volunteers, and a British strain in doses ranging from 10^3 to 10^6 EID_{50} to 30 volunteers. One month later 75 of the vaccinated volunteers were given 10^4 EID_{50} of the British vaccine. In addition, 55 volunteers with neutralizing antibody were given 10^7 EID_{50} of the Iksha vaccine by drops or spray, and 53 volunteers were given the same dose of vaccine inactivated with formalin.

Of the volunteers without antibody 41% had mild respiratory symptoms after the first dose, and 8% after the challenging dose; 9% of the volunteers with antibody who were given live vaccine had similar symptoms.

Virus was recovered from 25% of the volunteers without antibody after the first dose of vaccine, and from 5% after the challenging dose. Immediately before the challenging dose of vaccine 21% of the volunteers showed fourfold or greater haemagglutination-inhibition antibody response and 21% a complement-fixation response; two weeks after the challenging dose the proportions were 29% and 19% respectively. A much higher proportion of volunteers showed an antibody response after live than after inactivated vaccine.

A dose of 10^4 EID_{50} of the British vaccine gave similar results to that of 10^6 or 10^7 EID_{50} of the Iksha vaccine. Except perhaps in volunteers with antibody there was little difference in efficacy of administration by nasal drops or spray.

We thank the volunteers of the Common Cold Research Unit, Salisbury, and at R.A.F. Technical College, Henlow, without whom these studies could not have been carried out. We are grateful to Dr. M. L. Bynoe and Miss E. Bullock, S.R.N. (Salisbury), and the Medical Officers at R.A.F. Henlow for their valuable help. We are indebted to the Director-General of the Royal Air Force Medical Services for the facilities to carry out this trial, and for permission to publish this report.

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Extensive Histological and Cytological Survey of Patients with Acute Leukaemia in "Complete Remission"*

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[WITH SPECIAL PLATE]

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Throughout the world only about 50 patients with acute leukaemia have had a remission lasting longer than five years (Burchenal and Murphy, 1965). In most patients with this disease the remissions that are called "complete" are invariably followed by a recurrence.

It can be questioned whether this recurrence is due to proliferation of the leukaemic cells that have persisted and whose presence has not been detected on routine examination. On the other hand, it is possible that the original leukaemic cells are destroyed and that the recurrence is due to the induction of a new leukaemia by a leukaemogenic factor which remains in the body. It would therefore be logical to obtain very detailed information about the state of the leukaemic cells during a so-called complete remission.

In practice we have made the following observations on patients during a remission:

1. A study of the cells present in the blood by means of a leucoconcentration technique (this had previously been shown to be able to demonstrate circulating cancer cells in patients with haemato-sarcomata who were not leukaemic (Festing, 1962)).

2. A cytological examination of six bone-marrow biopsies and a histological examination of one. (Bernard and Mathé (1951) have shown that when multiple bone-marrow biopsies are taken simultaneously from patients with acute leukaemia the cellular picture may vary from site to site.) The skeleton is also examined radiologically.

3. The central nervous system is examined by an analysis of the cerebrospinal fluid and by electroencephalography; there is a high frequency of leukaemic infiltration of the meninges and central nervous system during the course of an acute leukaemia (Wells and Silver, 1957).

4. Biopsies are taken from the kidney, liver, and testicles (see Special Plate).

In those patients in whom the investigations revealed the presence of leukaemic cells, the treatment which has led to the remission is continued at twice the dosage for a month. This treatment is generally with corticosteroids. After this period, those tests that revealed the presence of leukaemic cells are repeated.

Methods, and Patients Studied

The methods used for this investigation start when the patient's routine blood count and bone-marrow are found to be in the normal range both quantitatively and qualitatively. Thirty-one patients were studied in the present survey; details of their age, sex, classification according to cell type involved, history of the proliferative phase preceding the remission, and treatment during the proliferative phase are given in Table I.

