two different kinds of test should be used routinely in patients receiving phenothiazine treatment.

Increased production and excretion of pituitary luteinizing hormone is suggested as the possible cause of false-positive immunological and biological pregnancy tests in phenothiazine-treated subjects.

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Low-protein Purine-free Diet in Treatment of Acute Leukaemia in Children: Preliminary Communication

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When considering the clinical symptoms accompanying responses or resistance to therapy in acute leukaemia of children our attention was drawn to the behaviour of the liver. Cases with symptoms of hepatic dysfunction manifested by low pre-albumin and albumin levels, raised gamma-globulin, and low plasma cholesterol levels, persisting or developing in the course of treatment, were found to have a bad prognosis (Halikowski *et al.*, 1965; Mejbaum-Katzenellenbogen *et al.*, 1965).

In 26 cases of acute leukaemia in children treated by standard methods (1962–4) negative correlation was found between the size of the liver (in centimetres below the costal margin) at the beginning of treatment and the survival time (Fig. 1).

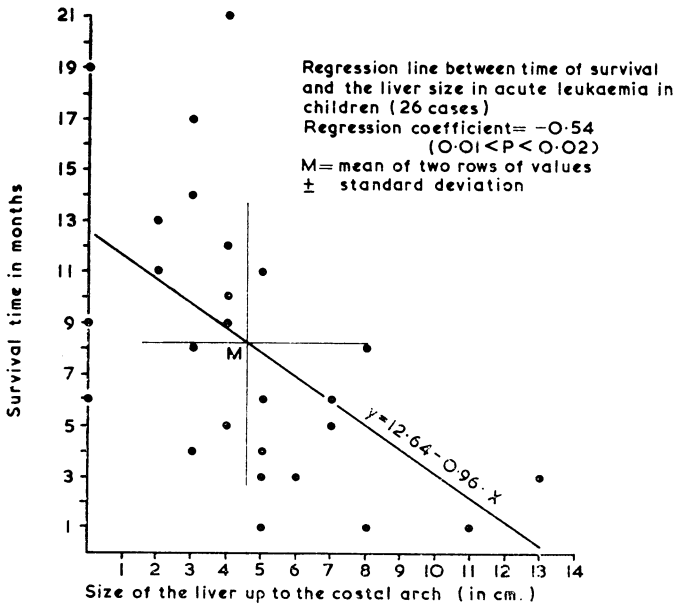

FIG. 1

Working Hypothesis

The hypothesis was proposed that response to treatment might be dependent on hepatic function. One of the disorders connected with hepatic dysfunction may consist in enzymatic block of the metabolic processes involved in syntheses occurring in the bone-marrow.

In acute leukaemia the outstanding feature of the clinical picture is bone-marrow insufficiency with aplastic symptoms. Low erythroblastosis, absence of normal development of the bone-marrow granulocytes, and pancytopenia in the peripheral blood are regularly met with. This raises the question whether these changes are not an expression of the basic lesion in acute leukaemia, while proliferation of pathologic cells is secondary.

The hypothesis of enzymatic blocking has already been proposed by Haddow (1954) and Osgood (1957).

In various countries the incidence of leukaemia and consumption of animal protein seem to be correlated. Statistical data tend to show a rise in the incidence of leukaemia in the higher income groups. This seems to be true not only in special population groups but also in different countries. It is widely known and generally accepted that protein consumption (especially animal protein) is a good indicator of the standard of living. The greater incidence of leukaemia in countries with a high protein consumption may be fortuitous, or it may be connected with a third factor or be due to a variety of causes. In the latter case high protein consumption, the content of certain amino-acids, or other factors associated with high protein consumption (purines) may be responsible.

The hypothesis of the part played by the liver in the disease, as well as the above-mentioned epidemiological observations, seems to make acceptable the possibility that some sort of "narrow throat" in metabolic pathways may, with other factors, be characteristic of a pre-leukaemic state. When excessive loading with the substrate occurs the full picture of the disease may develop.

