46% of the original group. The general acceptability of this regime may well vary in different localities.

Since the combination of streptomycin and isoniazid is more effective than P.A.S.+isoniazid it may be possible to reduce the length of treatment from the generally accepted period of 18 months. We are satisfied that the present results with daily streptomycin+daily isoniazid justify further trials to assess now the optimal duration of these drugs, including possibly an intermittent regime during the later stages of treatment.

Conclusions

Streptomycin 0.75 g. is an adequate dose and may represent the optimal dose in combination with isoniazid for the treatment of pulmonary tuberculosis.

This dose significantly reduces labyrinthine damage. Pantothenic acid has failed to prevent this complication.

High-dose isoniazid offers no advantage and is more toxic.

Summary

A total of 138 patients with active pulmonary tuberculosis were allotted by random selection to three regimes to examine the advantages of high-dose isoniazid, pantothenic acid to mitigate streptomycin-induced vestibular damage, and the effects of reduction of the daily dose of streptomycin from 1 to 0.75 g. A supplementary group of 37 patients who received the lower dose of streptomycin were added later. Patients were treated for 18 months and the results were analysed two years after stopping treatment.

Lowering of the daily dose of streptomycin to 0.75 g. reduced complicating vertigo from 30% to 7% without loss of therapeutic efficiency.

No long-term advantage was gained with high-dose isoniazid, which proved more toxic. Under the conditions of this trial pantothenic acid was of no value.

There is some evidence that daily streptomycin+isoniazid is not essential beyond a year, and an intermittent regime may be adequate in the later stages of treatment.

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Circulating Cancer Cells

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Two decades of advance in surgical techniques and management have permitted the cancer surgeon to perform massive local resections of tissue and increasingly extensive block dissections of the lymphatic pathways. In addition, with a more enlightened general public and greater diagnostic facilities, early diagnosis of cancer is becoming increasingly common. Nevertheless, the overall mortality from cancer remains very much the same as it was 25 years ago, the patients continuing to die from distant metastases. There is therefore increasing interest in the mechanisms of tumour dissemination and the means of preventing it.

Circulating cancer cells were first described by Ashworth in 1869, and further observations were made by Loeper and Louste (1904), Schleip (1906), Ward (1913), Marcus (1917),

Quensel (1921), and Pool and Dunlop (1934). Pool and Dunlop examined samples of peripheral blood from 40 patients with advanced cancer and recorded the presence of cancer cells in 17 of them.

Engell's (1955) description of cancer cells in the blood of patients with carcinoma of the large bowel aroused great interest, particularly in the U.S.A., and workers from several centres have described their results with the use of various techniques. Moore *et al.* (1957) identified cancer cells in peripheral blood samples from 93 (52%) of 179 cases. Roberts *et al.* (1958) found malignant cells in 16.7% of "curable" patients and in 31.1% of "incurable" patients, while Pruitt *et al.* (1958) described an incidence of 39% and Salgado *et al.* (1959) one of 57%.

We have attempted to repeat this work as part of an investigation into the exfoliation of cancer cells from various sites and the mechanisms by which they produce metastases, but, like other workers (Spriggs, 1960; Kuper *et al.*, 1961), we have been unable to confirm this high incidence.

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Technique

Two main problems are involved in the detection of cancer cells in the blood: (1) the separation of a few malignant cells from the enormous number of normal blood cells, and (2) the positive identification of a given cell or group of cells as malignant. Various techniques, recently described, attempt to overcome these problems and are concerned mainly with the extraction or destruction of the cellular elements normally present in circulating blood. These techniques are well reviewed by Griffiths (1961). Our experience is of four of these methods: those of Roberts *et al.* (1958), Malmgren *et al.* (1958), Seal (1959), and Kuper *et al.* (1961).