The technique used in counting the leukaemic blast cells after concentrating the blood is as follows: 30 ml. of blood is taken into A.C.D. (acid-citrate-dextrose, 1 vol. A.C.D. for 9 vol. blood) and allowed to stand at an angle of 45 degrees for three hours. The supernatant plasma containing the leucocytes is then removed and its volume measured. This supernatant is

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then slowly centrifuged at 100 r.p.m., and the leucocyte pellet obtained is spread and stained with May-Grünwald-Giemsa. Thirty-three normal bloods were examined by this method; statistical analysis of the results indicated that it is abnormal for more than 22 blast cells per 10,000 leucocytes to be present.

TABLE I.—*Clinical Classification of Acute Leukaemic Patients Studied During a Complete Remission*

Type of Leukaemia		*Age of Patients*	
Lymphoblastic	26	1–4 years	3
Myeloblastic	3	5–14 „	15
Monoblastic	2	15–20 „	7
		+20 „	6
No. of Remission		*Sex of Patients*	
First	24	Male	16
Second	6	Female	15
Third	1		
Treatment During Proliferative Phase Preceding Remission			
Δ-1 cortisone	25	Methotrexate	1
Leurocristine	2	Azathiopurine	1
6MP	2		

The six bone-marrow samples for cytological examination were taken at the same time from the following sites: sternum, spine, left and right iliac crests, and left and right posterior iliac crests. The criterion for abnormality was when the blast cells numbered more than 6%. When they were between 4 and 6% a further biopsy was taken from that particular site.

Bone-marrow biopsy specimens for histological examination were taken from the iliac crest with a Waitz (1953) trocar, the technique of Ceoara *et al.* (1958) being used. Renal biopsies were taken by percutaneous puncture; in children the kidney was exposed surgically. Liver biopsy specimens were obtained by the Menghini (1959) technique and a Menghini needle. Testicular biopsy specimens were taken under general anaesthesia. The fragments obtained were studied histologically to determine whether any leukaemic infiltration was present.

Results

The results of these investigations are summarized in Table II. In 12 of the 31 patients investigated during a period of "complete remission," leukaemic cells were found in one or more sites. In six patients a single test was positive—leucoconcentration, bone-marrow from one site, cerebrospinal-fluid cytology, liver biopsy, marrow histology, or *x*-ray film of the skeleton. In four patients two of the tests were positive—leucoconcentration and bone-marrow, leucoconcentration and encephalogram, bone-marrow and encephalogram, bone-marrow histology and hepatic biopsy. In two patients three of the tests were positive—renal, hepatic, and testicular biopsies in one patient and renal and hepatic biopsies and encephalogram in the other.

The frequency with which positive results were obtained and the number of times each test was made are shown in Table III. None of these new investigations gave persistently negative results.

After one month's treatment at double the usual dose the examination for malignant cells gave negative results in five patients. Those who had lymphoblastic cells in the cerebrospinal fluid were given intrathecal methotrexate in conjunction with the intensified systematic therapy. One patient died of an intercurrent infection before the repetition of the tests. In six patients, despite the increased dosage, the active phase of the disease returned in less than one month. This early recurrence was experienced by only one of the 19 patients who showed negative results in all the tests. This difference is statistically significant ($\chi^2=7.93$ for 1 d.f.; $P<0.01$). The principal factor determining the outcome for the patients would appear to be the multiplicity and extent of the visceral leukaemic infiltration. In the three patients with signs of infiltration in three different sites there was a very rapid recurrence of the disease. Of the four patients with abnormal bone-marrows, three had a rapid recurrence.

TABLE III.—*Results of Tests*

	Tests Made	Positive Results
Leucoconcentration	27	2
Bone-marrow smears, 6 sites	31	4
Cytology of C.S.F.	30	1
Electroencephalogram	31	3
Renal biopsy	31	2
Hepatic „	29	4
Testicular „	14	1
Bone-marrow histology	12	2
X-ray examination of skeleton	13	1

Discussion

These results give a more realistic picture of the extent to which the leukaemia regressed in patients whose remissions were said to be "complete." Twelve out of 31 patients, considered to be in complete remission from the study of their peripheral blood and a single sample of bone-marrow, were shown to have a persistent leukaemic infiltration in some area of the study.

