THE CHEMODIAGNOSIS OF MALIGNANCY

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In the present stage of cancer therapeutics the time elapsing between the onset of malignancy and the institution of treatment is a decisive factor influencing the success or failure of the latter. To be able to diagnose cancer unfailingly at an early stage, preferably in the precancerous phase, would be of immense help in combating the disease.

It is natural that the resources of biochemistry should be enlisted in the endeavour to perfect a diagnostic aid based upon the detection of specific chemical changes in the blood or tissues of the patient. The multiplicity of the methods investigated suggests that none has the required efficiency. Most of them have been relegated to obscurity, but during the past few years much prominence has been given to several.

The present review comprises: (1) a short summary of methods advocated by various workers for serodiagnosis; (2) some general observations and considerations which the writer, as a result of his researches, believes to be pertinent to the subject of cancer serum tests; (3) an account of the more recent serodiagnostic studies carried out at the Birmingham Centre. The conflicting results claimed by other investigators will not be discussed and summaries will be confined to the findings of the writer and his collaborators.

Varieties of Proposed Diagnostic Reactions

Abstracts of reports on cancer tests have been given in the AMERICAN JOURNAL OF CANCER during the past nine years. More extensive descriptions and reviews have been published by Boyland (3), Lavedan (14), Bing and Marangos (2), and Rondoni (18).

The various biochemical diagnostic reactions brought forward fall into several groups.

Group 1

Group 1 includes tests involving analyses for specific chemical constituents of blood, e.g., fibrin, calcium, magnesium, cystine, lipins, glutathione. To these may be added tests involving precipitation of protein or other blood components by physical and chemical agents and depending largely on variations of buffer capacity and relative amounts of albumin, globulin, etc. The following are examples:

Link's Test: Estimations are made of the haemoglobin content of the blood, and the potassium and magnesium content of three successive portions of serum which extrude from the clot; the values so derived are combined in an arbitrary formula to provide the diagnostic index.
**Dannmeyer’s Test:** In this test the blood fats and lipids are examined with regard to quantity, acid value, and saponification value. The number of cubic centimeters of alkali used in titrating for the acid value, plus the number of cubic centimeters used in determining the saponification value, divided by the quantity of fat used for the analysis, gives a figure which is termed the “cancer number.” In healthy persons this is usually less than 100, in cancer it may rise to 140.

**Kahn’s Albumin “A” Test:** In this reaction 0.07 of blood is absorbed into shreds of filter paper and dried for fifteen minutes in a desiccator containing phosphorus pentoxide. The material is then extracted at 27° C. with 7 c.c. of 37.2 per cent ammonium sulphate. After removal of the paper the clear fluid is boiled on a water bath and then cooled; the amount of cloud in the solution is computed by using an opalimeter. A negative reaction is indicated by a definite clouding of the liquid. It is suggested that the test measures the hydrophile portion of the blood albumin, which Kahn believed to be much reduced in malignancy.

**Verne’s Test:** If normal human serum is distributed into a number of tubes and a suitable protein-precipitating reagent is added in increasing proportions, the resulting turbidity does not increase in a regular manner with the quantity of reagent but presents fluctuations of heightened and diminished intensity. If the degree of turbidity be measured by a photometer, a curve may be charted which will follow an undulating course, with possibly several maxima. The wave form of such a curve will exhibit marked changes from the normal if a pathological serum is under test. For examining blood in tuberculous cases resorcinol was found to be the most delicate reagent, while for cancer tests a fairly concentrated copper acetate proved most suitable.

**Wigand’s Test:** A series of tubes is set up, each containing 2 c.c. of serum in progressive dilutions; 1 c.c. of a 1 per cent solution of tannic acid and three drops of carbol fuchsin are added. Precipitation is observed in certain dilutions in cancer sera while normal sera remain clear after a lapse of twenty-four hours.

**Brossa’s Test:** This test is based on the instability of blood colloids and the formation of a flocculum when whole blood, laked in distilled water containing congo-red, is placed in contact with an electrolyte together with a 2 per cent solution of quinine hydrochloride. Cancer blood becomes turbid within forty-eight hours.

**Bendien’s Test:** Serum is distributed in tubes and flocculated with certain concentrations of sodium vanadate in acetic acid. Diagnosis is made from a comparison of the degree of precipitation and from spectroscopic examination of the precipitate.

**Cronin Lowe’s Three-phase Test:** The Bendien test is extended by repeating the reaction with serum heated to 56° C. and with a further sample extracted with ether, thus giving three series. A ratio representing the amount of change in the flocculation due to these two treatments enables the diagnosis to be made, the ratio C–A/A–B being used, where A, B and C are the values of protein precipitates in the three series at a selected point.

**Bruer’s Test:** This test involves the direct determination of the albumin-globulin ratio. Serum is diluted with saline and magnesium chloride and
heated on a water-bath for fifteen minutes. Those tubes which have a high globulin content (30 per cent or more of the total protein) show a precipitation, while normal sera show no precipitate when the dilutions exceed 1 in 4.

