleen which was persistently 3-5 cms. below the costal margin.

On October 8, 1954, she was admitted as an acute emergency in marked respiratory distress. She was febrile and anaemic, and the spleen had become much bigger. The liver was also enlarged. A blood examination showed:- haemoglobin 7.5 G., white cells 39,000 per c.mm., differential: myelocytes 51%, metamyelocytes 2%, neutrophils 30%, lymphocytes 1%. 3 normoblasts per 100 white cells were present and much polychromasia and basephilic stippling was observed. Treatment with 6-MP, 150 mg. daily, antibiotics and oxygen was given but she succumbed to the respiratory infection, four days after admission, on October 12, 1954.

Post-mortem revealed evidence of chronic myeloid leukaemia in the bone marrow, liver (1930 grammes), spleen (1970 grammes) and mediastinal lymph glands. A fleshy mass 4 cm. x 2.5 cm. was found extending round behind the

manubrium and histologically was found to be

A suppurative bronchitis and considerable pulmonary oedema was also observed.

Summary and conclusion

A female, aged 42 years, with chronic myeloid leukaemia was treated for sixteen months with urethane. Thereafter she was found to respond to 6-MP and was maintained in a satisfactory clinical and haematological state for six months. For six months prior to 6-MP treatment, she had severe suppurative bronchitis. It seemed doubtful whether the 6-MP was related to the temporary improvement in the chest condition. Antibiotics, prolonged oxygen therapy and nursing care apart from the change in season were more likely explanations.

Death from respiratory infection occurred seven months after 6-MP therapy was instituted and was preceded by an exacerbation of the leukaemic state.

Case No. 34.

A female aged 34 years was admitted to the Leeds General Infirmary on March 8, 1955, complaining of pain in the left hypochondrium of one week's duration. Three days prior to admission she began to feel weak and complained of faintness and on the day of admission vomited about a pint of dark red blood.

On admission she was found to be anaemic and the spleen was enormously enlarged, the lower pole extending below the brim of the pelvis on the left side and the right border 4 cms. to the right of the umbilicus. A few small glands were palpable in both inguinal regions and in the left axilla.

Blood examination gave the following results: haemoglobin 4.9 G., red cells 2,200,000 per c.mm., colour index 0.7, platelets 270,000 per c.mm., white cells 204,000, differential white cell count: blasts 1%, myelocytes 11%, metamyelocytes 21%, polymorphs 60%, lymphocytes 6%. Bleeding time 2 minutes, clotting time 4 minutes. A blood film showed 2 nucleated red cells per 100 white cells.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment (Fig. 42)

A transfusion of whole blood was given on March 9, raising the haemoglobin to 7.5 G.

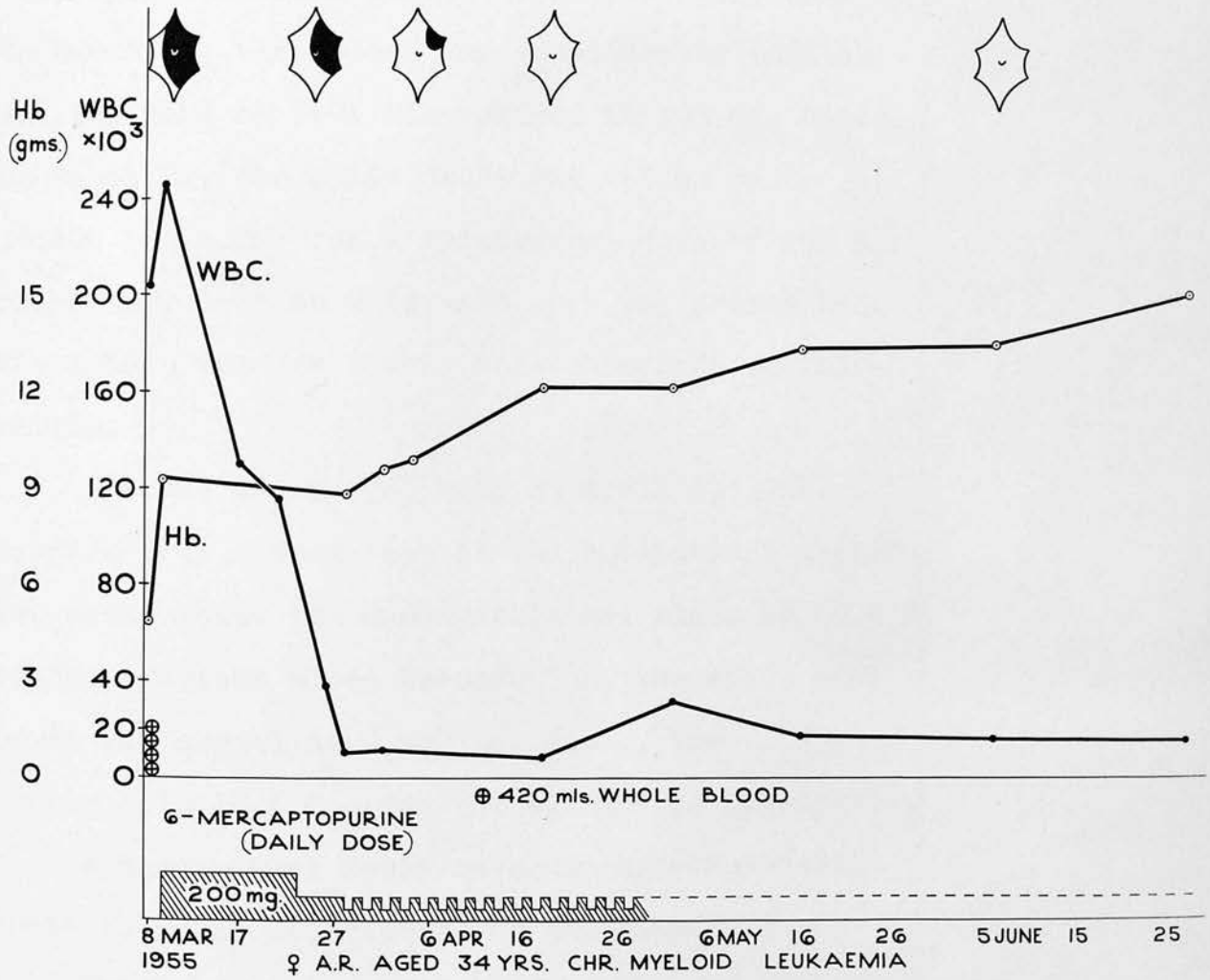

Hb (gms.)
WBC ×10³
240
200
160
120
80
40
20
0
15
12
9
6
3
0
WBC.
Hb.
⊕ 420 mls. WHOLE BLOOD
6-MERCAPTOPURINE (DAILY DOSE)
200 mg.
8 MAR 17 27 6 APR 16 26 6 MAY 16 26 5 JUNE 15 25
1955
♀ A.R. AGED 34 YRS. CHR. MYELOID LEUKAEMIA

FIG. 42.

Treatment with 6-MP, 200 mg. daily, was started on March 11. Six days later she was feeling improved, no further haematemesis had occurred and the leucocyte count had fallen to 129,600 per c.mm. On March 23, the spleen was considerably smaller and the dose of 6-MP was reduced to 100 mg. daily. On March 28, the white cells had fallen to 10,300 per c.mm. and a maintenance dose of 100 mg. and 50 mg. 6-MP on alternate days was prescribed. The spleen was now 5 cms. below the left costal margin.

She was discharged home on April 6, 1955, symptom free. When seen at the out-patient clinic two weeks later the haemoglobin had risen to 12.3 G without further blood transfusion, the white cell count was normal at 8,800 per c.mm., the haemoglobin 13.5 G., and the spleen was impalpable.

A barium meal x-ray revealed no abnormality in the oesophagus, stomach or duodenum.

She has continued in good health and the blood picture has remained satisfactory up to the present date. On September 12, 1955, the haemoglobin was 13.6 G. and white cells 6,300 per c.mm. The spleen was impalpable.

Comment

A female aged 34 years with chronic myeloid leukaemia responded satisfactorily to 6-MP therapy.

The blood picture returned to normal and an enormously enlarged spleen became impalpable. On maintenance therapy she continues in good health six months later.

Case No. 35.

A female patient, aged 62 years, was admitted to Leeds General Infirmary on February 25, 1955. She gave a history ofvvague symptoms of ill-health extending over a year with recurrent attacks of pain in the left loin of three months' duration. During the twenty-four hour period before admission haematuria was observed.

On admission she was found to be anaemic, underweight and confined to bed. A few lymph glands were palpable in the cervical and axillary regions and the spleen extended almost to the pubis.

