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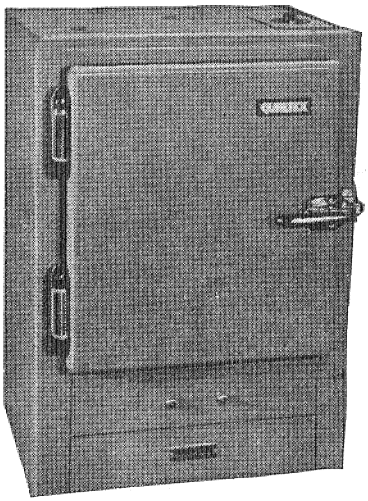

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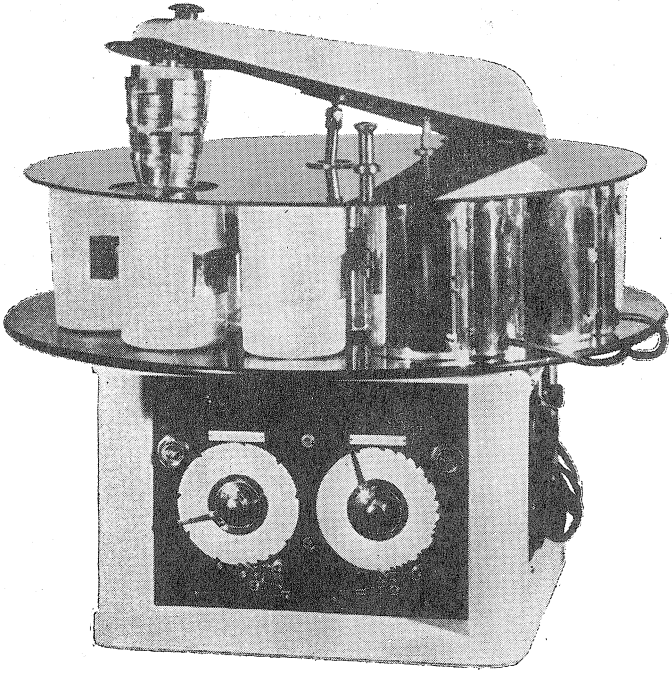

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LEUKAEMIA

R. C. Bridger

Paper read at the Bacteriologists Conference at Christchurch, July, 1953.

(Public Hospital, Timaru)

Leukaemia, perhaps more than any other haematological subject, is clouded by controversy. Authorities still argue at length, not only over its nomenclature and course, but more important, over its aetiology. How does it arrive? How is it instigated?

Now there are two sharply divided schools of thought concerning this latter problem of the leukaemic course—those holding the infection theory and those holding the neoplastic theory.

Proponents of the infection theory back their hypothesis with the characteristic clinical symptoms of the disease, namely—chills, fever, leucocytosis, mouth infection and, occasionally, septicaemia. The obvious deterrents to this theory are, lack of evidence for transmissability of the disease and the fact that there has been no demonstrable passage of the disease from leukaemic mother to foetus.

Against this stands the neoplastic theory. Ever since Banti in 1904 stated that: "A tissue with a typical structure that has a tendency to spread from the involved to neighbouring organs, cannot be called hyperplastic; it belongs, instead, in the group of new growths." Authoritative opinion has slowly but surely strengthened to this view, until now, a man like Custer, in his 'Atlas of Blood and Bone Marrow,' includes the words " . . . it (leukaemia) is in every sense a malignant neoplasm," and Willis in his book, 'Pathology of Tumours,' discusses leukaemia under the heading, "Neoplasms of Haemopeotic Tissues."

The evidence for this theory grows daily. It includes the fact that the infiltrative process of leukaemia is analogous to the invasive quality of other neoplastic cells and also, the work done on transplanting of malignant leukaemic cells into test animals to produce the disease. In this latter connection comes the research done on the hereditary factor of the disease in lower animals, particularly mice. This, however, cannot be evaluated, as yet, in man,

because of conflicting clinical data. Another point sustaining the theory is the refractory nature of the disease and fact that all research has led only to palliative treatment. These then, and many other features, especially concerning the pathology of the disease, predispose current opinion towards the neoplastic theory of leukaemia.

Regarding the age, sex and incidence of leukaemia, much has been reported. Again, there is conflict or opinion, in so far as there is much in the way of exception to contravene any set limits. Certain elastic limits have, however, been found to apply. Acute leukaemia predominates in childhood and young adult life. Chronic leukaemia is more typical of the middle and later decades, being exceedingly rare in childhood. Stem cell leukaemia is seen more often in children than adults, in common with acute lymphatic leukaemia; acute myelogenous leukaemia also occurs with frequency before puberty. Chronic myelogenous leukaemia has its highest incidence between the ages of thirty and sixty years, while chronic lymphatic leukaemia predominates beyond this point. Monocytic leukaemia is most commonly found in persons over thirty years of age. The sex incidence is about equal between the ages of ten and forty years. Before and after this period, the disease predominates in males. A recent survey of 647 cases carried out in Scotland over a fourteen-year period supports these limits with certain minor amendments. Two important points arising out of this survey are that there has been a twofold increase in the overall incidence over the period of the survey, not, however, as a result of increased facilities for diagnosis, and that, in particular, an increase of the lymphatic type. These points weaken the conclusions of Panton who carried out the last major survey of leukaemia in England in 1929.

The aetiology has already been mentioned as obscure and that, as yet, there has been no demonstrable familial linkage in human beings. A number of authentic cases of congenital leukaemia have been reported and, according to some investigators is probably commoner than has previously suggested, owing to the obvious difficulties of diagnosis in very young subjects and over subsequent autopsy. The reported cases of these have been mainly myelogeneous in type with characteristic clinical and haematological features. In particular, four cases reported by Bernhard et. al., lived from nineteen hours to several weeks and died of classical leukaemia. An interesting point that accrues from all recorded cases is that, in none of them, was the mother a leukaemic subject.