Botelho's Test: When acidified with citric acid cancer serum yields precipitates with a lower concentration of iodine in potassium iodide reagent than do normal sera. This, obviously, is to be correlated with the quantity of protein present. In order to avoid certain false reactions, Botelho analysed the sera for protein by determining the refractive index and converted them so as to have a normal content by diluting or concentrating as necessary.

As a readily available standard for observing the degree of opalescence, the reaction was termed positive if the degree of precipitation was such as to permit the filament of an electric bulb to be distinguished clearly when viewed through the reaction tubes. Itchikawa examined the content of albumin and globulin as part of the test and considered that a Botelho positive in the presence of a high albumin value is certainly correct. If the reaction is negative in the presence of high albumin this should be reduced to normal and the reaction rechecked. If the reaction is positive in the presence of a high protein content, the globulin value should be reduced to normal and the test repeated.

Group 2

Group 2 is made up of physico-chemical determinations directly or indirectly measuring surface tension, pH value of serum, sedimentation rate of corpuscles, electric resistances of cells, etc.

Kopazewski's Test: Two c.c. serum are mixed with 0.2 c.c. of pure lactic acid and the time required for gelatinisation so that the tube can be inverted without escape of the mixture is observed. The "neoplastic index" is the time required for normal sera divided by that for the test serum. Non-cancer sera were usually gelatinised in eight to twenty-four hours and cancer sera in less than eight hours.

Waltman's Test: Dilutions of calcium chloride ranging from 1.0 per cent to 0.1 per cent are added to serum and the position of the coagulation zone noted. In cancer there is a "shift to the left."

Roffo's Test: For Roffo's test 0.26 c.c. of a 1:10,000 solution of Grübler's neutral red is added to 1 c.c. of serum removed from blood which has been collected under liquid paraffin. A positive reaction is indicated by a red tint, negative by a yellow tint.

Meistagmin Test: Ascoli and Izar proposed to diagnose malignancy by measurements of the lowering of the surface tension of serum when incubated with a suitable antigen produced from tumor material. The determinations could be carried out with the dropping pipette or stalagmometer. Izar more recently described a modified technique, employing the so called meistagmin precipitant reagent. This consists of 1 part of ricinoleic acid diluted to 10 per cent in methyl alcohol, 7 parts of normal saline, and 2 parts of distilled water. Of this reagent 1 c.c. is added to 2 c.c. of fresh, unhaemolysed serum and incubated at 37° C. for twenty-four hours. At the end of this period non-cancer serum presents only a slight opalescence while malignant serum shows a clear fluid containing suspended floccules.
Group 3

Group 3 may be divided into two subgroups. Group 3a is made up of reactions of the antigen-antibody type. These tests utilise extracts of tumours, embryonic tissues, etc., and involve clumping of cells, flocculation of extract constituents, cell cytolysis, cutaneous reactions. With these are closely associated reactions dependent on enzyme changes, such as lipolysis, proteolytic reactions, phosphatase estimations, etc., forming Group 3b.

Examples of Group 3a are as follows:

*Abderhalden's Reaction*: The theory of this test postulates that the introduction into the blood stream of abnormal elements in a molecular condition unsuitable for assimilation by the body cells, or elimination by the kidneys, provokes the appearance of digestive ferments which hydrolyse the undesirable protein to albuminoses, peptides, or amino-acids. Applied at first to pregnancy diagnosis, the test was later adopted for detection of cancer.

The serum under examination is incubated for twenty-four hours at 37° C. with the coagulated protein derived from placental or tumor tissue and the polypeptides produced by the interaction are detected after dialysis of the fluid by means of the ninhydrin reagent, or, alternatively, the reaction between serum and peptides extracted from tumour tissue is followed by polaroscopic analysis.

*Hirsch's Test*: In this modification of the preceding test the serum is incubated at 37° C. with 5 mg. of the tumor antigen. The fluid is centrifuged and placed in one cell of an interferometer. A portion of the untreated serum is placed in the companion cell and the change in composition is estimated in interferometer refractive index units. A reading of 10–30 units constitutes a definite "positive."

*Lehmann-Facius Test*: This reaction postulates that serum albumin is fixed by an antibody present in guinea-pig serum and forms an alcohol-soluble decomposition product which can be detected by the ninhydrin reagent. The component in normal human serum is inactivated by heating to 63° C., but the component of cancer serum remains and can be detected after heat treatment.

*Sivori's Test*: This is a further variation of the Abderhalden test. Tumour material is digested with pepsin until the albuminose reaction disappears and traces of amino-acids appear. The resulting solution is diluted until the ninhydrin reaction is no longer given. A positive reaction is indicated if, on standing for twenty-four hours with the antigen solution, a portion of serum gives a blue colour with the ninhydrin reagent.