Blood examination was as follows: haemoglobin 8.3 G., red cells 3,310,000 per c.mm., white cells 275,200 per c.mm., differential white cell count: myelocytes 21%, metamyelocytes 29%, polymorphonuclear cells 41%, lymphocytes 9%, 3 nucleated red cells per 100 white cells were present in the peripheral blood.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment (Fig. 43)

Treatment with 6-MP, 200 mg. daily, was begun on the day of admission. No significant changes in the clinical or blood picture during the first week of treatment. On March 8, the leucocytes had

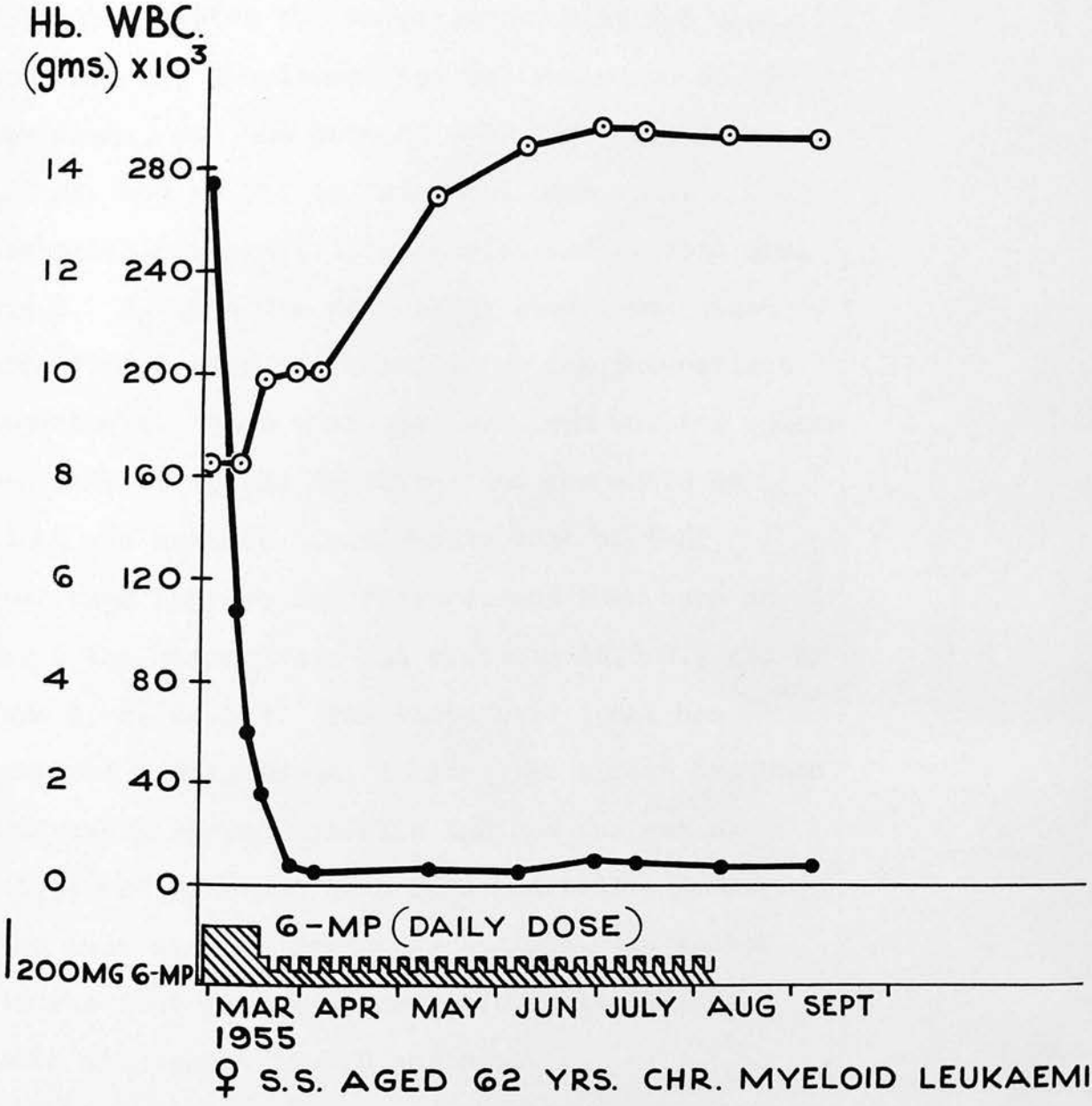

Hb. WBC.
(gms.) x10³
14 280
12 240
10 200
8 160
6 120
4 80
2 40
0 0
6-MP (DAILY DOSE)
200MG 6-MP
MAR APR MAY JUN JULY AUG SEPT
1955
♀ S.S. AGED 62 YRS. CHR. MYELOID LEUKAEMIA

FIG. 43.

fallen to 108,000 and six days later the spleen was definitely becoming smaller and the patient felt much better and was walking about the ward. On March 18, the leucocytes had fallen to 35,600 per c.mm., and the dose of 6-MP was reduced to 100 mg. and 50 mg. on alternate days. The haemoglobin was starting to rise and at this time was 9.9 G. She was now strong enough to return home with a view to follow-up at the out-patient department. When next seen on March 28, the spleen had receded to the umbilicus and the white cell count was normal. On the same dose of 6-MP continued improvement followed and when seen on May 2 the haemoglobin had risen to 13.5 G., and by June 6, to 14.5 G. The white cell count has remained within normal limits, the spleen has been remarkably reduced in size and she has put on weight and returned to a full and active life. When last seen on September 5, 1955, the spleen tip was just palpable, haemoglobin 14.1 G., and white cell count 10,800 per c.mm.

Comment

A patient with chronic myeloid leukaemia responded satisfactorily to 6-MP therapy. She regained her normal strength and became symptom free, the leucocytes fell to normal and the haemoglobin without blood transfusion rose from

8.3 G., on admission, to 14.5 G., approximately three months later. The spleen, which had given rise to discomfort and pain before treatment, was considerably reduced in size. Seven months after treatment was instituted the blood picture is normal and the spleen tip just palpable. She has received a maintenance dose of 6-MP of 50/100 mg. on alternate days.

Case No. 36.

A 56 year old housewife with a history of chronic myeloid leukaemia of seven years' duration, for which she had received several courses of deep x-ray therapy, the last in October, 1953, was admitted to St. James's Hospital, Leeds, on December 18, 1953. Her main complaints were repeated attacks of vomiting, a dragging sensation in the left side of the abdomen, loss of weight, sweating and epistaxis for which she had been confined to bed for three weeks prior to admission.

On examination she was extremely ill, sweating profusely and clinically anaemic. The spleen was firm and tender and extended 15 cms. below the left costal margin and the liver was palpable 2 cms. below the right costal margin.

Blood examination showed the following findings: haemoglobin 8.2 G., red cells 2,300,000 per c.mm., platelets 225,000 per c.mm., white cells 152,000 per c.mm. Differential white cell count: blast cells 8.5%, myelocytes 25.5%, metamyelocytes 8.0%, polymorphs 38.5%, lymphocytes 17.5%, monocytes 2%. The sternal bone marrow was hypercellular and showed blast cells 74.0%, myeloid cells 21.6%, erythroid cells 3.2%, lymphocytes 0.8%, other cells 0.4%. The blood findings were characteristic

of the acute terminal phase of chronic myeloid leukaemia.