Separation

Roberts and his colleagues have described a method based on the different densities of cancer cells and white blood cells. The red cells are first sedimented by a specially prepared bovine fibrinogen solution, and the supernatant fluid is centrifuged over a physiological preparation of Armour's fraction V albumin, and adjusted to a specific gravity midway between the densities of cancer cells and leucocytes. As they are heavier, the polymorphonuclear leucocytes sink to the bottom of the tube and the cancer cells remain at the interface. We have used this method successfully, but find it expensive and time-consuming. Large numbers of the polymorphs may remain at the interface, so that several slides may need to be prepared and subsequently screened.

A similar specific-gravity method has been described by Seal using a mixture of silicones instead of albumin. In our hands this has proved unreliable.

Malmgren and his group destroy the red cells and polymorphonuclear leucocytes with streptolysin O and pass the diluted residue through membrane filters to catch the cancer cells. However, streptolysin O is expensive and deteriorates rapidly even if refrigerated. The method involves repeated centrifugation and resuspension, which we thought might damage the cells and break up any cell clumps present. Often the polymorphonuclear leucocytes were only partly destroyed, increasing the difficulties of cytological examination.

We now prefer the method described by Kuper *et al.* (1961), and all of our patients in this series have been investigated by this method.

A 5-ml. sample of blood is incubated with a pinch of carbonyl iron, which is phagocytosed by the polymorphonuclear leucocytes. Saponin is then added to destroy the red cells, and the polymorphs are removed with a magnet. The sample is then centrifuged and the deposit resuspended in saline. Fixative is added and the suspension passed through a single millipore membrane.

This method is simple, cheap, and quick to perform; it is quantitative because all the cells are caught on the membrane filter.

Preparation for Microscopy

Any method using wet smear or streak preparations may involve the loss of cells during processing, so that false-negative results may occur and the method cannot be quantitative. We therefore prefer millipore membranes (47 mm.). The fixed suspension is drawn gently through the membrane at a negative pressure of 10 mm. of mercury and the membrane itself stained and finally cleared in xylene. It is then mounted on a 7.5 by 5-cm. glass microscope slide with a copious excess of mounting fluid. Many authors recommend the use of the Papanicolaou staining technique, but we are now using the more simple haematoxylin-and-eosin method, which gives equally good results in this type of investigation.

Recognition of Malignancy

The characteristic nuclear appearances of malignancy are well known, and are simply and clearly described by Griffiths (1961); but it must be emphasized that these are changes of degree rather than of kind, and they appear to a greater or less extent in any actively dividing colony of cells. They may all be recognized in primitive cells from normal bone-marrow which has been suitably stained. Certain mature cells which are occasionally found under normal conditions in circulating blood may also show bizarre arrangements of chromatin within the nucleus. In particular, megakaryocytes and plasma cells may be a source of false-positive results, as pointed out by Alexander and Spriggs (1960).

In these circumstances we find ourselves unable to state whether any given single cell is malignant or not, and all samples in which only one or two doubtful cells have been found are regarded as negative. In order to diagnose malignancy we require either a group of cells or a number of single cells which can be considered together as a group, in which abnormalities of nuclear form and structure are pronounced.

Results and Discussion

Peripheral Blood Samples

Our results from 5-ml. peripheral blood samples are given in Tables I, II, and III. It will be seen that the recovery of cells which were confidently recognized as malignant occurred in only 11% of patients examined. Only 50 (6.5%) of 773

TABLE I.—*Cancer Cells in the Peripheral Blood. Numbers of Patients*

Site of Primary Tumour	No. of Patients	No. Positive
Bronchus	72	3 (4%)
Breast	93	7 (7.5%)
Bladder	95	20 (21%)
Other sites	93	9 (9.5%)
Total	353	39 (11%)

TABLE II.—*Cancer Cells in the Peripheral Blood. Numbers of Samples*

Site of Primary Tumour	No. of Samples	No. Positive
Bronchus	98	4 (4%)
Breast	197	8 (4%)
Bladder	285	27 (9%)
Other sites	193	11 (6%)
Total	773	50 (6.5%)