*Hirszfeld's Test*: This is a test for complement fixation (or deviation) on the principle of the Wassermann reaction, employing a cholesterinised extract of malignant tissue. The difference in reaction between normal and malignant sera is partly dependent on the increased content of easily precipitable globulin in the latter.

*Freund-Kaminer Cytolysis Test*: This test is based on the belief that the blood of non-cancerous persons contains an agent able to destroy the cancer cell, unlike the previous tests, which suppose that cancer sera contain special ferments capable of decomposing cancer proteins. The present test is dependent on the fact that the normal anti-cancer ferment of healthy serum is
reduced in the cancerous state. The reaction is carried out by incubating at 40° C. an emulsion of cancer cells with the test serum. After twenty-four hours the number of intact cells is observed microscopically and if this has been reduced to 50 per cent of the original number the presence of cancer is inferred.

**Freund-Kaminer Skin Reaction:** The agent responsible for cell cytolysis is said to be specially abundant in the young thymus and in the intestine. Chemically it is a fatty acid and its injection into the skin of a cancer patient results in the production of a definite nodule.

**Gruskin's Skin Test:** This test assumes that a foreign protein of embryonic character is present in neoplastic diseases. When 0.2 c.c. of an antigen derived from embryonic tissue is injected into a cancer patient an allergic reaction shows the response of the fixed cells to the embryonic protein. An inflammatory zone is visible around the injection site within fifteen minutes.

**Moppett's Test:** The antibodies present in cancer sera prevent the normal development of outgrowing cells when a tissue culture of an animal tumour is implanted in such serum. The cell development is compared in a special apparatus with that of a similar culture growing in normal serum.

**Citelli-Piazzi Reaction:** A drop of 500 or more in the leucocyte count of the blood occurring twenty to thirty minutes following a subcutaneous injection into the patient of 1 c.c. of malignant tumor extract is said to be diagnostic of cancer.

**Aron's Urine Test:** An extract of urine from cancer patients, when injected into rabbits, is said to cause specific and characteristic changes in the lipoid zones of the adrenals.

The following are examples of Group IIIb:

**Waldschmidt-Leitz Test:** The serum of cancer patients is said to activate the enzyme papain less vigorously than normal serum.

**Shaw-Mackenzie's Test:** Serum of cancer patients augments the lipolytic activity of pancreatic extracts towards olive oil emulsion in a less degree than does normal serum.

**Fuchs Test:** Proteolysis ensues when cancer serum is incubated with normal blood fibrin or coagulated serum protein. The degree of proteolysis resulting from the interaction of normal serum and normal substrates is much less. Similarly normal serum and cancer protein interact, liberating peptones and amino-acids which can be estimated by micro Kjeldahl or other sensitive methods of analysis for non-protein-nitrogen.

**Group 4**

The Group 4 tests are those involving hormone assays, especially of the prolin excreted in certain types of malignancy of the genitalia. They are the analogues of the well established biological pregnancy tests. The methods involve the injection of suitable amounts of urine or urine extracts into certain test animals. The techniques chiefly used are the examination for maturation phenomena of the ovaries of the immature mouse (Aschheim-Zondek), for extrusion of blood into the follicles of the ovaries of the mature rabbit (Friedman), or for the extrusion of eggs by the South African "toad" following in-
jections of urine, urine extracts, or the isolated hormones. Chemical tests—e.g., the Voge bromine test, the Kappeller-Adler histidine reaction, and the phenylhydrazin-methyl cyanide test of Visscher and Bowman—have been suggested for the detection of these hormones or other substances produced in early pregnancy, but up to the present they have not been found as reliable as the biological methods.

**GENERAL CONSIDERATIONS IN CANCER SERODIAGNOSIS**

In the first place, it may be of value to enquire whether any universal test, biochemical, physiological, or biological, is likely to be forthcoming for malignancy. Is there a rational basis for a "cancer reaction"? Some evidence in favour of the possibility and some discountenancing it can be brought forward.

It is true that the vital processes of abnormal as well as normal cells must be translatable into terms of chemical and physical interchange, but, with one or two special exceptions, it has been impossible up to the present to detect chemically any specific substances elaborated only by malignant tissue. It is to be expected that quantitative changes will be minimised by the relatively huge mass of normal host tissue. A few years ago hopeful lines of research were opened by Warburg and his pupils in their studies of metabolic glycolysis of normal and tumour tissues under various conditions, but it is certain that the observed differences, apparently striking on the first examination, are closely imitated by the behaviour of embryonic and some other non-cancerous tissues in which metabolism is proceeding at a relatively high intensity.

The resemblance to embryonic cells in this and other respects has led some workers to formulate a hypothesis concerning the nature of the cancer cell which assumes that there is a close affinity between the two. Most authorities would deny, however, that the similarities are fundamental. For the latter belief the work of A. Fischer (7) is of importance. This worker produced malignant tumours in ordinary strain mice by a process of repeated transplantation of normal tissue (in particular, mammary gland) into various sites on the same animal. Malignant neoplasms developed in periods up to one hundred days. The author suggests that nothing new is impressed on the cells but that, as a result of the experimental process, certain members are enabled to display their inherent propensity for growth.