Progress and Treatment (Fig. 44)

Treatment with 6-MP, 200 mg. daily, was instituted on December 18, 1953, and on December 23 a blood transfusion was given which elevated the haemoglobin to 11.3 G. A fall in the leucocyte count was observed after three days treatment and after two weeks a level of 13,000 had been reached, the patient was feeling stronger, the vomiting had ceased and there was no sweating. No abdominal discomfort was complained of, the liver was now impalpable and the spleen was 9 cms. below the left costal margin. The dose of 6-MP was reduced to 100 mg. daily on January 2, and continued until January 14, when treatment was stopped. A total of 4.1 G. of 6-MP had been given in twenty seven days. At this stage the white cells had fallen to 300 per c.mm. and the haemoglobin to 7.5 G., and the spleen was just palpable. A further blood transfusion was given on January 22 which raised the haemoglobin to 11.7 G. On the same day bone marrow examination showed considerable haematological improvement:- blast cells 8.5%, myeloid cells 47.5%, erythroid cells 18.5%, lymphocytes 24%, other cells 1.5%. She was now well enough to get out of bed and although the

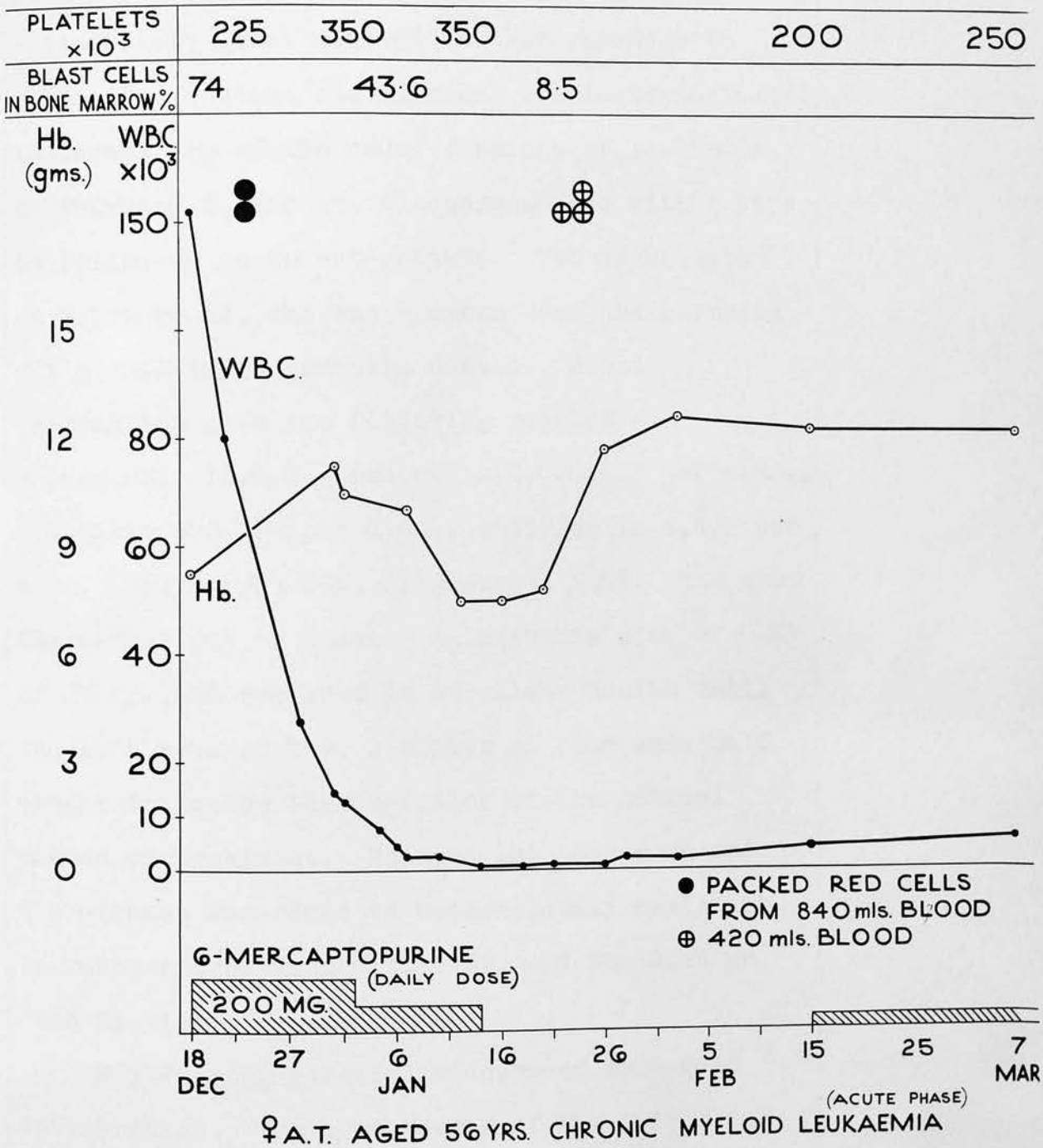

PLATELETS ×10³
225
350
350
200
250
BLAST CELLS IN BONE MARROW %
74
43·6
8·5
Hb. (gms.)
W.B.C. ×10³
150
15
12
80
9
60
6
40
3
20
10
0
0
WBC.
Hb.
● PACKED RED CELLS FROM 840 mls. BLOOD
⊕ 420 mls. BLOOD
6-MERCAPTOPURINE (DAILY DOSE)
200 MG.
18 DEC
27
6 JAN
16
26
5 FEB
15
25
7 MAR
(ACUTE PHASE)
♀ A.T. AGED 56 YRS. CHRONIC MYELOID LEUKAEMIA

FIG. 44.

leucopenia persisted for a month no complications resulted. The haemoglobin was maintained at a satisfactory level without further recourse to blood transfusions and clinical examinations failed to reveal any of the usual findings of leukaemia. On February 2, she was discharged home with a view to follow-up as an out-patient. Two weeks later on February 15, she was symptom free and carrying out a full day's domestic duties. Blood examination gave the following results:- haemoglobin 12.0 G., red cells 4,000,000 per c.mm., platelets 200,000 per c.mm., white cells 4,400 per c.mm. (polymorphs 80%, lymphocytes 20%). She was thereafter put on a daily maintenance dose of 6-MP of 50 mg. and remained in excellent health until the last week of May, a matter of four anda half months following the cessation of the initial course of treatment. Relapse then occurred and the disease was found to be completely resistant to further treatment with 6-MP, and she died on June 26, 1954.

Post-mortem revealed widespread leukaemic infiltration. The bone marrow of the left femur and lumbar vertebrae was soft and opaque pinkish and the spleen was enlarged (840 G.). The dark red cut surface showed recognisable Malphigian bodies. The lymph nodes were not noticeably

enlarged. Superficial ulceration of the soft palate and isolated areas of sloughing necrosis and ulceration of the mucosa of the small intestine was observed, with a moderate number of mucosal petechiae. The liver was enlarged (2350 G.), smooth, brick red and showed whitish mottling. Both kidneys showed alternating red and white streaking of the cortex, and the left renal pelvis contained numerous orange coloured, minute, calculi. The right lung showed pronounced oedema and the myocardium fatty change. On microscopic examination there were proliferating leukaemic cells in the bone marrow, and there were leukaemic infiltrations in the renal cortex and portal tracts.

Comment

A 56 year old female in the acute terminal phase of chronic myeloid leukaemia showed a remarkable response to 6-MP therapy. The clinical and haematological state returned to normal, and she remained in good health for nearly five months when relapse occurred. She was then found to be resistant to further therapy with 6-MP.

Case No. 37

A spinster aged 58 years was diagnosed as having chronic myeloid leukaemia in March, 1952. She received no treatment during the following year but due to the considerable enlargement and discomfort of the spleen a splenectomy was performed in April, 1953. Thereafter deep x-rays were given with temporary improvement. On December 4, 1953, she was admitted to the General Infirmary, Leeds, complaining of tiredness, loss of appetite, abdominal pain and distension, and swelling of the ankles.

On clinical examination the liver was enlarged 12 cms. below the right costal margin and small discrete lymph glands were palpable in the axillae and inguinal regions. There was no obvious clinical anaemia.

Blood examination revealed the following findings: haemoglobin 12.0 G., red cells 3,700,000 per c.mm., platelets 210,000 per c.mm., white cells 128,000 per c.mm. Differential white cell count: myelocytes 3%, metamyelocytes 12%, neutrophils 84%, lymphocytes 1%.

Progress and Treatment (**Fig. 45**)

6-MP, 200 mg. daily, was begun on December 5. A delay of nine days elapsed before treatment became effective. After twelve days the white cell