Our results agree with those reported by Nies *et al.* (1965). These workers studied post-mortem histological material from 15 leukaemic patients who had died while in apparently complete remission. They were able to demonstrate that leukaemic infiltration was present in one or more organs in 10 of these patients. The low frequency of our positive cases seems to indicate that certain visceral localizations of leukaemic cells may escape detection, even in an intensive investigation such as ours. Our results clearly indicate that leukaemic cells may exist in the meninges when remission was thought to be complete. Indeed, these additional investigations are sufficient to alter considerably the usual concepts about remissions in acute leukaemia. The role of these nests of leukaemic cells in the natural evolution of the disease and their part in causing a rapid recurrence are apparent from our results. Only one of the 19 negative patients had an early recurrence, while this occurred in 6 of the 12 positive patients, despite the increased dosage of chemotherapy.

This investigation provides information which is useful as a basis for an opinion regarding the patient's prognosis. Even more important it provides possibilities of further progress in

TABLE II.—*Summary of Results*

Case No.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	–	–	–	–	–	–	–	–	–	+	–	–	–		–	–	–	–	–	–	–	–	–		–		–	–	+	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+
3	–	–	–	–		+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
4	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
6		–	–	–	+	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	+	+	–	+	–	–	–	–	–	–
7	–		–	–						–	–	–		–	–				–	–	–		+	–		–					
8																			–	+	–		–	–	+	–	–	–	–	–	–
9																			–	–	–	–	–	–	–	–	+	–	–	–	–
PP (m)*	>14	2	>13	>13	1	9	1	>12	>12	8	3	5	>8	<1	>8	3	5	4	>5	5	2	<1	<1	2	<1	<1	>2	>2	<1	<1	>1

1. = No. of blast cells in peripheral blood (leucoconcentration). 2. = Bone-marrow smears from six sites. 3. = Cytological examination of cerebrospinal fluid. 4. = Electroencephalogram. 5. = Renal biopsy. 6. = Hepatic biopsy. 7. = Testicular biopsy. 8. = Bone-marrow histology. 9. = *X*-ray examination of skeleton.
* PP (m) = Time in months to recurrence of proliferative phase.

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the treatment of acute leukaemia. Nests of leukaemic cells may remain in the tissues of patients who had been thought to be in a satisfactory state of remission, and treatment must therefore be persisted with until these nests have been eradicated. This should be the ultimate aim (Mathé, 1965). To achieve this it is necessary that on two successive occasions an extensive search must be made for such nests. This search should be followed up by what Dameshek *et al.* (1965) have called "a new use of old remedies."

Clinical trials are now in progress in many American centres on the simultaneous use of several antimitotic agents—as yet, it is too early to judge the results (Freireich and Frei, 1964; Freireich *et al.*, 1964).

The clinical procedures that we have adopted in view of our recent results are as follows. The main aim is to induce in our positive cases a state of "true" remission by stepping up the therapeutic dosage which had induced the apparent remission. This concept is based on the hope that the resistance of the nests of leukaemic cells might be due to various extracellular factors, such as anatomical site or vascular distribution, rather than intracellular factors. In all cases we use a treatment that comprises a two-months course for each type of chemotherapy at maximal doses, alternating with a month's treatment with corticosteroids. This intensive therapy is preceded by a systematic irradiation of the meninges at 1,000 rads. The first course of chemotherapy to be given was methotrexate, which is administered both intrathecally and systemically.

The first results that we obtained in the attempt to eradicate the leukaemic cells completely have been encouraging (Mathé, 1965). Full details will be published later.