The malignant cell according to this hypothesis is a species of normal tissue cell which has intrinsically the ability to grow and multiply. The transplantation favours a natural selection of those individual cells which are most capable of surviving and proliferating, a process which also occurs, it is suggested, in the many experimental methods of inducing malignancy—by radiation, by application of carcinogenic chemicals, or by hormone "stimulus."

Such and other evidence forces us to the conclusion that it is difficult to state a fundamental difference between the normal and the "malignant" cell in biochemical terms. Accordingly, one would not expect the host to react against the "malignant" cell as though it were an alien, by the evocation of antibodies or any other specialised mechanism which would be detectable by agglutination, cytolytic, or similar sero-chemical reaction.
It cannot be denied, however, that experimental and clinical evidence can be produced in support of the belief that some form of antibody defence mechanism may be elaborated in cancer. Certain diagnostic tests are based on this assumption. Unfortunately, most of the experimental data are derived from observations upon transplanted tumours, and it is undoubtedly fallacious to assume similar reactions in spontaneous cancer. It has long been known that animals may show a natural resistance or may develop an acquired immunity to certain types of implanted malignant neoplasms. Grafts invariably fail in the immunised animals, although successful in non-immunised individuals of similar strain. Also, an acquired immunity may result from implantation of somewhat devitalised tumour grafts. Following the resorption or the surgical removal of the tumour the animal is resistant to future inoculations of the same tumour strain. There appear to be immunising and non-immunising types of neoplasm. When induced, the immunity remains specific for the particular tumour which evoked it and is not proof against other, even histologically similar, tumours. Moreover, an immunity reaction capable of preventing grafts from "taking" may be produced by injections, not of tumour cells, but of normal embryonic tissue. This immunity, therefore, must be considered as an anti-protein immunity and not an anti-tumour immunity, similar to the "anti-species" immunity which prevents the tissue of one animal from developing in the body of another animal of a foreign species, although in the form of an in vitro tissue culture it may flourish in plasma withdrawn from that animal.

Lumsden (16) maintains that his researches, which are intricate and laborious, demonstrate that specialised anti-cancer bodies are produced as well as anti-species and anti-protein bodies. This field of work is a centre of much controversy and, in any event, it is dangerous to suggest that similar reactions are likely to occur in spontaneous cancer.

Reviewing the groups of reactions outlined previously, we can now evaluate more adequately their possibilities.

**Group 1:** Detectable changes in the constitution of the blood serum undoubtedly occur in malignancy, but they are probably attributable to the secondary effects of the disease. Concomitant anaemia, toxaemia due to infective processes, absorption of cell disintegration products, etc., are mainly or wholly responsible. Similar changes will also be produced by non-malignant affections.

The occurrence of Bence-Jones protein in the urine of patients suffering from multiple myeloma is an isolated example of a protein body with special characteristics resulting from a neoplastic process. No specific changes in the gross chemistry of the blood protein constituents have been observed in other forms of neoplasia, though, as in many other pathologic conditions, the quantity of protein and the ratio of albumin and globulin may alter markedly.\(^1\)

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\(^1\) Recently (1939) Kögl and Erxleben (12) have produced evidence that some tumour tissues contain demonstrable quantities of the dextro-rotary form of amino-acids, particularly glutamic, leucin, and hydroxy glutamic acids, which occur in normal proteins almost entirely in the laevorotatory modification. Their observations have had no satisfactory confirmation as yet. Also Outhouse (17) has isolated (1937) from malignant tissue amino-ethyl-phosphoric ester to the extent of 36 mg. per cent, which he demonstrated to be present in normal tissues in a proportion less than 1 mg. per cent.
alkali reserve also may become depleted. Theoretically, one would expect, therefore, that tests based on these factors would be of little value for differentiating malignant from non-malignant processes. Nevertheless, as a result of the claims of one test depending on such changes, investigations which are described later were made by the writer utilising the technique of protein flocculation by means of the vanadate reagent introduced by Bendien.

**Group 2:** All such physico-chemical reactions will probably be quite non-specific since similar changes occur in a wide variety of diseases.

**Group 3a:** Since the evidence for antibody production in spontaneous cancer is open to criticism, the possibility of satisfactory tests based on this assumption must be highly speculative.

**Groups 3b and 4:** Two of the tests which have been specially examined at this Centre fall into Group 3b. One of them, the Fuchs reaction, has given encouraging results; the other, based on the lipolytic augmentation test, did not give satisfactory correspondence in a series of diagnosed cases.