Other aetiological agents include—radiant energy, and this with strong evidence because the incidence of leukaemia in radiologists has been shown to be 8 to 10 times that of any non-radiologic group; intoxication with benzol, analine dyes and related compounds; trauma—regarded as exciting factor in the development of leukaemia by some authors. Endogenous substances extracted

from urine of patients with myelogenous and lymphatic leukaemia have been separated into carbinol (lymphatic) and non-carbinol (myelogeneous) fractions, each a stimulator of the respective cells when injected into guinea pigs; it has been postulated that imbalance of these substances may determine the occurrence of leukaemia.

Symptomatology of leukaemia is well known. The disease itself is insidious and the clinical symptoms exceedingly variable. The latter fall into two categories; 1 non-specific manifestations common to many disease processes, and 2. signs and symptoms directly related to the proliferation of neoplastic cells. Haemorrhagic phenomena occupy a position midway between the two and may confuse rather than clarify the diagnosis, as in the case of aleukaemic leukaemia. The syndromes themselves often confuse the diagnosis considerably and can present as a wide range of unrelated conditions, e.g., neuritis, arthritis, abdominal conditions —both acute and chronic, toxic goitre and haematological conditions other than leukaemia.

A point of diagnosis not generally used in this country but used to some extent in England and America is the estimation of the Basal Metabolic Rate. The figure is generally raised in leukaemia and usually about the range of +20 to +30 and occasionally even to +70 to +80. The cause of this is not definitely known, but it is considered to be due to the fact that the oxygen consumption of the patient is increased by the leukaemic cells in the body requiring their own metabolism. It is considered that the evaluation of the B.M.R. is often more accurate as an index of the patient's condition than either a total white cell count or a differential count and it has been found that a high B.M.R. figure which persists despite continued irradiation suggests a very poor prognosis.

Significant laboratory data includes—an increase in total blood volume; possible elevation of the C.S.F. pressure and increase in protein content; urine containing, possibly, albumin, casts and R.B.Cs. and, terminally, often a frank haematuria; Bence-Jones protein has been noted in a few cases; B.M.R. elevation has already been noted; uric acid and total nitrogen levels of blood are elevated and the nitrogen balance of the blood may be negative, especially in acute leukaemia; generally a decrease in total plasma proteins and sometimes an inversion of the albumin-globulin ratio, although hyperproteinemia has been reported. Total lipoids of the blood are generally increased, but cholesterol is usually normal or decreased; blood phosphorus is high in chronic leukaemia, increases with treatment and reverts to normal in remission; serum phosphatase is also increased over normal.

The haematological studies of leukaemia are well-known to you and these, I shall not elaborate, except in the matter of monocytic leukaemia. Now, in the matter of monocytic leukaemia,

Downey recognises two types:—1. The Schilling or histiomonocytic type which shows monocytes derived from the reticuloendothelial system, and 2 The Naegeli or myelomonocytic type with monocytes developing presumably from myeloblasts, making this latter type a variant of myelogeneous leukaemia. Although the mature monocytes are identical in both forms of the disease, their origin is thought to differ. In the Naegeli variety, the peripheral blood contains a predominance of monocytes in various stages of development in association with a number of myeloblasts and young myelocytes, whereas, in the Schilling type, monocytes, usually fairly mature, predominate and larger histiocytes may be found. In the latter variety, myeloblasts are absent from the peripheral blood, but an occasional myelocyte may be found.

But authority still holds widely divergent views about the actual existence of monocytic leukaemia. Forkner admits to its existence as a distinct disease; Willis refers to it as a variant of myeloid leukaemia exhibiting monocytoid myeloblasts; Naegeli speaks of it as a reactive monocytosis and White calls it merely a variant of lymphatic leukaemia. Cecil, in his 'Textbook of Medicine' says: "There is no agreement concerning the origin of the monocytes but there is strong evidence that they are an independent strain of cells. Undoubtedly, a leukaemia in acute, subacute, chronic and aleukaemic phases with predominance of monocytes does exist." This is the liberal view, the view held by conservative authorities and the one practised in the majority of routine laboratories today. It is with this view in mind that I pass on to the case of monocytic leukaemia in point.

The subject, a youth of sixteen years of age, was seen by an outside practitioner on 22nd April last year. The presenting symptoms were vague and comprised pallor, tiredness and a cough of a fortnight's duration resultant from an earlier cold. A blood count was done and recorded thus:—

R.B.Cs	2,150,000	W.B.Cs	123,400
Hb	7.5 gms (52%)		
C.I.	1.2	Polys	7% — 8,600
P.C.V.	19	Myelos	nil.
M.C.V.	95 cu. mic.	Lymphos	2% — 2,400
M.C.H.C.	39%	Monos	91% — 112,300
I.I.	Normal		
E.S.R.	75 Grade : 5	Monocytes almost exclusively adult forms.	
Platelets:	Moderately reduced.		
R.B.Cs showed moderate anisocytosis. No nucleated R.B.C.s seen.			

A bone marrow was performed on the patient two days later, as an outpatient. The marrow films confirmed the peripheral blood

findings and a diagnosis of monocytic leukaemia was made. Marrow findings, incidentally, showed an almost total replacement of the normal marrow cells by monocytes in various stages of development. There were 6% of polymorphs up to the myelocyte stage, but no myeloblasts in 500 cells counted.

The patient was admitted to hospital late on the day of the 28th April. Added symptoms at this stage included several small areas of bruising on the legs and a septic infection of the forearm resulting from a scratch four days previously. Further elicited history here told of a progressive weight loss over several weeks of about five pounds. His appetite was fair and he suffered no indigestion. Latterly his cough had become slightly painful. The examination produced nothing of particular significance except enlarged glands in the (L) axilla and the inguinal region. Both the spleen and liver were palpable but not obviously enlarged.