Summary

In 31 patients with acute leukaemia who were stated to be in "complete remission," as indicated by a normal peripheral blood and bone-marrow, an extensive histological and cytological investigation was carried out. This investigation comprised the counting of blast cells in the circulating blood, examination of bone-marrow from six sites, examination of the cytology of the C.S.F., an electroencephalogram, renal, hepatic, and testicular biopsies, bone-marrow histology, and *x*-ray examination of the skeleton.

Nests of leukaemic cells were found in 12 patients. None of the tests used gave consistently negative results.

The lessons to be learnt from this study, as regards both prognosis and the treatment designed to eradicate these leukaemic foci, are discussed.

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Osteoporosis, Scurvy, and Siderosis in Johannesburg Bantu*

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[WITH SPECIAL PLATE]

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It is not generally recognized that osteoporosis is a common and disabling disease among middle-aged Bantu in Johannesburg. There have been only two previous studies of the condition. Grusin and Kincaid-Smith (1954) and Grusin and Samuel (1957) were the first to document the disease as a clinical entity, and showed that it was often associated with scurvy. Grobbelaar noted its association with severe siderosis in necropsy material, but in a subsequent post-mortem study of Bantu with varying degrees of siderosis Walker, Strydom, Reynolds, and Grobbelaar (1955) were unable to demonstrate a correlation between the iron content of vertebral bodies and their mineral composition or mineral density. After 1957 interest in the condition lapsed, and unawareness of the existence of such a disorder has resulted in its frequently being misdiagnosed as myeloma, secondary carcinoma, or tuberculosis.

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Present Investigation

In 1962 we began to study the condition again, and the objects of the present paper are to describe the clinical, radiological, biochemical, and pathological characteristics in 32 patients with the disease, to present further data on its association with siderosis and scurvy, and to discuss its possible aetiology and pathogenesis.

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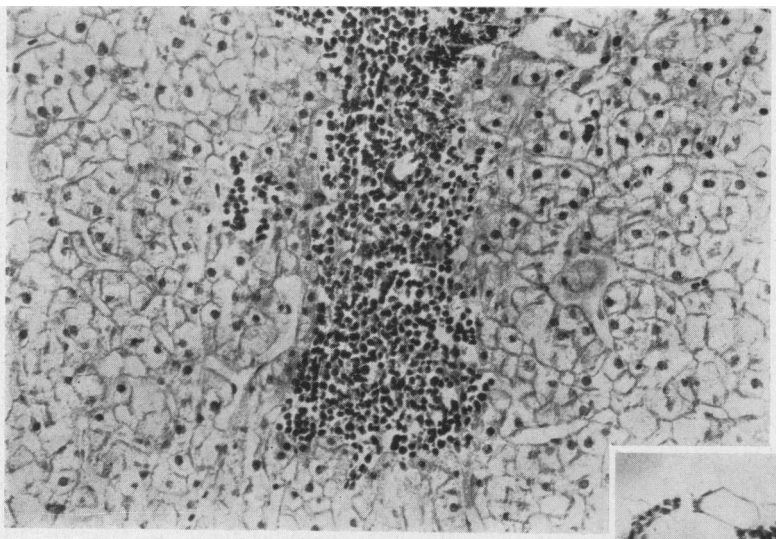

FIG. 1.—Infiltrates of leukaemic cells in the liver.