Enzymes and hormones belong to the lesser understood regions of biological chemistry. Indeed, whether strictly chemical entities are involved in the so-called enzyme processes has been questioned, and the mechanism has been ascribed to physico-chemical colloidal phenomena and catalysis effects rather than to true intermediaries. On the other hand, although they are of recent emergence into formulated physiological chemistry, the composition of the hormones has been very thoroughly explored in several instances.

By means of urine assays for prolan in suspected cases of chorionepitelioma, valuable evidence can be provided for diagnosis of the condition in females and of chorionepithelioma of the testis in males. The detection of 4 mouse units per ml. in the urine of a non-pregnant female or of 1 mouse unit in the male can be considered as strong evidence of the presence of a chorionepitheliomatous lesion.

Under special circumstances, therefore, a biological test (dependent on specific chemical cell products) gives trustworthy evidence otherwise difficult to obtain of the presence of a malignant process. Other forms of cancer naturally do not give this reaction, nor have analyses for other hormones, e.g., oestrogens, so far afforded any definite help for such diagnosis. The exceptional accuracy of the routine Aschheim-Zondek test for pregnancy, i.e., for a "normal" kind of growth, does, however, afford ground for hope that in due course some kind of test may be possible for malignant growth. Various forerunners of the established pregnancy test were proposed with very indifferent success. Some of these followed closely upon the lines of those which are suggested for detection of cancer. Further research for a cancer test may result in a more successful issue than appears at the present.

By similar reasoning it would seem logical to believe that enzyme processes may be capable of affording diagnostic evidence. It was because of this possibility that researches into two of them were carried out at this Centre: first, the effects of cancer and non-cancer serum on the lipolytic activity of pancreatic lipase; secondly, the proteolysis test elaborated by Fuchs (8), which apparently had achieved a high degree of success in the hands of a number of independent investigators.
THE CHEMODIAGNOSIS OF MALIGNANCY

RESULTS AND CONCLUSIONS FROM SPECIAL INVESTIGATIONS

The following reactions will be dealt with in this section: (a) lipolysis tests; (b) vanadate reaction; (c) Fuchs test.

Since the details of the techniques of these tests are readily available in the several publications cited below, only a sufficient account of each will be given for the appreciation of the results of the investigations.

(a) **Lipolysis Tests**: Aqueous or glycerol extracts of animal pancreas contain a very active enzyme capable of decomposing fats of animal and vegetable origin, liberating free fatty acids which may be estimated by simple alkali titration. Purified olive oil, emulsified with sodium oleate to ensure intimate contact with the enzyme, forms a satisfactory medium for testing the splitting capacity under the specified conditions, which must be standard as regards the quantity of extract and substrate, the temperature, and the period of incubation.

It was observed by a number of experimenters that various agents, including blood serum, had an augmenting effect on pancreatic lipolysis, and the experiments of Shaw-Mackenzie (21), in particular, showed that the augmenting effect of cancer serum was, in certain instances, less than that of normal sera. This was also demonstrated by Corran and Lewis (6) in a small series of cases in which twenty malignant sera showed a definitely smaller augmentation than six normal sera. Two other cancer sera, however, closely approached the normal augmentation, while one value exceeded it. As no controls from other pathological conditions were included, the diagnostic value of the procedure was undecided. In order to throw light upon the subject, an investigation of the details of the test was entered upon at this Centre.

It was decided, in the first place, that in order to obtain intimate contact between enzyme and substrate it was important that the oily emulsion be retained in fine division during the entire incubation period of sixteen to eighteen hours. In order to effect this a shaking apparatus working within the incubator was devised so that the contents of the bottles were in violent agitation throughout. Duplicate analyses showed practically identical values of acid titer when the lipolysis was conducted under these conditions.

In the second place, it was desired to magnify any difference in augmenting power which might be detectable between various sera by making the activating effect of the serum as pronounced as possible. This was done by separating the lipase extract into two portions, each of which was inactive by itself: the thermolabile portion was designated "prolipase"; the thermostable portion "co-enzyme." This separation had been previously described by Rosenheim (19), who also showed that blood serum could act as the activating co-enzyme. Some difficulty was encountered in producing the required prolipase until a simple method was evolved by flocculating the aqueous extract in the presence of a trace of lactic acid. When this flocculated prolipase is incubated with the fatty substrate no lipolysis occurs, but the addition of blood serum activates the latent enzyme and the co-enzyme value of serum can be observed.

The following protocol will illustrate the type of reaction observed (Woodhouse, 23).
**Action of Serum on Pro-lipase:** 0.5 c.c. of prolipase extract, 4.0 c.c. of olive oil substrate, 0.5 c.c. of serum, together with water to bring to total of 6.5 c.c.; incubation period eighteen hours, at 37° C. The following result was obtained:

<table>
<thead>
<tr>
<th>Prolipase c.c.</th>
<th>Serum c.c.</th>
<th>Oil Emulsion c.c.</th>
<th>Titre N/10 NaOH</th>
<th>Augmentation c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>—</td>
<td>4.0</td>
<td>1.2</td>
<td>—</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>4.0</td>
<td>38.8</td>
<td>37.6</td>
</tr>
</tbody>
</table>

The lipase values of Corran and Lewis (6) were usually approximately 20 c.c. N/10 NaOH in the absence of serum, and in the presence of normal serum 38–40 c.c., i.e., an augmentation of 18–20 c.c. With the revised technique, the differential augmentation is much more apparent, being thirty times instead of about twice the initial value.