Treatment was instituted on the 2nd May—A.C.T.H. by intravenous drip, 10 mgms/24 hours, but on the 7th May patient developed severe tachycardia and tachypnoea with gallop rhythm, relapsed into coma and died in a few hours. An autopsy was performed and the findings were consistent with acute monocytic leukaemia, showing marked infiltration of monocytes throughout the body organs.

Unfortunately, the laboratory was not informed of the patient's admission and further tests could not be done in consequence.

Perhaps the most interesting point of this case is the brevity of the final acute stage. Despite the fact that the patient was presumably leukaemic before being seen by the outside practitioner, he worked and led a perfectly normal life until ten days before his death. Another point worthy of note is the patient's age. He is, by the conclusions of much authoritative opinion, something of an exception as regards age incidence. The obvious question arises here. Could he have been saved with earlier diagnosis and treatment? Just as obviously, there is no means of telling. In cases such as this the aim is to hold the patient with treatment in the hope of a spontaneous remission. These do occur. Literature records many such cases. But they are indefinite, unheralded and run to no set pattern. Treatment today is merely palliative. It is divided into two categories:—1. Measures designed to improve the general physical condition of the patient and protect him from infection, and, 2. Measures designed to destroy the leukaemic cells or inhibit their proliferation.

In the first category, transfusion of whole blood, the fresher the better, is the most important. The antiheparin substances, toluidin blue and protamine, are said to help occasionally in controlling haemorrhage. In acute leukaemia, the free use of antibiotics and chemotherapeutic agents is strongly recommended to

avoid ulcerative and gangrenous lesions of the mouth and throat. A high caloric intake should be prescribed, along with sufficient sedation to keep the patient comfortable.

In the second category, radiant energy in the form of X-rays and radioactive isotopes are the choice in the chronic forms of leukaemia to destroy and inhibit leukaemic cells; chemical agents for use in chronic leukaemia include Fowler's solution, urethane and nitrogen mustards. Regarding acute leukaemia, the folic acid antagonists such as aminopterin, amethopterin and aminoanfol have been used with varying results, none of them with any great degree of success. Latterly, A.C.T.H. has been used and as yet, the results have not been fully evaluated and correlated. One other more hopeful note was published in a recent journal where remissions in leukaemia were reported in leukaemic patients becoming infected with glandular fever during the course of their illness. Regarding this, however, there is as yet, scant evidence, but further research is in progress along these lines at the moment.

This then, is a brief picture of leukaemia—in so many ways, an anomalous and unsatisfactory picture. Much work remains to be done, much research by many qualified people. That work is being done, in laboratories throughout the world. It is only to be hoped that through the efforts of these people will come the information so vitally necessary for the final defeat of leukaemia—the disease as we know it today.

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SERUM PROTEIN FRACTIONS

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Estimation of serum proteins presents two distinct problems, partition and quantitative determination. There is hardly any constituent of serum which may be estimated in so many different ways, and there is still a growing literature on new methods and improvements. The standard method of the quantitative estimation in the past was the classical Kjeldahl, while lately there is a tendency to use simpler colorimetry, or to dispense with chemical reaction altogether, and use turbidimetry. Physical methods include calculation of protein concentrations from the refractive index, the specific gravity, or viscosity of the serum.

There are essentially two main procedures for fractionation of proteins. 1. Precipitation with (a) strong salt solutions at 37 C. or room temperature, (b) ethanol or methanol at low temperature; depending on different solubilities of the fractions.
2. Electrophoresis, depending on different mobilities of fractions in an electric field. The first salting out method was discovered a hundred years ago. Procedures 1 b, and 2 have been applied to serum proteins during the last 15 years.

Albumin

The routine procedure for separation of albumin and globulin is still that of Howe, either in its original form or as modified by Kingsley, using 21.5 percent sodium sulphate solution at 37 C., and the method of Campbell and Hanna, using sodium sulphite at room temperature. One of the striking results of early electrophoretic experiments was to show that both these methods are inaccurate, giving a too high albumin and a too low globulin value. Not only albumin, but most of the α globulin remain in solution. The error is even more marked if the albumin/globulin ratio is calculated. This is almost double of that arrived at with the "true albumin" methods.

Several salting out methods have been developed, giving an albumin value 10 to 20 percent lower than Howe's. The technique is essentially the same, except that different concentrations are used—26 percent sodium sulphate or 26.9 percent sodium sulphite. (Kibrick and Blonstein, 1948; Wolfson, et. al. 1948). The concentrations represent weight/volume relations after addition of the serum to the salt solution. The concentrations of the salt solutions are actually higher, 27-28 percent depending on the dilution used.

Different techniques are utilised in the methanol, and in the immunologic true albumin procedure.

Most textbooks do not describe these methods yet. It is argued that 1. for clinical purposes it is immaterial what method is used, as long as the normal range for this particular method is known, and 2. the normal range for the "true" methods is not sufficiently defined yet. On the other hand several arguments could be brought forward to support the introduction of these tests: 1. There is good agreement between values arrived at by electrophoresis and the true albumin methods. 2. Information on the normal range has been available for years. 3. The more abnormal the albumin concentration of the particular sample, the larger the error of Howe's method (especially if α globulin is increased at the same time). (Table I.) 4. No new technique is necessary. 5. One of the aims of laboratory work is to apply accurate procedures. The situation is somewhat similar to the true sugar problem. Clinically it is irrelevant, whether or not the glucose level is ten percent lower, as long as the normal range is defined for that particular method. Nevertheless, the so-called "true sugar" methods have been generally adopted.

TABLE I.

Total Protein	% Albumin		% γ globulin	% Error	A/G Ratio	
	Howe	true			Howe	true
7	4.6	3.8	0.8	17	1.9	1.2
4	2.0	1.0	1.0	50	1.0	0.4

The Howe and true A/G ratio in a normal and abnormal serum.