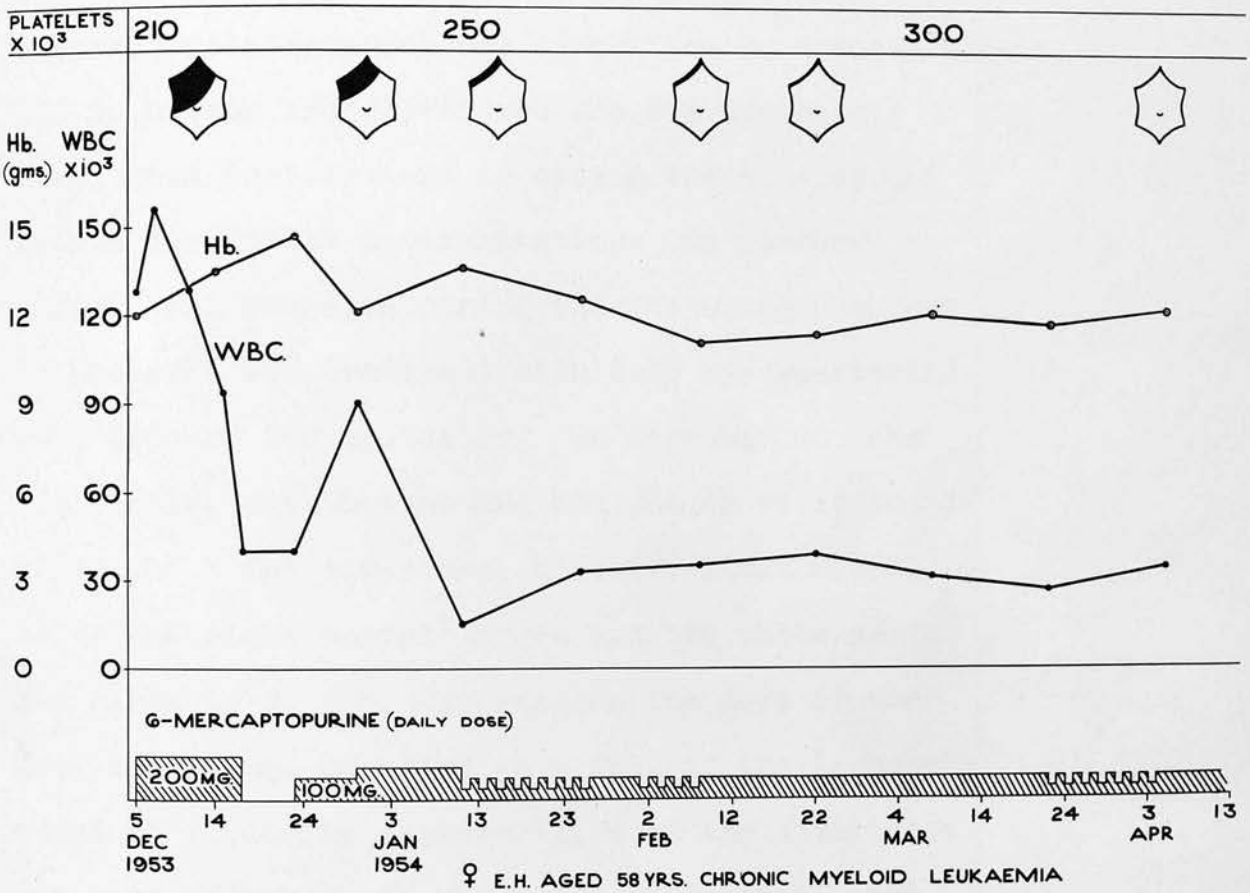

PLATELETS X 10³
210
250
300
Hb. (gms.)
WBC X10³
15
12
9
6
3
0
150
120
90
60
30
0
Hb.
WBC.
6-MERCAPTOPURINE (DAILY DOSE)
200MG.
100MG.
5 DEC 1953
14
24
3 JAN 1954
13
23
2 FEB
12
22
4 MAR
14
24
3 APR
13
♀ E.H. AGED 58 YRS. CHRONIC MYELOID LEUKAEMIA

FIG. 45.

count had fallen to 39,600 per c.mm., the patient was feeling improved and the liver was reduced in size, being palpable 6 cms. below the right costal margin. Treatment was now discontinued, a total of 2.6 G. having been given and she was discharged home, with instructions to attend the out-patient clinic for follow-up examination. No further improvement occurred during the following week and on December 23, treatment with 6-MP was re-started at a dose of 100 mg. daily. On December 30, she was feeling much better and had put on seven pounds in weight. The liver was, however, still 6 cms. below the right costal margin and the white cells had risen to 91,200. Increasing the dose of the drug to 150 mg. resulted in a fall of the leucocyte count to 15,400 by January 11, when the liver edge was just palpable and she was symptom free. The haemoglobin on this date was 13.5 G. and plbtelets 250,000. No immature white cells were observed in the peripheral blood, but a preponderance of granular cells was present.

This patient was maintained on a daily dose of 6-MP of 50 or 100 mg. for nineteen months during which time she was in good health, symptom free and fully active. No objective signs of disease re-a peared and the blood picture was satisfactory, the white cells remaining in the region of 30,000

per c.mm., together with a normal haemoglobin level. In May, 1955, she developed enlarged lymph glands in the neck with absecess formation. Biopsy of a gland showed it to be the seat of tuberculous infection. Anaemia developed but the leucocyte count and the absence of other findings on clinical examination suggested that the leukaemic process was still under control. Blood transfusion, surgical operation and anti-tuberculous treatment was undertaken with improvement. She was later discharged home, and continued to take 6-MP.

Case No. 38.

A female, aged 48 years, was admitted to St. James's Hospital, Leeds, on March 17, 1955, with congestive heart failure. A history of heart trouble since childhood was obtained.

On examination on admission she was found to be anaemic. Oedema of the ankles and sacrum was present and she was fibrillating. A mid-diastolic murmur was audible in the mitral area, in keeping with mitral stenosis. The liver was enlarged 6 cms. below the right costal margin and the spleen was palpable 5 cms. below the left costal margin.

Examination of the blood gave the following findings: haemoglobin 7.4 G., red cells 2,520,000 per c.mm., colour index 0.98, platelets 150,000 per c.mm., white cells 202,000 per c.mm. Differential white cell count:- blasts 4%, promyelocytes 10%, myelocytes 29%, metamyelocytes 28%, neutrophils 25%, basophils 1%, lymphocytes 3%. 2 normoblasts per 100 white cells were observed.

A diagnosis of chronic myeloid leukaemia was made in addition to rheumatic heart disease and congestive heart failure.

Progress and Treatment

The heart failure responded satisfactorily to digitalis, diuretics and a low salt diet.

Treatment with 6-MP was started on March, 31,

following a transfusion of packed red cells. An initial dose of 200 mg. daily was given, but reduced four days later to 100 mg. daily. After an initial delay of eleven days, improvement occurred with reduction in size of the spleen and liver, a fall in white cells and maintenance of a satisfactory haemoglobin level. Progress was interrupted by a severe attack of Herpes Zoster which was associated with much pain. On April 20, the leucocytes had fallen to 24,000 per c.mm. She was now convalescing and on April 30 was allowed home with a view to follow-up as an out-patient. The blood picture on discharge was: haemoglobin 10.5 G., white cells 8,000 per c.mm. (polymorphs 74%, lymphocytes 20%, monocytes 6%) pltelets 130,000 per c.mm. The liver was impalpable and the spleen 2 cms. below the costal margin. A maintenance dose of 100 mg. and 50 mg. 6-MP on alternate days was recommended.

When seen at the out-patient clinic two months later she was feeling well and able to carry out her domestic duties. The haemoglobin was 11.8 G. and white cells 16,300. The same maintenance dose was prescribed and when seen on August 13, 1955, she was symptom free apart from occasional post-herpetic pain. The haemoglobin level had risen to 12.3 G., and the leucocyte count was 15,300 per c.mm.

Comment

A 48 year old female patient with chronic myeloid leukaemia responded satisfactorily to 6-MP therapy. Clinical evidence of the disease regressed, associated with a fall in leucocytes and a rise in the haemoglobin level. The improvement continues five months after the institution of treatment.

Case No. 39.

A male, aged 49 years, was admitted to the Leeds General Infirmary on July 24, 1953, with a history of recurrent ulceration of the left leg, loss in weight and general ill health of fourteen months' duration.

On admission the temperature was 101.4°F., he was anaemic and lymph glands, 1-2 cms. in diameter, were palpable in the cervical, axillary and inguinal regions. The spleen was enormously enlarged, occupying the whole of the left side of the abdomen, the lower pole extending into the pelvis. The liver edge was palpable 4½ cms. below the right costal margin. An ulcer measuring 4 cms. x 2 cms. was present over the medial aspect of the left calf.

Blood examination gave the following result: haemoglobin 7.7 G., red cells 2,300,000 per c.mm., platelets 150,000 per c.mm., white cells 112,400 per c.mm. Differential white cell count:- blast cells 1%, promyelocytes 4%, myelocytes 42%, metamyelocytes 7%, polymorphonuclear cells 33%, lymphocytes 13%. Sternal bone marrow revealed changes in keeping with the diagnosis of chronic myelogenous leukaemia, erythropoiesis being markedly depressed.

A biopsy taken from the edge of the leg ulcer

showed leukaemic infiltration to be present in both dermis and subcutis.