When the test was made in this manner, it became evident that the diminished augmentation value of cancer sera is not specific. The figures from a series of normal and non-cancerous pathological sera showed an augmentation average of 36 c.c. In some instances the augmentation produced by cancer sera actually exceeded the figures for non-malignant pathological sera.

**(b) Vanadate Flocculation Method of Cronin Lowe:** Investigations on this reaction have been carried out at the Birmingham Centre over a number of years. The technic is briefly outlined here. For fuller accounts the communications of Bendien (1), Cronin Lowe (15), Jones and Woodhouse (10), Hunt and Woodhouse (9) may be consulted.

A series of 10 solutions of sodium ortho-vanadate are produced by diluting the standardised N/10 vanadate reagent with N/10 acetic acid so that the final quantities range from N/60 to N/100. On addition of 1 c.c. of these to 10 samples of 0.10 serum plus 0.10 c.c. distilled water the colloidal vanadate ions neutralize the opposite electric charge on the protein ions and a flocculation ensues in those tubes in which the vanadate exceeds a critical value, depending on the quantity and type of protein present. Serum globulin has a protecting effect on albumin fraction of the serum, while other factors such as pH, salt, and lipin content also influence the precipitation.

A second series of serum samples is treated similarly, the serum having been previously heated to 56° C. for thirty minutes. In this process the denaturation alters the protein complex and precipitation is more difficult. In a third series the lipins are removed by ether extraction, which causes enhanced flocculation. Analysis of the amount of precipitate in the three series, A, B, and C, gives, therefore, a value for the effects of denaturation and lipin removal. It is suggested that the "heat effect" is proportional to the patient's resistance to a pathological process and the "ether effect" represents the extent of the pathological change. Thus the ratio \( C-A/A-B \) at the point of the precipitation, i.e., of greatest lability, gives a figure which in malignancy is found to exceed unity (Cronin Lowe, 15). The point at which flocculation ensues is usually tube 27 for a healthy person, and a deviation from this position indicates an abnormal serum. This tube is the 8th in ascending order and the serum in it is treated with reagent containing vanadate 27 N/2000.
Three groups of tests were made:

**Group I:** In this series the ratio from which the diagnosis is made was calculated from estimations of the amounts of precipitate which occurred in those tubes containing the first definite sedimentation, and the units were determined by nephelometric comparison with a casein standard.

The results in 296 classified cases (Jones and Woodhouse, 10) may be summarized as follows:

Corresponding to clinical diagnosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Malignant</th>
<th>Non-malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant disease</td>
<td>131 (74.8 per cent)</td>
<td></td>
</tr>
<tr>
<td>Non-malignant disease</td>
<td>90 (74.4 per cent)</td>
<td></td>
</tr>
</tbody>
</table>

Not corresponding to clinical diagnosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Malignant</th>
<th>Non-malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant disease</td>
<td>44 (25.2 per cent)</td>
<td></td>
</tr>
<tr>
<td>Non-malignant disease</td>
<td>31 (25.6 per cent)</td>
<td></td>
</tr>
</tbody>
</table>

The following additional results were obtained in certain special conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Result of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent ulcer</td>
<td>2</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>1</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>4</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>5</td>
</tr>
<tr>
<td>Malignant</td>
<td>7</td>
</tr>
<tr>
<td>Non-Malignant</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Thus in both categories of patients, at least 25 per cent of incorrect results were apparent. Continued experience and endeavour did not result in the elimination of this quota of false returns nor could a reasonable explanation be obtained why, in some instances, repeated examinations of obviously malignant cases should give uniformly negative reactions and *vice versa*.

**Group II:** In a second series of examinations the analyses of the flocculated protein were carried out in a number of tubes in each of the A, B, and C series of serum samples. From these values complete charts of the behaviour of serum over the precipitation zone were drawn. It had been suggested by Coke (5) that the deviation from the normal sedimentation tube number as well as the ratio previously calculated should be considered in making a diagnosis, and that the whole picture thus produced by the three curves should be studied.

The type of chart obtained is illustrated by three typical reproductions, one of a normal healthy serum, one of a pathological non-malignant serum, and one of serum from a definitely malignant case (Charts 1, 2 and 3). The deviation values are arbitrary steps of ± 10 per cent for each tube to right or left of No. 27. The value of 40 units of precipitate is taken as the point of critical flocculation and the sedimentation ratio is calculated from the chart at the point where the A line has that value (A', Chart 1). The three lines depict the sedimentation in the three phases throughout the test. The area between the A and B lines represents the resources of the body in opposing the pathological condition while the area between the A and C lines indicates the degree to which the pathological change has progressed.