Globulins

It has been known for some time that sera with abnormal protein pattern may have a normal A/G ratio, and sera with widely differing protein pattern may have identical ratios. Some leading authorities have suggested that the concept of A/G ratio should be abandoned altogether as it may give misleading results. Better information may be gained by supplementing the A/G estimation with determination of globulin fractions. A number of simple flocculation and turbidity tests are in routine use, giving an approximate indication of globulin abnormalities, and are of practical value, especially in liver disease. They do not provide, however, a picture of the whole globulin pattern. The only flocculation test approximating this is the Weltmann, especially in its nephelometric modification (Wuhrmann and Wunderly, 1947).

The most comprehensive pattern is given by the solubility curve of Butler and Montgomery (1933), using thirty different concentrations of potassium phosphate. Neither this, nor the classical electrophoretic procedure is, however, suitable for routine use.

Applying the latter method as standard of reference, simple salting out methods have been developed.

Gamma globulin.—Of the globulin fractions gamma is of the greatest clinical significance. The simplest yet sufficiently accurate procedure is that of Huerga and Popper (1950) also referred to as ammonium sulphate or Popper's test. The technique is as follows:

Reagent: One hundred eighty nine grams of ammonium sulphate and 29.3 grams of sodium chloride are dissolved in distilled water and the volume is made to one litre. Test: 0.1 ml. of serum is transferred to a test tube or cuvette and 5 ml. of the reagent added. After allowing the mixture to stand for 30 minutes the tube is inverted twice without shaking, and the turbidity read in a spectrophotometer or photelometer, using a wavelength of 650 mu or a suitable red filter. The reagent serves as a blank. The results may be expressed in units, or in grams γ globulin per 100 ml.

Some confusion is caused by different calibration curves used by different authors. Popper and most American authors use the curve of Shank and Hoagland. The standard is prepared by diluting 3.0 ml. of a 0.0962 normal barium chloride solution to volume in a 100 ml. flask by adding O.2 N. sulphuric acid at 10 C. 0.3 ml. of 0.2 N. sulphuric acid added to 2.7 ml. of barium sulphate suspension represents 20 units. Units may be converted into grams.

$$\text{Gamma globulin grams per 100 ml.} = \frac{\text{units}}{5.8} + 0.02\ (20\text{-units})$$

The upper normal limit with this standard is 5.6 units or 1.25 grams per 100 ml. MacLagan, the British authority (1951, 1952) prefers the salicylsulphonic acid standard for all turbidity tests. His upper normal limit for Popper's test is two, and for the zinc sulphate test 4 units. American authors give the upper normal limit for the zinc sulphate test variously as 8, 12 and 16.

Alternate methods of γ globulin estimation are those using sodium sulphate (Kibrick and Blonstein, 1948), or the ammonium sulphate-sodium chloride solution, with subsequent quantitative estimation by the biuret method (Wolfson, et.al.1948). All these tests separate the γ globulin in one step, and are therefore less accurate than the low temperature-alcohol procedure. They are, however, less time-consuming, and sufficiently accurate for clinical purposes.

Other globulin fractions.—The scheme is as follows: 1. Total protein, and 2. albumin is determined as usual by Howe's method. In addition 3. true albumin and 4. γ globulin is determined. Alpha globulin is calculated by subtracting the true albumin figure from Howe's albumin. Beta globulin equals total protein minus Howe's

albumin minus gamma globulin. The whole estimation does not take up much more time than the usual A/G method.

Simplification of the classical electrophoretic method by the ingenious idea of replacing the Tiselius cell with a strip of filter paper provides a new, relatively simple alternative. It is a matter of opinion which method is preferable for routine use. The salting out procedure is quicker, less expensive, and does not require the learning of a new technique. Filter paper electrophoresis requires several steps with special apparatus. The partition takes at least six hours. Further procedures include dying of strips, eluting the dye and measuring the density, or in a simplified version direct densitometry of the strips, and plotting the curve. To obtain absolute values a total protein estimation has to be performed in addition by a chemical method. On the other hand it gives more subfractions, and can indicate presence of abnormal globulins with mobilities differing from those of the main fractions. This is not so obvious with the salting out method. In diseases such as multiple myeloma it seems superior to salting out partition (Griffiths and Brews, 1953).

CLINICAL APPLICATION

The empirical liver function tests are mainly an expression of γ globulin elevation, but other fractions may have some effect, either by contributing to a positive result, or preventing one (colloid-protective effect). Thus while they are of clinical value, they do not give such a clear picture of the protein pattern as the fractional procedures and are less often positive. It has been suggested that quantitative γ globulin estimation should be used as an additional liver function test and in other conditions where it may be elevated. It has also been proposed that the A/G ratio should be replaced by the Albumin/gamma globulin ratio.

Parallel thymol turbidity, zinc sulphate and Popper's tests carried out on 60 cases where liver involvement was suspected, in this laboratory, gave 24 positive Popper's, 20 zinc sulphate, and 17 tymol turbidity tests, with two borderline values each. It is noteworthy that in infectious hepatitis, which constituted the majority of positive cases, the three tests show close parallelism. Of the few cases where Popper's test was more sensitive, one was a convalescing infectious hepatitis, the other three suspected carcinoma or cirrhosis. This is in agreement with reported literature, that the γ globulin estimation is slightly more sensitive than the empirical tests in cirrhosis, especially without jaundice. It also has the tendency in some cases to appear earlier and become negative later than the empirical tests (Spellberg, et.al.1950, Popper, et.al.1950). MacLagan (1952) found the Popper test more often positive in multiple myeloma than the empirical tests.

It is still to be decided to what extent the whole protein pattern should be utilised as a routine procedure in other diseases where pattern shifts are known to occur. Among other conditions T.B. and rheumatoid arthritis are of special interest. The routine follow up test in both is the ESR. As this is mainly a test for plasma abnormalities, and it cannot differentiate between the rise due to fibrinogen, and globulin fractions, many clinics use one or more additional tests to indicate the extent of serum protein abnormality. Some Swiss clinics for example use the Weltmann and other empirical tests routinely, and consider them valuable both prognostically, and as a measure of activity in T.B. (Wuhrmann and Wunderly, 1947).