Progress and Treatment (Fig. 46)

6-MP, 200 mg. daily, was begun on August 7, and continued until August 20, when a total dose of 2.8 G. had been given. The patient's clinical status at the start of treatment showed no change from that on admission eleven days earlier and a swinging temperature persisted. The white cells had risen to 160,400 per c.mm. On August 20, the spleen showed a reduction in size and the leucocytes had fallen to 20,800 per c.mm. A fall in the haemoglobin to 5.2 G., however, was also observed and a blood transfusion was necessary. The leucocytes continued to diminish in number and on August 25 were 9,800. By this time he was feeling stronger, his appetite had improved and the temperature had settled. The leg ulcer was healing (Fig. 47), the liver edge had almost receded under the costal margin and the spleen had now reached a point midway between the pelvis and umbilicus. The white cells continued to fall for thirteen days after treatment was stopped, and on September 2 were 1,700 per c.mm. A further blood transfusion was given on that date raising the haemoglobin to 8.9 G. The platelet count was normal at 209,000 per c.mm. The leucocytes had risen to normal a week

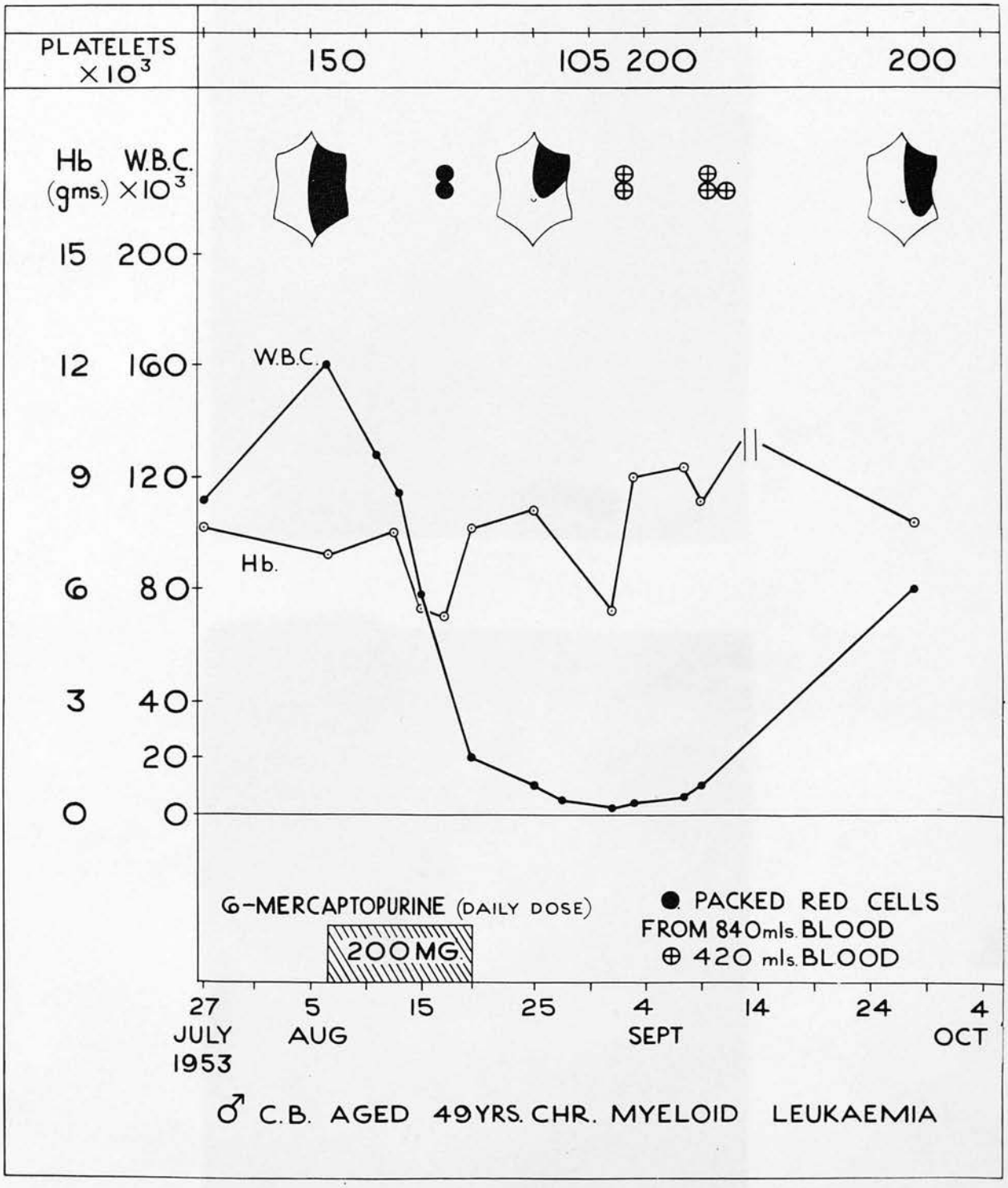


FIG. 46.

Note the relapse when treatment was stopped, indicating the necessity for continuous (maintenance) therapy.

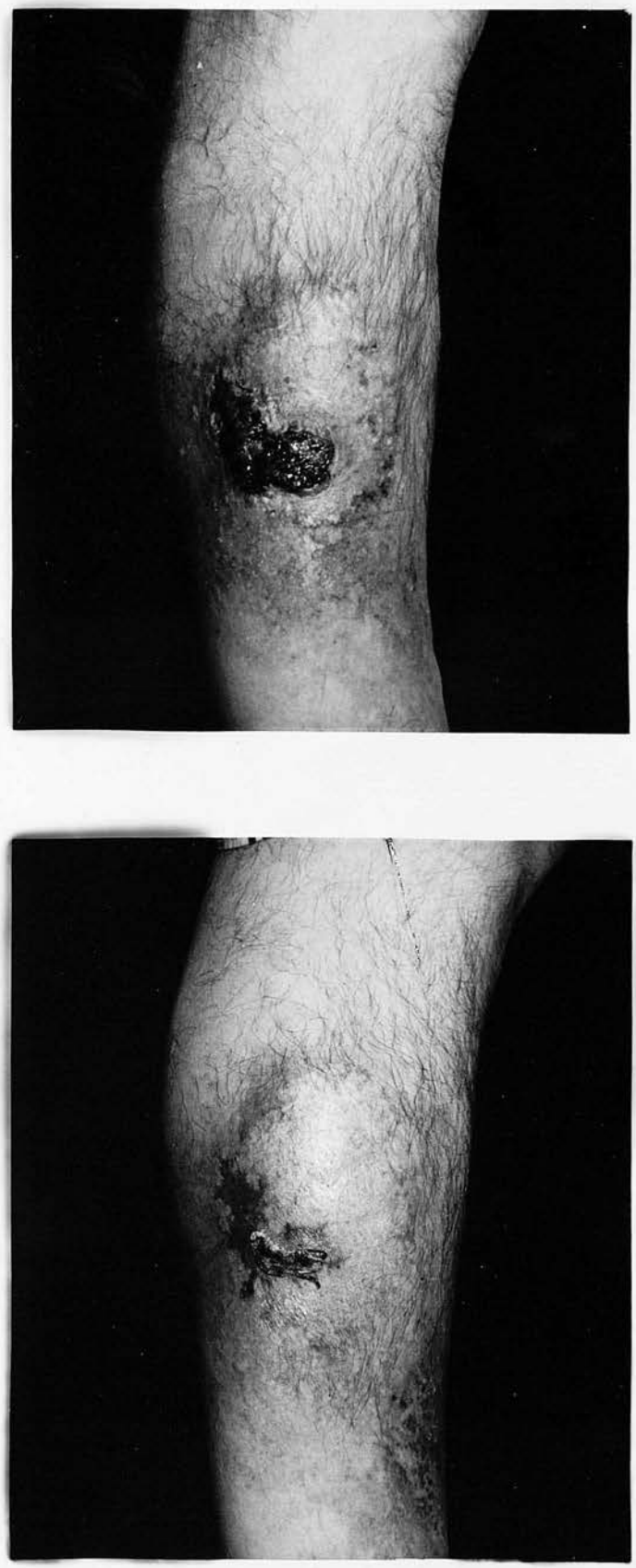


FIG. 47. Case No. 39. Photograph of left calf showing healing of ulcer following treatment with 6-MP.

later, the clinical evidence of the disease continued to regress and after a further transfusion he was discharged home on September 12. In view of his home being a considerable distance from Leeds he was not seen again until September 28. The leg ulcer had now completely healed, but the spleen was enlarging again and the white cells had risen to 80,000 per c.mm. After a second short course of 6-MP, 50 mg. daily, the leucocytes again fell but in view of the persistent anaemia, and marked splenic enlargement he was referred after further blood transfusions to the Radiotherapy Department for the benefit of deep x-ray treatment on November 2, 1953. Treatment with deep x-rays was given in November and December, 1953, and February and March, 1954. Only temporary symptomatic improvement followed with a fall in white cells and a slight reduction in size of the spleen - which at its best always reached the umbilicus. The refractory anaemia continued and transfusions were required during this period.