A series of 50 frankly malignant cases were charted by this method and a series of 25 pathological but clinically non-malignant cases. Since it had also
been suggested that the differential vanadate test was valuable for studying
the serum reactions in asthmatics, where allergic conditions frequently play
an important rôle, a series of 50 patients from the Birmingham General Hos-
pital Asthma Clinic were also examined. The results in these groups are col-
lected in the following table (Hunt and Woodhouse, 9).

<table>
<thead>
<tr>
<th></th>
<th>Malignant Cases (50)</th>
<th>Non-Malignant Cases (25)</th>
<th>Asthmatic Cases (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant type of reaction</td>
<td>42 (84%)</td>
<td>19 (76%)</td>
<td>20 (40%)</td>
</tr>
<tr>
<td>Non-malignant type of reaction</td>
<td>8 (16%)</td>
<td>6 (24%)</td>
<td>10 (60%)</td>
</tr>
</tbody>
</table>

It is probable that the higher return for "correspondence" in the collection
of malignant cases is accounted for by the fact that they constituted a series in
which the condition of many of the patients was advanced though not terminal.
The number of incorrect returns in the non-malignant cases is approximately
the same proportionately as in the previous series. The asthmatics, on the
other hand, constituted a very abnormal series, 40 percent giving the malig-
nant type of ratio while 15 cases, or 30 percent, showed marked negative
deviations, a feature not presented by either of the other groups.

**Group III:** In a further series of 54 unselected cases the analyses and
charts showed the following results:

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total malignant cases</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant type of reaction</td>
<td>20 (70 per cent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-malignant type of reaction</td>
<td>9 (30 per cent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total non-malignant cases</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant type of reaction</td>
<td>7 (28 per cent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-malignant type of reaction</td>
<td>18 (72 per cent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>38/54 (70.4 per cent)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The correlations are therefore similar to those given by the first series of
tests covering all types of cases.

An analysis of the total of 425 cases shows:
(c) **Fuchs Proteolysis Reaction**: The theory underlying the Fuchs reaction has features in common with the Abderhalden hypothesis. It contends that the organism can produce an antibody reaction to embryonal and malignant cells.

It is known that embryo serum, when injected into the mother animal, induces a definite antibody reaction; likewise, spermatozoa will evoke a reaction in the animal from which they are derived. Adult tissues of the same species will not do this. Fuchs (8) maintains that the malignant cell is one which has been arrested at a certain point in its development from its embryonal origin and that its proteins retain the power of antibody stimulation if they are absorbed in the body. These antibodies can be demonstrated because they enable the enzyme reaction between normal serum and protein derived from cancerous blood or, vice versa, between cancer serum and normal protein, to proceed to an extent which can be observed by delicate chemical analysis.

Previous to the present investigations the test had been examined by a number of workers and favourable reports were forthcoming from such investigators as Wright and Wolf (24), Bing and Marangos (2), Kafka (11).

Suitable substrates for the reaction must be prepared from the blood of healthy persons, from known cancer cases, and from patients with certain pathological conditions (e.g., tuberculosis), by precipitating the serum proteins with trichloracetic acid. The precipitate is well washed, dried, and powdered, and 5 mg. of each sample are used for incubating with exactly 1.0 c.c. of the serum of the patient under test. The non-protein nitrogen in the incubated sera and in blank tests is estimated by a micro-Kjeldahl method. The increase in "rest" nitrogen during the incubation period of sixteen to twenty-four hours enables the diagnosis to be made, since this is much greater when cancer serum is acting on normal protein than when it is acting on protein derived from cancer blood.

Because the accurate estimation of the slight change in non-protein nitrogen involved in the test presents some difficulty, methods for determining the extent of the interaction by utilising alternative techniques have been elaborated in some recent investigations; e.g., using *B. coli* to transform the nitrogenous end-products to indole, which is subsequently estimated by colouration with p-dimethyl-amino-benzaldehyde (Čížek, 4), the titration of the amino-acid content by the method of Minibeck (Rosenthal, 20), or even by utilising the polarograph for recording the cystin liberated in the reaction (Kotljar and Podroužek, 13). In all the experiments recorded in this article the colorimetric method described by Fuchs has been used without, however, employing his special colorimeter. In a few instances the non-protein nitrogen values were checked by means of the "Spekker" absorptiometer. The results are summarised under four headings:

1. **Series of 120 Cases with Known Diagnosis**: The following results were obtained in a series of 120 patients and have been previously reported in detail (Woodhouse, 22).
Malignant cases ............................................ 53
Correct result ........................................ 50 (94 per cent)
Incorrect result ...................................... 3 (6 per cent)
Non-malignant cases ................................. 42
Correct result ........................................ 38 (90.3 per cent)
Incorrect result ...................................... 4 (9.5 per cent)
Total correct results ................................. 88/95 (92.6 per cent)

In three cases the reaction was indeterminate. Twenty-two malignant cases receiving roentgen radiation gave positive reactions in 2 cases only and were therefore anomalous.