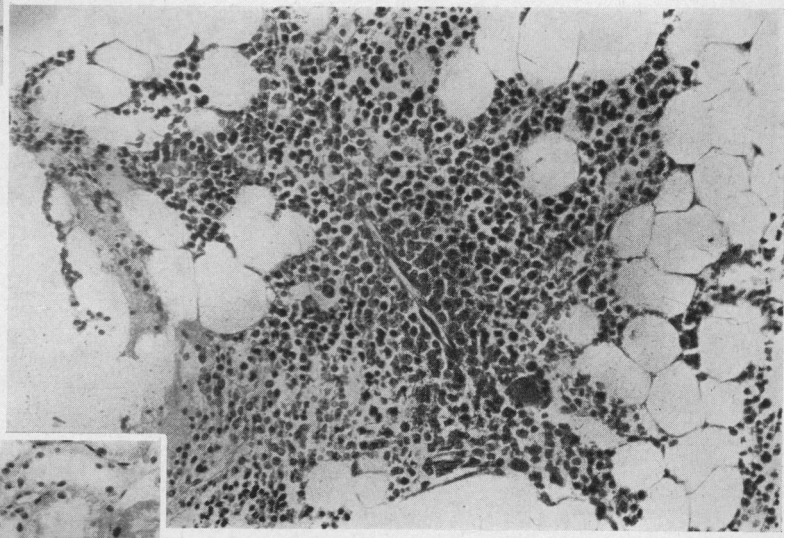

FIG. 2.—Small pericapillary infiltrates of leukaemic cells in the bone-marrow.

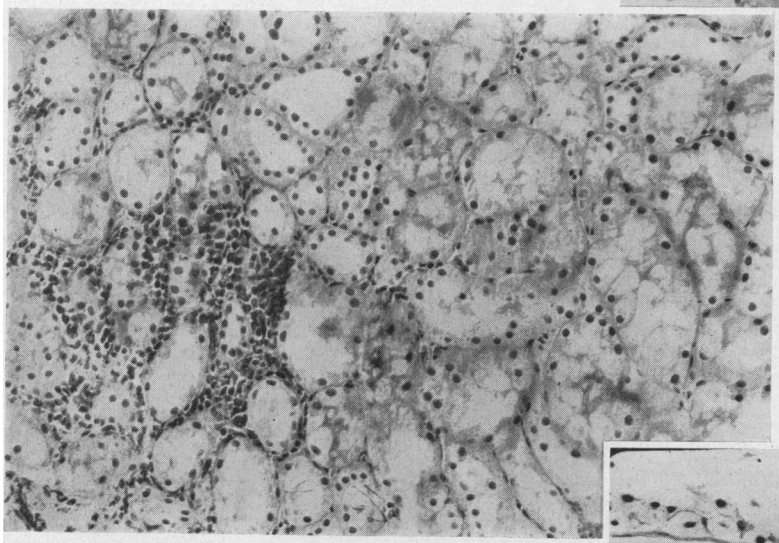

FIG. 3.—Infiltrates of leukaemic cells in the kidney.

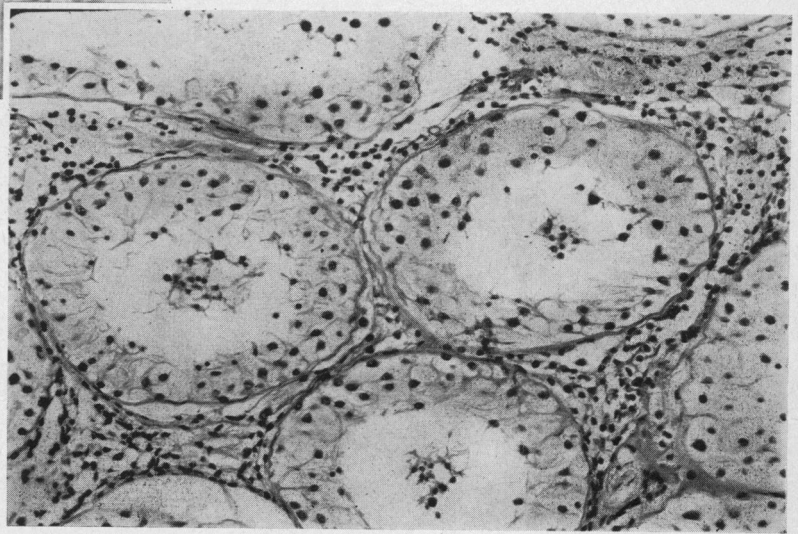

FIG. 4.—Leukaemic-cell infiltration in the intertubular tissues of the testes.

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