He was re-admitted to hospital on June 1, 1954 with profound anaemia, congestive heart failure, massive splenomegaly, lymphadenopathy and hepatomegaly. The haemoglobin was 3.2 G., white cells 198,400, platelets 100,000 per c.mm. Blood transfusions were given on admission, digitalis,

diuretics and a low sodium diet. In view of the poor response to deep x-rays it was decided to try the effect again of 6-MP. In view of his poor condition an initial dose of 1 G. 6-MP was given during the 24 hour period on June 2. On June 3, the white cells had fallen to 51,200 per c.mm. 6-MP, 200 mg. daily, was continued until June 9, when the white cells were 7,900 per c.mm., and then discontinued. The clinical evidence of leukaemia (lymph glands, spleen and liver) started to regress, but a further blood transfusion was necessary on June 14, which raised the haemoglobin to 9.3 G. His general condition now improved, the signs of heart failure cleared and he was allowed up. On June 17, a maintenance dose of 6-MP, 100 mg. and 50 mg. on alternate days was prescribed, and nine days later he was judged fit enough to return home. When seen on July 5, the spleen had receded almost to the umbilicus and the haemoglobin level had been maintained. A slight rise in white cells to 36,800 led to an increase of 6-MP to 100 mg. daily. He was thereafter seen at monthly intervals. The leucocytes remained at a satisfactory level and the haemoglobin, although never reaching the normal figure, climbed to 12.1 G. He was symptom free, fully active and the spleen which had previously extended into the pelvis was now just palpable on

inspiration. This happy state continued for nine and a half months, during which time he carried out a light job. On March 28, 1955, the haemoglobin began to fall and he was given a blood transfusion. The spleen started to enlarge again and he complained of weakness, anorexia, and general ill health. 6-MP, 150 mg. daily, was continued but further improvement did not occur. Further transfusions were necessary in early May, but only temporary improvement followed, and he died in June, 1955 Latterly Myeleran was prescribed but it proved ineffective.

Comment

A 49 year old male patient with chronic myeloid leukaemia showed a response to an initial single course of 6-MP. There was clinical improvement; a chronic ulcer of the leg with leukaemic infiltration healed, the spleen, liver and lymph glands regressed in size and his general condition improved. A refractory anaemia persisted however. Relapse occurred just over a month after stopping 6-MP therapy, and it was later decided to try the effect of deep x-rays. Four courses in the following seven months only resulted in temporary clinical improvement after each course, a fall in leucocyte count and a reduced size of the spleen. The anaemia persisted and further transfusions

were required. A second course of 6-MP followed by maintenance treatment gave considerable relief. For the first time since diagnosis the haemoglobin rose spontaneously to over 12 G., the spleen receded to the left costal margin and he regained almost normal health. No transfusions were necessary for over nine months when relapse occurred. Thereafter the disease proved refractory to treatment with 6-MP and Myeleran.

Case No. 40.

A male, aged 55 years, was admitted to the Leeds General Infirmary on May 21, 1955, with a history of dyspnoea on exertion of one year's duration, intermittent claudication for three years and pain in the right shoulder for eight months.

On admission he was found to be anaemic, the spleen was palpable mid-way between the left costal margin and the umbilicus, but there was no glandular enlargement. Pulsations were absent in the arteries of the legs and feet and movement around the right shoulder joint was considerably restricted.

Blood examination gave the following findings: haemoglobin 10.9 G., red cells 3.5 million per c.mm., white cells 58,400 per c.mm. Differential white cell count showed:- myelocytes 6%, metamyelocytes 14%, neutrophils 64%, eosinophils 1%, basophils 7%, lymphocytes 8%.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment

Treatment with 6-MP was started on May 25, a dose of 150 mg. daily being prescribed. No effect of therapy was noted for over two weeks, but on

June 13, the spleen had become impalpable and the leucocyte count was 14,700 per c.mm. The haemoglobin level, however, remained unchanged. A maintenance dose of 100 mg. and 50 mg. 6-MP on alternate days was given which has controlled the leukaemic state up to the present time. When seen on September 5, 1955, the haemoglobin had risen to 13.0 G. and the white cells were 15,800 per c.mm. The spleen was impalpable and his only complaint was a persistent pain in the right shoulder.

Comment

A male patient with chronic myeloid leukaemia after treatment with 6-MP showed a fall in the leucocyte count, a disappearance of splenomegaly, and gradual rise in haemoglobin level. On maintenance therapy he continues to do well five months after the start of treatment.

Case No. 41.

A male aged 48 years was admitted to the Leeds General Infirmary on April 27, 1955, with a history of lassitude, dyspnoea on exertion and swelling of the ankles of six weeks' duration.

On examination on admission he was found to be anaemic, the spleen was enlarged 4 cms. below the umbilicus and the liver 6 cms. below the right costal margin. A few shotty lymph glands were palpable in both inguinal regions.

Blood examination gave the following results: haemoglobin 7.5 G., red cells 2,500,000 per c.mm., white cells 364,800 per c.mm., differential count: myelocytes 27%, metamyelocytes 25%, polymorphs 51%, lymphocytes 2%.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment (Fig. 48)

6-MP, 200 mg. daily, was started on April 30, 1955. No change in the clinical or haematological state occurred during the next twelve days. On May 12, the leucocytes had fallen to 118,400 per c.mm., but the haemoglobin was low at 7.3 G. and he was consequently given a transfusion of packed red cells which raised the haemoglobin to 9.0 G. on May 16. By May 23, the leucocyte count was normal at 11,600

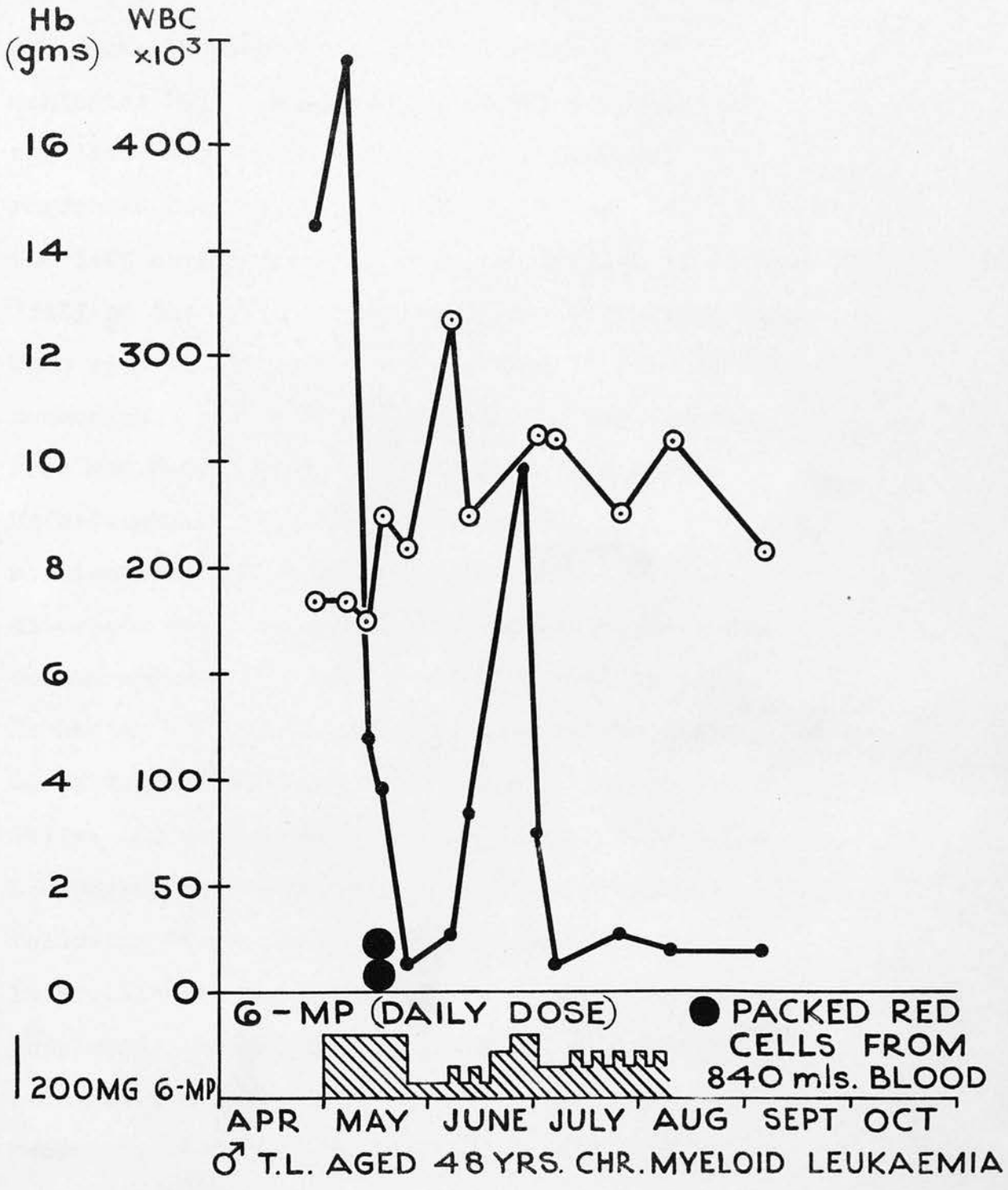

Hb (gms)
WBC ×10³
16
14
12
10
8
6
4
2
0
400
300
200
100
50
0
6-MP (DAILY DOSE)
PACKED RED CELLS FROM 840 mls. BLOOD
200MG 6-MP
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
♂ T.L. AGED 48 YRS. CHR. MYELOID LEUKAEMIA