As a result of a reduced supply of substrate, low-protein purine-free diet may diminish the effects of enzymatic block and thus act as a factor in the maintenance or resumption of normal development of bone-marrow cells.

Clinical Trials with Low-protein Purine-free Diet

On the basis of this working hypothesis low-protein purine-free diet has since October 1964 been introduced as an adjunct to the routine therapy of acute leukaemia in children.

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The basic therapy consisted in the administration of Encorton (1.5–9 mg./kg. body weight/24 hr.), mercaptopurine (2.5 mg./kg./24 or 48 hr.), or methotrexate (2.5–5 mg./24 or 48 hr.), small doses of vitamins, antibiotics as indicated, and (rarely) blood transfusions.

The new diet consisted in restriction of animal proteins and total elimination of purines. During the first three days a diet free from animal protein was given. After that, protein was restricted to about 1 g./kg. body weight (including about 0.5 g./kg. of animal protein in the form of milk, cheese, and eggs) and the caloric requirements were fulfilled according to the child's age.

To date, 13 children aged 2–11 years have been treated with this diet, including four in the initial phase of the disease and nine in later phases. The control group consists of 36 cases of acute leukaemia treated in the three years from September 1961, before introduction of the diet, and 9 cases at present on the diet, in which the clinical course before and after augmentation of the diet is being compared. We consider the observations in the last-mentioned group to be of special importance.

The clinical observations indicate marked differences in the dynamics of the disease and its symptoms in individual patients. On the other hand, the course of the disease during exacerbations in the same child follows a stereotyped pattern. For this reason "longitudinal study" in each particular case appears to be of greater value than statistical evaluation of all the cases at a given time. Knowledge of individual characteristics of acute leukaemia is essential when evaluating therapeutic results, allowing prediction of the course of the disease in the event of the new therapeutic factor not being introduced.

Specificity of characteristics, however, is subject to the reservation that the meaning of a symptom observed at different times in the course of the disease may not be the same. The response to treatment varies at different times, and each successive relapse is associated with increasing resistance to therapy.

Analysis of the course of the disease requires the co-operation of the child's parents. Children discharged from the Clinic during remission were examined at intervals of two to three weeks, and the parents were asked to report immediately any new symptoms observed. In this way, oligosymptomatic as well as asymptomatic relapses of the disease were detected at once.

In four children the new diet was given from the onset of illness. The percentages of parablasts in the bone-marrow at admission were 72, 85.5, 88.5, and 95.5%, and after two weeks of treatment dropped to 1, 4.5, 12, and 0.5% respectively. At the same time pathological cells disappeared from the peripheral blood, although pancytopenia continued. In two cases complete regression of pancytopenia was achieved after 10 weeks. Greater benefit of combined treatment with low-protein diet and routine methods was observed in the two remaining children, in whom full remissions in the peripheral blood were achieved after five weeks.

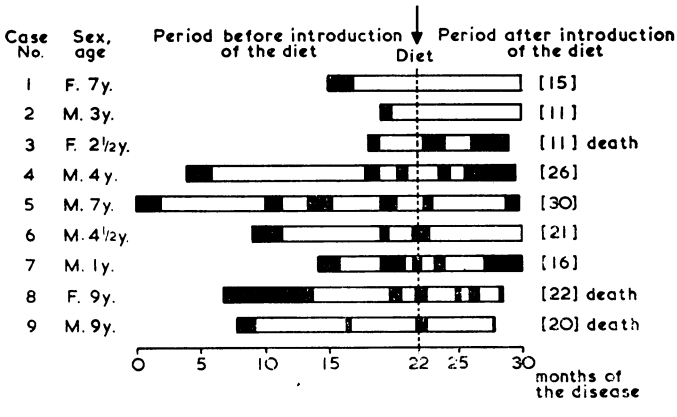

FIG. 2.—Showing remissions (white) and exacerbations (black).

In nine children the diet was started in later stages of the disease. The course of the disease before and after introduction of the diet in these cases is illustrated in Fig. 2.