It has been suggested that as in liver disease, the fractional protein pattern gives more quantitative and reliable information than the empirical tests. This has been shown first by the classical electrophoretic experiments, however application of this knowledge was not practicable, until simpler procedures were evolved. In rheumatoid arthritis an early change is rise of α globulin, followed later by γ globulin. This is not so obvious, if only the ESR, or the ESR and empirical tests are performed.

Marrack and Hoch (1949) and MacLagan (1951 point out that the empirical tests may eventually be replaced by more quantitative estimations.

SUMMARY

Techniques for "true" albumin, and the globulin fractions are described. The clinical significance of these procedures both in hepatic and non-hepatic conditions is discussed.

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The author is grateful to Dr. F. H. Sims for very valuable criticism of the paper.

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THE GROWTH OF POLIOMYELITIS VIRUSES IN TISSUE CULTURE

A. M. Murphy

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A paper given at the 8th Science Congress held in Auckland from May 17th to 21st, 1954.

With the advent of antibiotics and their consequent reduction of epidemics resulting from bacteria, research into infectious disease has tended, during the last decade, to concentrate on the study of viruses. A considerable amount of knowledge has accumulated during this time, regarding the epidemiology of virus diseases, but less progress has been made regarding their method of multiplication and their method of cellular destruction. There does not seem to be any sign at the moment of an antibiotic capable of affecting virus multiplication in the human body. A fundamental characteristic of viruses, in contrast to bacteria, is that they require living tissue for multiplication and that they in some way interfere with fundamental cellular metabolic processes. Burnet, in Australia, with his studies on influenza, has perhaps contributed most along these lines. He has shown that the influenza virus can attach itself to muco-protein like substances on the surface of the epithelial cells, of the mucous membrane, and is then absorbed into the cell where it appears to break up into smaller non infective units. These units then grow at the expense of the host cell, into fully infective virus, and with the breakdown and rupture of the cell, are liberated to infect surrounding cells. This process, probably can be applied, with slight modifications, to all animal viruses.

Probably more is known about the life history of influenza virus than any other because of the early discovery that it would grow in the developing fertile hen's egg. This problem of finding a suitable medium in which to study viral processes, is one of the greatest problems confronting the virologist. Animals of course can be used for the multiplication of most known human viruses but they have many disadvantages in housing and feeding, and of course they have natural protective mechanisms against disease which renders them resistant to small doses. In short it may be said that virology is at the stage bacteriology had reached at the turn of the century—before the advent of good culture media. It was therefore of considerable interest when Enders[1] and his co-workers in Boston made the discovery that the virus of poliomyelitis would multiply in fibroblasts grown from human or simian tissue of non nervous origin. This was a somewhat surprising discovery and upset many of the current theories regarding poliomyelitis, and it is chiefly about this advance and its consequent impact on polio research that I wish to speak.

Previously monkeys were the only susceptible laboratory animal, and this retarded research considerably. One strain of polio (Lansing), had been adapted to mice, but the other two serological types remained non pathogenic to mice. This technical advance allows much simpler laboratory manipulation and promises to contribute greatly to our knowledge of the spread of epidemics, diagnosis, serological surveys and also provides a basis for the manufacture of a prophylatic vaccine. A great deal of literature has accumulated in the last few years on this subject, and a certain amount has appeared in the lay press so that most people have some knowledge of what is involved. However, it is my intention to describe, perhaps a little more fully, some of the technical details and the possible uses to which this technique can be put.

The technique of tissue culture which allows direct observation of the tissues for the so-called cytopathic effect has become almost universal and has replaced the earlier suspended cell culture.

Four to six pieces of tissue about 1 mm. in diameter are placed in a test tube and held in place by clotting plasma around them. 0.5c.c.1c.c. of a nutrient fluid is then added. The test tube is then placed in an incubator. In 3-7 days depending on the type of tissue there is an outgrowth of fibroblasts.

Nutrient Fluid

A considerable amount of work has been carried out on the composition of the nutrient fluid. Little is known about the actual growth factors required by proliferating fibroblasts, so that methods are of trial and error nature. The original medium as used by Enders consisted of Hanks's balanced salt solution, horse serum, chick embryo extract and serum ultrafiltrate. The latter is simply ox serum which has been filtered through a fine collodion membrane, thereby removing the protein and leaving amino acids and soluble salts, etc.

Lately this medium has been altered somewhat in the interests of simplicity and the balanced salt solution and ultrafiltrate have been replaced by bovine amniotic fluid.

This is obtainable from the abattoirs. The gravid uterus is obtained and the amniotic fluid removed under aseptic conditions and is stored at low temperature. This method saves a considerable amount of time in making up salt solutions.