FIG. 48.

per c.mm. (polymorphs 83%, lymphocytes 15%, monocytes 2%). The patient was not complaining, the liver was impalpable and the spleen had regressed considerably being now 3 cms. below the left costal margin. 6-MP was reduced to 50 mg. daily on May 24 on which day he was discharged home. When seen at the out-patient clinic on June 6, the haemoglobin had risen to 12.7 G., he was symptom free and had started to work again as a miner. Unfortunately, in spite of increasing the maintenance dose of 6-MP to 100 and 50 mg. on alternate days the leucocyte count rose, he again became anaemic and the spleen increased in size. He had no complaints, however, and it was decided to continue 6-MP therapy at doses of 100 to 150 mg. daily. An improvement followed and by July 7 the leucocytes had returned to normal. During the following three months he has worked only intermittently and a refractory anaemia has persisted, the highest haemoglobin level attained being 10.4 G Blood transfusions have been necessary, but the leucocytes have remained within normal limits.

Comment

A 48 year old male patient with chronic myeloid leukaemia showed a poor response to 6-MP

therapy. The clinical evidence of the disease regressed and the leucocytes returned to normal, but a refractory anaemia persists five months after treatment was begun.

Case No. 42.

A female, aged 58 years, was admitted to Leeds General Infirmary on February 4, 1954. A diagnosis of chronic myeloid leukaemia had been made one year previously. Improvement lasting two months had followed a course of deep x-ray treatment in April, 1953, but another course of treatment in December, 1953, had little effect on the patient's general health. Her main complaints now were dyspnoea on exertion, swelling of the ankles and abdomen, and loss of energy which had confined her to bed.

On admission oedema of the ankles and sacrum was present, enlargement of the neck veins, massive enlargement of the spleen down to the pubis, and lymphadenopathy and the liver was palpable below the right costal margin.

Blood examination was as follows: haemoglobin 7.7 G., red cells 2,500,000 per c.mm., platelets 110,000 per c.mm., white cells 10,000 per c.mm., differential white cell count:- myelocytes 22%, polymorphs 22%, lymphocytes 21%, monocytes 9%. The sternal bone marrow showed a preponderance of myeloid cells, 79.5% being of that series with 14.1% lymphoid and 6.4% erythroid cells.

Progress and Treatment

Treatment with 6-MP was begun on February 6

and continued until February 17, a total dose of 2.4 G. being given. The congestive heart failure responded gradually to treatment with digitalis, diuretics, etc. 6-MP failed, however, to have the desired effect. A fall in the leucocytes first observed nine days after the start of treatment was associated with considerable reduction in size of the liver, spleen and lymph glands, but no improvement in her general condition occurred. She developed severe bed sores which healed very slowly, and the haemoglobin failed to rise necessitating repeated blood transfusions. As no further benefit from treatment was expected she was allowed to return home on April 14, 1954. Although 6-MP therapy was not given after the initial course the leucocyte count remained extremely low. Two months after treatment was stopped, being only 2,300 per c.mm. On May 13, she was re-admitted with advanced congestive heart failure and died a few hours later.

Post-mortem revealed the characteristic changes of chronic myeloid leukaemia and congestive heart failure.

Comment

An adult female with chronic myeloid leukaemia who failed to improve after deep x-ray therapy also

showed no benefit from treatment with 6-MP. A fall in the leucocyte count with diminution in size of the spleen, etc. occurred but her general condition was not affected and the haemoglobin failed to rise. The white cells, initially low, fell to a leucopenic level and failed to rise before discharge two months later.

Case No. 43.

A 69 year old male was admitted to the Leeds General Infirmary on December 3, 1953, with a history of dyspnoea on exertion of six months' duration, cough and recent haemoptysis.

On admission there were signs of congestive heart failure, enlarged lymph glands were palpable in the neck and massive hepato-splenomegaly, the liver edge being 15 cms. below the right costal margin and the tip of spleen 26 cms. below the left costal margin. Purpura was present on the legs.

The blood findings were typical of advanced chronic myeloid leukaemia:- haemoglobin 10.5 G., platelets 85,000 per c.mm., white cells 198,400 per c.mm. Differential white cell count: blast cells 14%, myelocytes 41%, metamyelocytes 19%, neutrophils 26%.

Progress and Treatment

6-MP, 200 mg. daily, was begun on December 5. On the evening of December 6, he suddenly collapsed and died a few hours later of a cerebro-vascular accident. Autopsy showed the presence of chronic myeloid leukaemia, a right anterior cerebral haemorrhage and a lung abscess situated in the right lower lobe.

Comment

A male aged 69 years with advanced chronic myeloid leukaemia died following a cerebral haemorrhage a day following the start of 6-MP therapy.

Case No. 44.

An adult female, aged 56 years, was admitted to the Leeds General Infirmary on February 5, 1954, complaining of dyspnoea on exertion, palpitations and increasing pallor for six months. In October, 1953, a diagnosis of chronic lymphatic leukaemia was made at another hospital, the leucocyte count at that time being 51,000 per c.mm. of which 92% were lymphocytes. In December, 1953, the haemoglobin had fallen to 3.7 G. and a blood transfusion was given. A further transfusion was given in January, 1954.

On admission she was found to be anaemic and numerous petichael haemorrhages were observed. Lymph glands were palpable in the cervical, axillary and inguinal regions. The spleen was enlarged 10 cms. below the left costal margin.

Blood examination gave the following findings: haemoglobin 10.2 G., red cells 3,500,000 per c.mm., platelets 32,000 per c.mm., white cells 70,200 per c.mm. Differential white cell count:- neutrophils 4%, lymphocytes 96%. Bleeding time 10½ minutes.

Bone marrow examination showed:- lymphocytes 94.8%, blast cells 2.8%, myeloid cells 2.4%. No red cell precursors or megakaryocytes were seen.

A diagnosis of chronic lymphatic leukaemia

in the terminal stage was made.

Progress and Treatment

6-MP, 200 mg. daily, was started on February 5 and continued for nineteen days without any effect on the clinical or haematological picture. Repeated blood transfusions were necessary and latterly A.C.T.H., 25 mg. 6 hourly, intramuscularly, was given in addition without effect, other than reducing the leucocyte count from 76,800 per c.mm. on February 19 to 17,600 per c.mm. three days later. The patient died on February 24 and post-mortem revealed findings characteristic of chronic lymphatic leukaemia.

Comment

A total dose of 3.8 G., 6-MP, given over a period of nineteen days to a patient with advanced chronic lymphatic leukaemia, failed to produce any clinical or haematological improvement.

Case No. 45.

A female, aged 73 years, was admitted to the Leeds General Infirmary on December 3, 1953, with a history of pain across the front of the chest, cough, dyspnoea and sweating of two days duration.

On admission the temperature was 101.4°F., and signs of consolidation were present over the right upper chest. The spleen was enlarged 2 cms. below the left costal margin, and lymph glands were palpable in the cervical and inguinal regions.

A radiograph of the chest showed evidence of consolidation of the upper lobe of the right lung in keeping with lobar pneumonia.

Blood examination gave the following results: haemoglobin 15.3 G., red cells 4,900,000 per c.mm., platelets 170,000 per c.mm., white cells 24,000 per c.mm. Differential white cell count:- polymorphonuclear cells 11%, lymphocytes 89%. Sternal bone marrow showed 55.8% lymphocytes, 35.8% myeloid cells, 8.4% erythroid cells and other cell types 0.8%.