On the time scale the moment of the introduction of the diet is indicated as well as the preceding and subsequent remissions (white) and exacerbations (black). The survival time in months up to the end of August 1965 or to an earlier date if death occurred is shown in brackets.

In five cases the diet was begun during remissions, and in four cases during relapses.

In three cases (Nos. 3, 4, and 5) treated during remissions, relapses were not prevented. In two patients (Cases 7 and 8), in whom the diet was begun during relapses, resulting in improvement, the subsequent remissions were not prolonged and further exacerbations were not prevented. The observations in these five cases indicate that low-protein purine-free diet, together with mercaptopurine administered during remission, does not prevent relapses or prolong the period of remission.

A different picture was observed in relapses of the disease. In four cases in which the diet was begun during exacerbation a strikingly quick drop in the numbers of bone-marrow parablasts was observed. In Case 5 relatively rapid improvement in the myelogram was noted, the number of parablasts being reduced from 75% to 8% in the course of 21 days.[1] In this case the exacerbation was the fifth in the course of the disease—that is, at a stage in which remissions are usually rare, especially as the duration of the illness was 23 months in this patient. In another patient (Case 8) remission was obtained after 21 days of the fifth exacerbation and 20 months' duration of illness. In this case the remission was the third after beginning the diet. It should be noted, however, that the improvement in bone-marrow (fall in the percentages of parablasts from 78.5% to 4.2%, and from 56.8% to 8.5%) during the third and fourth exacerbations was obtained after unusually short intervals—12 and 9 days. In two children (Cases 7 and 9) remissions were obtained after 14 days during the fourth exacerbation; the duration of the disease was 17 months in Case 7, and 15 months in Case 9.

The third exacerbation in the mongoloid child (Case 3) (8th month of the disease), the fifth one in Case 4 (22nd month of the disease), and the fifth one in Case 7 (13th month of the disease) were unusually prolonged and of strikingly moderate course. Nevertheless in Case 3 death occurred as a consequence of the third relapse. This child seemed to be resistant to the influence of the diet.

As to the two children (Cases 5 and 8) treated during the sixth exacerbation, the former is progressively improving, and the second died a few days after the onset of the relapse.

The new diet was applied during 14 exacerbations of the disease: in one case during the second exacerbation, in four cases during the third, in three cases during the fourth, in four cases during the fifth, and in two cases during the sixth exacerbation.

The evaluation of the results was based mainly on the rapidity of regression of pathological bone-marrow cell proliferation, not including other criteria of remission. Discrepancies between the haematological and clinical states, as well as between the myelogram and the peripheral blood picture, are known to occur. Nevertheless, reduction in the percentages of parablasts in the myelogram is likely to be a fundamental condition of return to normal blood regeneration.

[1] During the fifth exacerbation in this case the dose of Encorton was increased to 8 mg./kg/24 hr. During the fourth exacerbation the dose of Encorton had been increased from 1.5 mg. to 5 mg./kg./24 hr. In spite of this, the drop in the percentage of bone-marrow parablasts from 75% to 12% did not occur until after 36 days of treatment.

Our observations justify the conclusion that low-protein purine-free diet is a factor in the disappearance of parablasts from the bone-marrow and therefore contributes to earlier remission. The observation of remission after the fifth and even the sixth exacerbation of long-standing disease also supports this conclusion.

The prolonged and mild course of the relapses, such as was observed in three children, may possibly also add to the effect of the diet. Low-protein purine-free diet may therefore play a part in the induction of late remissions as well as in provoking slower and milder clinical progress in subsequent relapses and consequently in prolongation of the survival time of children with acute leukaemia.

Discussion

The encouraging results of our observations require confirmation on a larger series of cases. Although the diet described herein may not be the optimal one, it is apparent that it exerts an influence on exacerbations of acute leukaemia in children.

In general the percentages of remission in successive exacerbations of acute leukaemia can be presented as in Fig. 3 (calculated on the basis of the data of Bernard *et al.*, 1962).