TABLE III.—*Peripheral Blood Samples. Other Cancers*

Type of Tumour	No. of Cases	No. Positive	Type of Tumour	No. of Cases	No. Positive
Ca. oesophagus ..	5	1	Ca. kidney	10	2
„ stomach	7	1	„ ureter	1	0
„ pancreas	5	1	„ prostate	3	0
„ colon	4	0	„ testes	7	0
„ rectum	9	1	Carcinomatosis ..	1	0
„ uterus	1	0	Malignant glioma ..	7	0
„ cervix	4	0	Epithelioma	3	1
„ vagina	1	0	Malignant melanoma	13	1
„ ovary	2	0	Various sarcomata ..	9	1
„ suprarenal ..	1	0	Total	93	9 (9.5%)

samples were positive. This incidence is much lower than that recorded by various other workers (Moore *et al.*, 1957; Roberts *et al.*, 1958; Pruitt *et al.*, 1958; Salgado *et al.*, 1959), but agrees well with the findings of Spriggs (1960) and Scheinin (1962). We presume that these differences are due to the difficulties of cytological interpretation, our method having been shown to be quantitative (Kuper *et al.*, 1961). Ritchie and Webster (1961), who described an incidence of 64%, stated that often only one tumour cell was found in a sample. Using our criteria, such samples would be regarded as negative.

Table I appears to indicate a relatively high incidence of circulating tumour cells in patients with cancer of the bladder. The data in Table II suggest, however, that this observation is due to the large number of samples examined from each patient.

The numbers in Table III are too small for any statistical analysis, but it is of interest that we found tumour cells in only one out of 13 patients with malignant melanoma.

It is perhaps surprising that large clumps of cells, such as those shown in the illustration, should appear in a peripheral vein; this implies that at least two capillary systems have been negotiated, presumably through arteriovenous shunts.

Clump of cancer cells isolated from the peripheral blood of a patient with cancer of the bladder. ($\times 1,100$.)

We conclude that the incidence of circulating cancer cells in small samples of peripheral blood is extremely low. Moreover, such cells are present most commonly in patients in whom the disease has progressed to such an extent that the diagnosis is clinically obvious, and the disease is often so advanced that palliation is the most for which one can hope.

Local Blood Samples

The results from local blood samples are shown in Tables IV and V, and indicate a higher incidence of tumour cells in blood

TABLE IV.—Local Blood Samples. Numbers of Patients

Site of Primary Tumour	No. of Patients	No. Positive
Bronchus	44	6 (14%)
Breast	20	4 (20%)
Bladder	7	1 (14%)
Other sites	24	3 (12.5%)
Total	95	14 (15%)

TABLE V.—Local Blood Samples. Numbers of Samples

Site of Primary Tumour	No. of Samples	No. Positive
Bronchus	56	7 (12.5%)
Breast	65	4 (6%)
Bladder	8	1 (12.5%)
Other sites	27	3 (11%)
Total	156	15 (10%)

obtained from veins draining the tumours than occurs in the peripheral circulation.

The detection of tumour cells in the local venous blood is of no diagnostic value because such samples are seldom obtainable except at operation.

Engell (1959) concludes from his own five- to nine-year follow-up figures that patients in whom circulating cancer cells were found have the same prognosis as those in whom they were not. A further analysis of his figures indicated to us, however, that the outlook was, in fact, significantly worse for those patients in whom circulating tumour cells were originally found. Of 41 patients with cancer of the rectum from whom cancer cells were isolated only 12 (29%) were alive after five to nine years, whereas 22 of 35 patients in whom cancer cells were not found (63%) were alive after the same period.