A diagnosis of chronic lymphatic leukaemia was also made.

Progress and Treatment

Penicillin rapidly controlled the pneumonic process. In view of a rising leucocyte count (51,200 per c.mm.) and falling haemoglobin (12.2 G.)

it was decided to try the effect of 6-MP, starting on December 11. A total dose of 3.8 G. was given which failed to produce any improvement in the distribution of leucocytes in the peripheral blood, the splenomegaly remained unchanged and the lymphadenopathy persisted. The leucocytes fell to 12,400 per c.mm. by December 31, but over 90% were lymphocytes, and as the count remained around 20,000 per c.mm. during the following five months, without further treatment, it seemed likely that the initial rise may have been associated in some way with the infection and the fall not due to 6-MP but a consequence of the clearing of the pneumonic process.

It was decided in view of the patient's excellent condition not to press further treatment and possibly produce complications due to bone marrow depression.

Comment

A case of chronic lymphatic leukaemia failed to show any haematological or clinical improvement when treated with 6-MP.

Case No. 46.

A 63 year old female was admitted to St. James's Hospital, Leeds, on January 19, 1954, with a history of back pain of ten months' duration. For six months she had noticed a lump on the head which was gradually increasing in size.

On admission she was found to be anaemic and a large soft swelling, 7.5 cms. in diameter, was present over the left parietal region. There was no splenomegaly or lymphadenopathy.

Blood examination gave the following findings: haemoglobin 7.4 G., red cells 2,370,000 per c.mm., white cells 5,400 per c.mm. (polymorphs 50%, lymphocytes 41%, monocytes 9%), platelets 295,000 per c.mm.

Sternal bone marrow showed a preponderance of plasma cells.

Other investigations were as follows: E.S.R. (Westergren) 150 in 1 hour, plasma proteins 11.9 G.% (Alb. 4.25 G.%, Glob. 7.65 G.%), alkaline phosphatase 5.5 units. X-ray of skeleton showed generalised decalcification and also punched out areas in keeping with a diagnosis of multiple myelomatosis.

Progress and Treatment

Treatment with 6-MP, 200 mg. daily, was begun on January 23, 1954. Thereafter weekly clinical,

haematological and biochemical examinations were performed. No improvement occurred and she died on February 14, 1954, after three weeks' treatment.

Comment

A case of multiple myeloma failed to show any clinical, haematological or biochemical improvement after three weeks' treatment with 6-MP.

Case No. 47.

A female aged 64 years was admitted to an orthopaedic ward at St. James's Hospital, Leeds, on January 8, 1954, with a history of recurrent pain in the lumbar-sacral region and left sciatica of sixteen months duration.

On admission there was evidence of dorso-lumbar kyphosis, wasting of the left thigh and calf muscles and an absent left ankle jerk. The temperature was 98.6°F. and she was anaemic. There was no splenomegaly.

Blood examination gave the following findings: haemoglobin 8.1 G., red cells 2,700,000 per c.mm., platelets 200,000 per c.mm., white cells 5,000 per c.mm. (polymorphs 65%, lymphocytes 32%, monocytes 3%). The sternal bone marrow showed the presence of 30% plasma cells, many of which appeared immature. Other investigations included: x-ray of skull, which showed multiple punched out areas; plasma proteins 12 G.% (Alb. 3.2 G.%, Glob. 8.8 G.%) urine - negative for Bence-Jones proteose; alkaline phosphatase 5.0 units; E.S.R. (Westergren) 10 mm. in 1 hour.

Apart from the inexplicably low E.S.R. the findings were characteristic of multiple myelomatosis.

Progress and Treatment

It was decided to try the effect of 6-MP and treatment was begun on January 26, 1954, a dose of 200 mg. daily being prescribed. Thereafter clinical examination and a complete haematological and biochemical investigation was carried out at weekly intervals. No improvement occurred. Definite evidence of depression of marrow function was observed and on February 17 the peripheral blood picture was as follows: haemoglobin 4.2 G., white cells 2,200 per c.mm., platelets 96,000 per c.mm. Irrespective of blood transfusions, the patient rapidly went downhill andafter developing purpura died on February 21, 1954.

Comment

A case of multiple myeloma showing no response to 6-MP therapy. Profound depression of marrow function occurred and death occurred one month after starting treatment.

Case No. 46.

A 32 year old male was admitted to the Leeds General Infirmary on July 11, 1955, with a history of recurrent pain in the left shoulder and loss of energy of four months duration.

On admission his colour was good, and the temperature was normal. The lower pole of the spleen was palpable one inch below the umbilicus and the right border extended across the mid-line. There was no hepatomegaly, but a few enlarged lymph glands were present in the inguinal and axillary regions.

Blood examination gave the following results: haemoglobin 11.5 G., red cells 4,200,000 per c.mm., white cells 262,400 per c.mm. A blood film showed numerous myelocytes and metamyelocytes.

A diagnosis of chronic myeloid leukaemia was made.

Progress and Treatment

6-MP, 200 mg. daily, was begun on the day of admission. No response occurred for fourteen days when the leucocyte count fell to 73,600 per c.mm. He was feeling well and was discharged on a maintenance dose of 100 mg. 6-MP daily. When seen at the out-patient clinic on August 12, after one month's treatment, the leucocyte count had fallen

to 23,400 per c.mm., but by September 5, the count had risen to approximately 100,000 per c.mm., and the spleen extended to the umbilicus, and he was feeling depressed. Thereafter a maintenance dose of 100 mg. and 150 mg. 6-MP on alternate days was given. With this regime he has been maintained in good health. The spleen became much smaller, the haemoglobin rose and he has had no further pain. The leucocytes have been consistently above normal, but in view of his good general health no attempt to inhibit the leukaemia process further was considered advisable.

When last seen on November 28, 1955, four and a half months after the start of treatment he was well and doing a full day's work. The haemoglobin was 13.5 G. and the leucocyte count 32,000 per c.mm.

Comment

A patient with chronic myeloid leukaemia showed a satisfactory response to 6-MP therapy. The symptoms cleared, the leucocyte count fell, the haemoglobin level rose and the spleen became smaller following treatment.

CASE RECORD NUMBERS

CASE NUMBER	HOSPITAL	RECORD NUMBER
1	LEEDS GENERAL INFIRMARY	276510
2.	do.	305675
3.	WAKEFIELD GENERAL INFIRMARY	34752
4.	do.	33962
5.	ST. JAMES' LEEDS	55/001434
6.	SEACROFT LEEDS	556686
7.	ST. JAMES' LEEDS	53/8231
8.	LEEDS GENERAL INFIRMARY	30806
9.	do	308384
10.	do.	313659
11.	PONTEFRACT GENERAL INFIRMARY	M 30771
12.	LEEDS GENERAL INFIRMARY	334809
13.	PONTEFRACT GENERAL INFIRMARY	M 28791
14.	LEEDS GENERAL INFIRMARY	341725
15.	do.	258234
16.	ST. JAMES' LEEDS	53/18002
17.	do.	54/1891
18.	LEEDS GENERAL INFIRMARY	301556
19	WAKEFIELD GENERAL INFIRMARY	33138

CASE NUMBER	HOSPITAL	RECORD NUMBER
20	PONTEFRACT GENERAL INFIRMARY	M 29245
21.	LEEDS GENERAL INFIRMARY	334233
22.	ST. JAMES' LEEDS	54/016066
23.	do.	55/6149
24.	do.	53/13736
25.	LEEDS GENERAL INFIRMARY	301789
26.	do.	309887
27.	ST. LUKE'S BRADFORD	54/15417
28.	do.	51/5379
29.	LEEDS GENERAL INFIRMARY	117672
30.	do.	283611
31.	ST. JAMES' LEEDS	54/011200
32.	LEEDS GENERAL INFIRMARY	304911
33.	do.	177864
34.	do.	340973
35.	do.	338231
36.	ST. JAMES' LEEDS	53/17797
37.	LEEDS GENERAL INFIRMARY	227067
38.	ST. JAMES' LEEDS	55/4249
39.	LEEDS GENERAL INFIRMARY	285586

CASE NUMBER	HOSPITAL	RECORD NUMBER
40.	LEEDS GENERAL INFIRMARY	253676
41.	do.	347511
42.	do.	270808
43.	do.	304902
44.	do.	303547
45.	do.	296747
46.	ST. JAMES' LEEDS	53/16145
47.	do.	54/408
48.	LEEDS GENERAL INFIRMARY	360624
49.	do.	289506
50.	ST. JAMES' LEEDS	52/14531

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