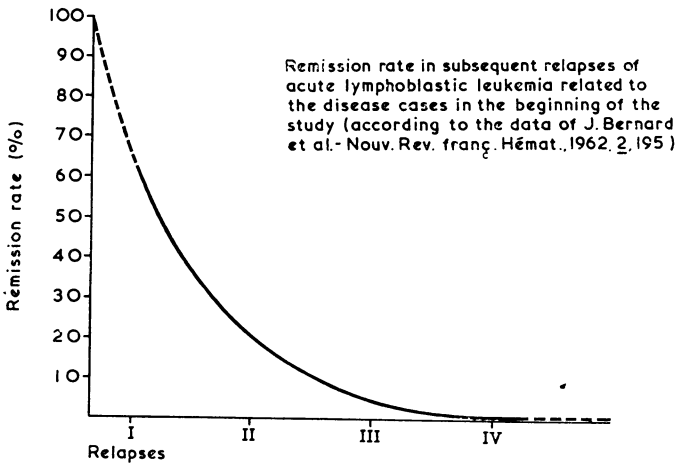

FIG. 3

Efforts to increase the response to therapy during late relapses of acute leukaemia have recently been made. Methods of restoring a declining sensitivity to treatment include high dosage of adrenocortical hormones, change of the antimetabolite or routes of its administration, and introduction of hypoxanthine or folic acid in an attempt to sensitize the patient to mercaptopurine and methotrexate respectively. To these methods may now be added that of a low-protein diet. Analogy may be found in the use of low-calorie diet to prevent leukaemia in mice (Saxton *et al.*, 1944) and in the treatment of leukaemia in chickens.

Recently, Allan *et al.* (1965) reported the results of supplementing the diet with L-phenylalanine and L-tyrosine in five cases of acute leukaemia in children. In one case dietetic treatment in itself produced improvement in the blood picture and reduction in the size of the enlarged liver and lymph nodes after eight days ; and in another case rapid remission was obtained by dietetic-plus-routine therapy.

Trials of the treatment of malignant tumours with low-calorie (low-protein ?) diet have been carried out, and reduction in the rate of development of the tumours or metastases has been reported (Tannenbaum, 1944).

We believe that the beneficial results in our cases and those observed by Allan *et al.* (1965) may be attributed to a common dietetic factor connected with the substrate of cellular metabolism. The effects obtained by Allan *et al.* and in our own cases do not refute the possibility of basic enzymatic blocking. On the contrary, they seem to support the hypothesis and point to a central role of the liver in the pathogenesis of acute leukaemia in children.

Assuming the development of tumours to be biphasic—that is, consisting of an initial phase of neoplastic transformation and of a prompting phase of proliferation (Rous and Kidd, 1941 ; Berenblum, 1941, 1964)—we reached the conclusion that low-protein purine-free diet exerts a clinical effect on the proliferative phase. However, this does not solve the problem of a causal relationship between proliferation and enzymatic blocking in the first phase, and is not an argument against the hypothesis of enzymatic blocking in the normal bone-marrow.

Formulation of a definite hypothesis at present, however, would be premature, and continued trials of various methods of treatment are justified.

Past experience shows that successful therapeutic trials often precede the discovery of the causes of diseases and their pathogenesis.

Summary

Response to therapy in acute leukaemia appears to be an individual characteristic showing variation among a population of patients and diminishing in most responsive cases with duration of the disease.

Resistance to therapy appears to be correlated with hepatic lesions and symptoms of hepatic dysfunction.

The relationship between hepatic function and the bone-marrow may consist in the dependence of normal production of cells in the bone-marrow upon normal hepatic function.

Low-protein purine-free diet was given to 13 children with acute leukaemia with the purpose of reducing the supply of substrate of the presumably blocked reactions. An increased response to therapy in successive and late relapses of the disease was observed.

The use of such a diet or of some modification of it in acute leukaemia, if our observations are confirmed, may contribute to the prolongation of the life-span of children suffering from acute leukaemia.

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