Type of Tissue

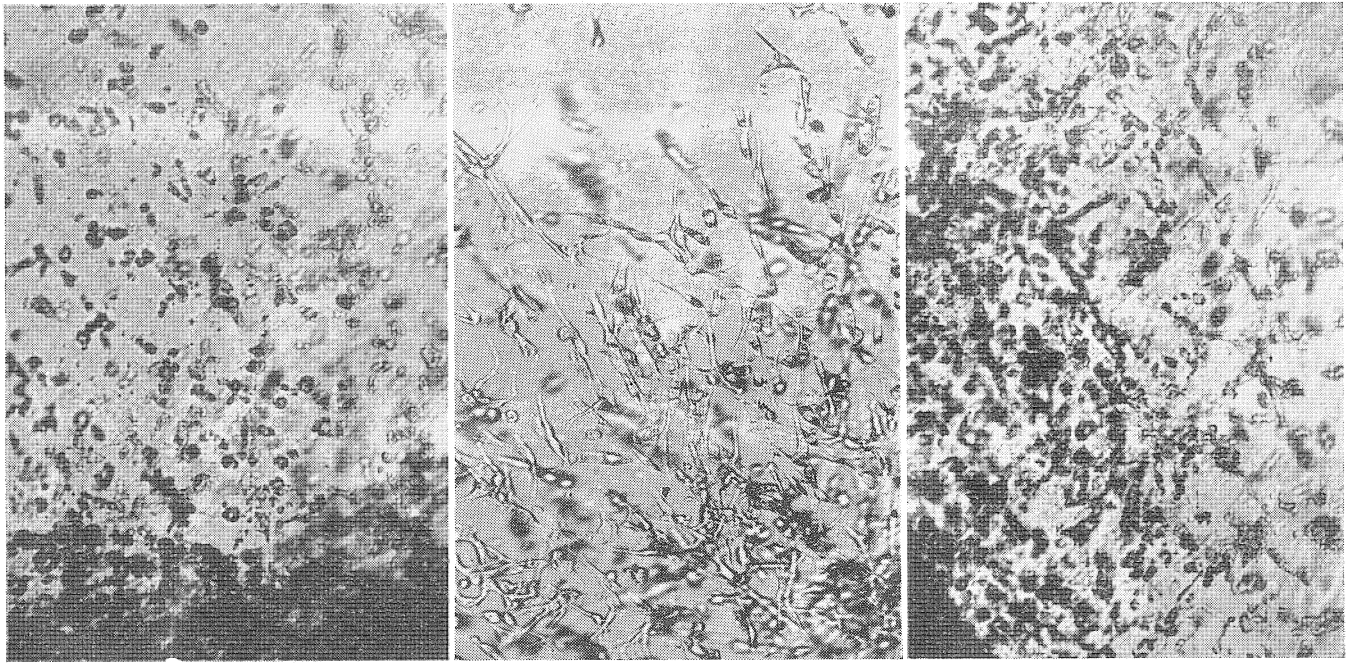
Enders originally used human embryonic tissue. This is relatively difficult to obtain and certainly cannot be obtained at the constant intervals demanded by a planned research programme. Most of the large American laboratories have changed to using monkey kidney or testicular tissue. We of course have no easy access to monkeys, so that up to now we have been largely experimenting with different types of human tissue which are removed at operation. The tissue to which we have devoted most of our

time has been tonsillar tissue. There are of course several serious objections to the use of this tissue, firstly it is an infected tissue and bacteria must be kept under control with penicillin and streptomycin. Secondly this tissue does not proliferate at nearly such a rapid rate as embryonic tissue. However, the constant supply of tissue to a certain extent offsets these difficulties. We have been unable to grow fibroblasts from tonsil tissue without the presence of human serum in the medium. This also has serious difficulties associated with it as this human serum must be replaced with a different type of medium without human serum at the time of inoculation, as antibodies which may neutralise the virus are quite likely to be present. Associated with this change of medium are non specific changes in the fibroblasts and this up to the present time has been one of our chief problems.

Recently, however, we have experimented with the subculturing of the tissue cultures of embryonic tissue. Very little work along these lines seems to have been attempted overseas except of course by the tissue culturists. It does not seem to have been considered by the virologists as a practical proposition. There are only two references in the literature, one from workers in the Pasteur Institute, who succeeded in keeping growing for three months a strain of fibroblasts of embryonic origin, and Sylverton and Scherer after three years' work have established a strain of epithelial cell, derived from a carcinoma which will support the growth of poliomyelitis virus. The piece of tissue with surrounding growth of fibroblasts is gently scraped from the wall of the test tube, placed on a sterile petrie dish and cut into two or three pieces and then placed in further test tubes with clotted plasma. In this way three cultures can be produced from one original, two of these being used for experimental purposes and the other to keep the strain going. In this way the laboratory is completely independent and does not have to rely on a source of supply from outside.

After incubation time of 5-7 days, when the outgrowth of fibroplasts is sufficient, the polio virus can be introduced. Multiplication of the virus results in degenerative changes taking place in these fibroblasts and finally complete destruction and disintegration. This occurs in 2 to 5 days depending on the amount of virus in the inoculum.

It would seem that this method will become the standard technique for isolation of the virus for diagnostic purposes. The virus appears in the faeces prior to the so-called "minor illness" and some 8-10 days before the onset of paralysis. Evidence is accumulating to show that it is present in the blood stream 4-5 days prior to paralysis. At the present state of our knowledge it is not definitely known what other viruses cause this cytopathic effect in human fibroblasts, so that any isolation must be proven by the use of type specific immune serum. That is to say, that, once an isolation has been made, inhibition of the cytopathic effect must



Demonstration of the destruction of human fibroblasts grown in tissue culture by Type II. (Lansing) poliomyelitis virus, and protection by homologous antiserum.

Fig. I., Type II. virus plus type I. antiserum; Fig. 2, Type II. virus plus type II. antiserum; Fig. 3, Type II. virus plus type III. antiserum.

These are cultures photographed in the test tube x100.

be demonstrated by the addition of a serum known to contain poliomyelitis neutralising antibodies. Furthermore this neutralisation is type specific and provides a method of actually typing the strain. Type II. and III. antisera will not inhibit the effect of type I. virus.

Again this neutralisation phenomenon can be reversed and used to test and titrate antibodies present in convalescent sera. The three known serological virus types can be mixed with an unknown serum and introduced into different tissue cultures. A serum containing say type III. antibodies will prevent the destruction of fibroblasts by type III. virus, but will have no effect on virus types I. and II. This method can therefore be used for the typing of epidemics by examination of convalescent sera and also for serological surveys of the population at large.

The fact that the viruses of poliomyelitis would multiply in fibroblasts was of considerable interest when first demonstrated as it showed that, contrary to the then accepted theory, multiplication would take place in tissue other than nervous tissue. This work has strengthened the current theories regarding the pathogenesis of polio. Bodian ([2]) has put forward the hypothesis that viraemia plays an important part in the progress of infection. He envisages three separate sites of multiplication occurring in the following sequence.