Summary

The techniques available for the extraction of cancer cells from the circulating blood are discussed and a preference is expressed for the iron-separation technique described by Kuper *et al.* (1961). The problems of screening a relatively large volume of fluid for a small number of cells and the particular difficulties involved in the recognition of cancer cells in blood are considered. In particular, stress is laid on the value of millipore membranes to obtain accurate quantitative results.

Considerable difficulty is experienced in cytological identification when small numbers of single atypical cells are present. Similar morphological features occur in cells from normal bone-marrow, and a definite diagnosis of malignancy is unreliable unless a group of cells is examined. In particular, megakaryocytes have been incriminated as a source of false-positive results.

Cancer cells were recognized in peripheral blood samples from only 11% of cancer patients. Only 6.5% of the total number of samples examined were positive. This figure is very much lower than those of several other workers, but is similar to those published by Spriggs (1960) and Kuper *et al.* (1961). Differences are almost certainly due to differences of cytological interpretation.

Examination of the blood for cancer cells is of no diagnostic value, as most of the patients from whom such cells are isolated already suffer from obvious advanced malignant disease. Blood samples taken from local veins draining tumours yield a somewhat higher incidence of cells (15% of patients), and might be of some value in studying the dissemination of cancer cells during surgical operations.

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Medical Memoranda

Screening Test for High Serum Salicylate Levels

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The differential diagnosis of coma is a pressing problem to the casualty department. Too often an inexperienced clinician has to make a diagnosis without adequate laboratory services. A simple and reliable screening test for salicylate intoxication would be of great assistance to the clinician. We describe here an investigation into the use of "phenistix" strips as a screening test for high levels of serum salicylate.

Rupe and Free (1958) showed that phenistix gave a colour reaction with salicylate. Johnson, Free, and Free (1962) described a series of cases of aspirin poisoning in children, in which phenistix strips were used to detect salicylates, both in urine and in plasma. They obtained a colour reaction with sera in which the salicylate level was greater than 20 mg./100 ml.

Salicylates are bound to the plasma proteins and at low concentrations this binding is almost complete. Sodium salicylate is 92% bound to the plasma proteins at a level of 5.8 mg./100 ml. Phenistix strips will react only with free salicylate, and it would appear that at about 20 mg./100 ml.

Results of Test				
Sample	Serum Salicylates (mg./100 ml.)	Phenistix Colour		
		No Colour	Faint Mauve	Strong Mauve
1	0	+		
2	1	+		
3	5	+		
4	9	+		
5	10	+		
6	10	+		
7	14	+		
8	15	+		
9	20		+	
10	33		+	
11	44		+	
12	50			+
13	75			+
14	89			+

the plasma-protein-binding mechanism is saturated and sufficient free salicylate is present. Trinder's (1954) ferric-chloride/mercuric-chloride method acts as a hydrolysing agent and a colour developer.

Patients.—These were cases of aspirin overdosage admitted to the casualty department over the past 12 months. Sera were also tested from four cases receiving salicylate therapy.

Methods.—The serum salicylates were estimated by Trinder's method. The phenistix strips were dipped into separated serum, not whole blood, and the colour was read immediately. This was graded as mauve or dense mauve.

Results.—These are given in the Table. It will be seen that a definite mauve colour is developed between 15 and 20 mg./100 ml. Case 14 drank oil of wintergreen (methyl salicylate), with a fatal outcome. A lumbar puncture had been performed, and, on testing, the cerebrospinal fluid gave a mauve colour with phenistix strips; the salicylate level was found to be 29 mg./100 ml.

In aqueous solution phenistix will detect salicylate levels down to 2.5 mg./100 ml.

COMMENT

These results support the findings of Johnson *et al.* (1962) and suggest that phenistix can detect high serum-salicylate levels, and are of use in the diagnosis of salicylate poisoning.

The different sensitivity of phenistix to salicylate in plasma and aqueous solution is due to the protein-binding. The interpretation of phenistix strip colours in cerebrospinal fluid must be made in this light, in view of the low level of protein in cerebrospinal fluid.

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