Firstly multiplication in the oropharynx and in the lower alimentary tract, followed by a spilling over of virus into the blood stream. This initiates the second or vascular phase, with multiplication probably occurring in the regional lymph nodes and spleen. The relatively high titres obtained from blood indicate multiplication in the reticulo-endothelial system rather than just leakage from the alimentary phase. The third phase is the neutral phase with multiplication in the central nervous system. The initiation of this phase either, from the blood stream direct, or along nerve pathways, appears to be equally possible. This remains one of the big unanswered questions at the moment.

These considerations lead to the hypothesis that poliomyelitis infections may be divided into 5 categories dependent on the presence of antibody in the blood stream and the rapidity of antibody response in the non-immune.

1. Silent infections in which only the alimentary phase takes place. The patient remains completely asymptomatic.
2. Abortive infections in which phases 1 and 2 occur but not stage 3. In other words the C.N.S. stage is prevented by antibody either present or rapidly manufactured in response to the two previous phases.
3. Non paralytic infections characterised by all three phases of multiplication but with C.N.S. involvement below the level of clinical recognition of paralysis.

4. Paralytic infections in which sufficient damage to the C.N.S. to cause clinical paralysis.
5. Paralytic infection resulting from accidental exposure of nerve endings as in tonsillectomy, and accidental virus injections. This is an abnormal infection and is still in the speculative category.

All these categories have been produced experimentally in chimpanzees and at the same time it has been shown that a comparativly low level of antibody in the blood stream is sufficient to prevent progress beyond the vascular phase.

Hammon([3]) and his co-workers have carried out large scale inoculations of gamma globulin (passive immunisation). Some 55,000 children were inoculated and it was shown that protection was obtained although it was short lived. The gamma globulin was excreted fairly rapidly and fell below effective protective level in 4-6 weeks. This passive immunisation is therefore not a practical proposition except in special cases. It would be impossible to manufacture sufficient gamma globulin to protect whole communities. The next step, is to induce active immunisation with a killed vaccine. Salk is attempting this at the present time. By using essentially the same tissue culture technique as described, the three serological types of virus are being grown separately. The supernatant fluids are collected and incorporated in a vaccine. Inactivation of the virus is carried out by the addition of formalin. Whether this vaccine will be a success remains to be seen. It may be that a killed vaccine will not produce sufficient antigenic stimulus for antibody production to reach a high enough level.

The next step would be the production of a living vaccine. This of course would be a rather difficult undertaking and would require several years of work. It is not, however, beyond the bounds of possibility. It would require the production of strains of polio which had lost much of their pathogenicity. Viruses are such labile organisms that such strains could undoubtedly be produced. Cox ([4]) has recently adapted strains to the chick embryo with resulting loss of pathogenicity. Also as the result of variants arising in tissue culture, both types I. and III. have been adapted to laboratory mice.

Finally this method of tissue culture can be used to study the more academic question of host parasite relationships and we can expect some answers to the questions of the method of entry of the virus into a susceptible cell and its method of multiplication once inside the cell.

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(2)Bodian, D., 1952. Amer. J. Hyg. *55*, 414.
(3)Hammon, W. McD., Conell, L. L., Wehrle, P. F., and Stokes, J. Jr., 1953, J.A.M.A. *151*, 1272.

THE BANISHED BACTERUM

We reprint here, for the benefit of those interested in bacteriological nomenclature, part of an article which appeared in recent edition of the Lancet (Oct. 17, 1953).

At meetings in Rome last month the International Committee on Bacteriological Nomenclature rejected the generic name *Bacterium* and the family name *Bacteriaceœ*. Thus these names are no longer available to systematists; but for a time at least *Bacterium* will almost certainly continue to appear on report forms.

Bacterium had had a long and checkered career. It was first used in 1828 by Ehrenberg for an organism, *Bacterium triloculare*, that cannot now be identified. It was once applied to spore-forming as well as to non-sporing rods; Lehmann and Neumann used it for most gram-negative rod-shaped bacteria, while in the latest edition of *Bergey's Manual*[1] it was the generic name for the dump-heap of unclassified rod-shaped bacteria, both gram-positive and gram-negative. In this country *Bacterium* was treated with rather more respect; medical bacteriologists used it for the lactose-fermenting coliform organisms which biochemists, knowing little of bacteriological taxonomy and nomenclature, called by the generic names used in *Bergey's Manual—Escherichia* and *Aerobacter. Some, including Topley and Wilson,*[2] used *Bacterium* for gram-negative rods that were rather like coliforms but, because of significant difference, had to be excluded from that group—for example, *Bacterium anitratum* and *Bacterium alcaligenes.*

The problems facing the systematist—and those will eventually affect all bacteriologists and even sanitarians and clinicians—concern the renaming of many well-known species, including *Bacterium coli* and *Bacterium maerogenes.* By the rules of bacterial nomenclature the oldest name has priority; but a number of requirements have to be satisfied and it is on the interpretation of these lesser considerations that doubt and debate may centre. Classification must precede naming; and the systematists, robbed of a convenient label for bacteria of uncertain taxonomic position, must now do some heart-searching and decide into what genera, existing or new, these organisms best fit. *Bacterium aerogenes*, an organism found in water, soil, faeces and urine, exemplifies the systematist's difficulties. Culturally it closely resembles Friedlander's bacillus. Some bacteriologists say they cannot distinguish the two; others separate them by origin (above or below the umbilicus); a few attempt to distinguish them by differences in, for example, the sliminess of colonies or the ability to ferment certain sugars, produce gas in fermentation, or produce the enzyme urease. Even those who recognise these organisms as separate species generally admit that they fall into the same genus. The next problem is their relation to other coliforms; should *Bacterium coli* be grouped within the same genus? This question is important, because on the answer depends the new generic name of *Bacterium coli.* Friedlander's bacillus and *Bacterium aerogenes* resemble *Bacterium coli* in being gram-negative rod-shaped

bacteria that ferment lactose; but they differ from *Bacterium coli* in such characters as motility, encapsulation, urease-production, and the end-products of glucose fermentation. If these differences were always clear-cut it would be easy to define two genera, but there are so many intergrading forms that generic differentiation is difficult. In general, British bacteriologists have grouped these species together in the genus *Bacterium*, whereas workers in other countries have split them among two or three genera. Serologists and biochemists can each separate these coliforms into two large groups, and the groups so formed by these workers of different outlook are surprisingly similar. A grouping arrived at by two distinct approaches has more to commend it than one achieved from a single angle; and it is likely that, for a time at least, this division into two groups will prevail.

Family names of bacteria are not often used; but one, *Enterobacteriaceœ*, has become popular as a convenient name for the coliform group of bacteria. Plant pathologists object to it because the bacteria pathogenic to plants and related to the coliforms have not been found in the intestinal canal of animals. As a family name it does not accord with the rules and recommendations of the bacteriological code, which says that the stem of a family name should be that of a contained genus —there is no genus *Enterobacterium*. By the rules the family name should be *Klebsiaceœ* or possibly *Escherichiaceœ;* so perhaps it would be best to accept *Enterobacteriaceœ* as an exception to the rules.

The newer names and combinations will seem strange to readers in this country; but however much we may regret the passing of the familiar name *Bacterium coli*, the use of the generic name *Bacterium* had, in other combinations, become an anachronism. Surely it is better to make a readjustment now than to continue to use *Bacterium* and be out of step with the rest of the world.

1 Bergey's Manual of Determinative Bacteriology. Edited by R. S. Breed, E. G. D. Murray, and A. Parker, Hitchins, Philadelphia and London, 1948

2 Topley and Wolson's Principles of Bacteriology and Immunity. Revised by G. S. Wilson and A. A. Miles. London, 1946

INTERMEDIATE EXAMINATION RESULTS

We congratulate the successful candidates in this examination.

They were:—

G. L. Cameron
Shirley A. Cook
D. W. Fitzgerald
Rosemary A. Graham
Carmel A. Hall
Verona A. Hamilton
M. G. Harper
Beverley D. Jackson
R. H. Kennedy
D. M. Liardet
Lucy Mackay
R. Mackenzie
B. McLean
J. C. Mann
Janet M. Munro
Valerie M. Toms
Jocelyn F. Tong

INTERMEDIATE EXAMINATION

Examiners: Dr. Burns, Mr. Reynolds.

WRITTEN PAPER—9.30 a.m. - 12.30 p.m.

1. You are asked to select a bottle of stored blood for transfusion to an adult male patient
   (a) in 24 hours time; (b) in 30 minutes time.
   Detail in each case the technical methods you would employ to prevent the possibility of transfusion of incompatible blood.
2. Describe the methods and principles underlying the laboratory sterilisation of:
   (a) Cotton wool plugged tubes of nutrient broth.
   (b) Screw-topped 1oz. bottles for specimen collection.
   (c) Contaminated winchester bottles.
   (d) Serum for preparation of Loeffler's medium.
   (e) Bark corked bottles for specimen collection.
3. What is the principle of Benedict's qualitative test for glucose in urine? what other substances reduce Benedict's solution besides glucose?
4. Given a 24-hour specimen of urine, describe fully the method you would adopt to demonstrate and confirm the presence of M. Tuberculosis.

PRACTICAL PAPER—2 p.m. - 5 p.m.

This consisted of 24 "spot" tests. Six minutes were allowed to report on each one.

1. Two bottles of blood typing antiserum were provided. One was labelled B serum and the other anti A. Write notes on the use of these for all A B O groupings.
2. Write notes on piece of apparatus provided (Westergrens blood sedimentation rack).
3. Construct a Wright's capsule.
4. Write notes on apparatus (thermostat capsule, mercury "make and break" thermostat).
5. Use of a method of testing piece of apparatus (Berkfeld filter).
6. Write notes on the reagent provided and read the test (Esbach's reagent).
7. Report on the three blood films.
8. Early red cells, infectious mononucleosis.
9. Lymphatic leukaemia.
10. Write notes on apparatus (maximum temp. thermometer).
11. Two bottles of 5% glucose for intravenous use were provided (one bottle was caramelised).
12. Write notes on apparatus (a desiccator).
13. Write notes on apparatus (special counting chamber for counting cells in C. S. F.'s etc.).
14. Write notes on apparatus (dark ground condenser and funnel stop).
15. Organism on agar plates and gram stain and a methylene blue stain were provided.
17. Organisms were B. subtilis, corynebacterium, B. prodigiosus, Enterococcus.
19. A bottle of trypsin broth plus penicillinase and P. amino benzoic acid was provided. Note to be written on use of constituents.
20. Use of and method of sterilising bottle of solution provided (anticoagulant for blood bank).

21-24. Four organisms were to be identified from sugar reactions, indole, motility, etc. They included Salmonella, Coliform and dysentery organisms.

Orals included antibiotics, microscopes, pipettes, refractive index, numerical aperture, sedimentation rate, solutions, definition of normal solution, postal regulations, first aid in the laboratory and various questions associated with the examination itself. Paul Bunnell. Stills.

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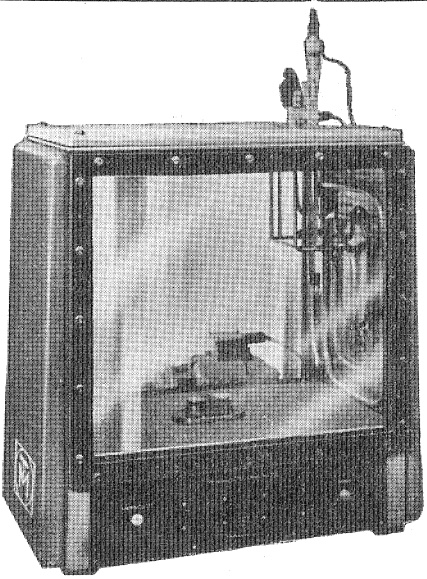

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