(2) Series of 230 Cases: In a further series of 230 consecutive cases the diagnosis of malignancy or non-malignancy has been established by re-checking after an interval of several months in all instances where histologic evidence was not available. The series included 205 patients in which the test was carried out before any radiation treatment was given, and 25 tested during or after courses of irradiation. Experience has shown that such treatment results in anomalous effects upon the serum when examined by this test.

The diagnostic accuracy of the test in this group of patients was as follows:

Malignant cases ............................................ 99
Correct result ........................................ 75 (76 per cent)
Incorrect result ...................................... 24 (24 per cent)
Non-malignant cases ................................. 106
Correct result ........................................ 85 (80.2 per cent)
Incorrect result ...................................... 21 (19.8 per cent)
Irradiated cases ........................................... 25
Non-malignant reactions .............................. 12

(3) Analysis of Results in 303 Non-irradiated Cases: In the non-irradiated cases included under headings (1) and (2), the correct results were as follows: total, 81.8 per cent; malignant cases, 82.0 per cent; non-malignant cases, 83.0 per cent.

(4) The Reaction Applied to Tissue Extracts: In order to obtain further insight into the reaction, a number of tests were carried out in which the serum in the usual technique was replaced with saline extracts of fresh tissue removed at operation. It was thought possible that the malignant tumours would yield fluids which would contain elements similar to those in cancer blood and would therefore give positive reactions, and that non-neoplastic tissue would give negative reactions. The tissues were minced, ground with sterile sand and four times their weight of normal saline; the fluid was centrifuged, filtered through a Seitz filter, and the clear filtrate incubated with the protein substrates as usual. The tissues examined included the following:

<table>
<thead>
<tr>
<th>Malignant Tissues Correctly Diagnosed</th>
<th>Non-Malignant Tissues Correctly Diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast ........................................ 16 cases</td>
<td>Breast ........................................ 8 cases</td>
</tr>
<tr>
<td>Ovary ........................................... 5</td>
<td>Uterus ........................................... 9</td>
</tr>
<tr>
<td>Colon ........................................... 4</td>
<td>Ovary ........................................... 7</td>
</tr>
<tr>
<td>Uterus .......................................... 2</td>
<td>Stomach .......................................... 3</td>
</tr>
<tr>
<td>Bladder .......................................... 2</td>
<td>Spleen ........................................... 2</td>
</tr>
<tr>
<td>Scrotum .......................................... 2</td>
<td>Parotid gland .................................... 2</td>
</tr>
<tr>
<td>Stomach .......................................... 1</td>
<td>Testis ........................................... 1</td>
</tr>
<tr>
<td>Testis ........................................... 1</td>
<td>Thyroid ........................................... 1</td>
</tr>
<tr>
<td>...............................................</td>
<td>Placenta ........................................ 1</td>
</tr>
<tr>
<td>...............................................</td>
<td>Gallbladder ..................................... 1</td>
</tr>
</tbody>
</table>
The following tissues were among those incorrectly diagnosed:

<table>
<thead>
<tr>
<th>Histologically Malignant Tissue</th>
<th>Histologically Non-Malignant Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>4 cases</td>
</tr>
<tr>
<td>Breast</td>
<td>1 case</td>
</tr>
<tr>
<td>Uterus</td>
<td>2</td>
</tr>
<tr>
<td>Ovary</td>
<td>1</td>
</tr>
<tr>
<td>Ovary</td>
<td>1</td>
</tr>
<tr>
<td>Cervical lymph nodes</td>
<td>1</td>
</tr>
</tbody>
</table>

The complete results were as follows:

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Positive</th>
<th>Negative</th>
<th>Indefinite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total examined: 112</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologically malignant: 57</td>
<td>41 (72%)</td>
<td>10 (18%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Histologically non-malignant: 55</td>
<td>3 (5.5%)</td>
<td>50 (91%)</td>
<td>2 (3.5%)</td>
</tr>
</tbody>
</table>

Thus in a large proportion (70 per cent) of the malignant tissues the extract gave a positive proteolysis reaction and 90 per cent of the non-malignant tissues gave a "correct" type of reaction, affording a very satisfactory measure of support to the Fuchs hypothesis. Excluding indefinite reactions, 80 per cent of correct diagnoses were obtained for the miscellaneous series of tissues.

**DISCUSSION OF RESULTS**

Reviewing the results obtained with the three methods examined, it is an obvious conclusion that none has the specificity for affording the assistance desired. The fact that so high a percentage of non-malignant cases gave false positive reactions is especially to be deplored. The Fuchs reaction gives the most favourable figures and the fact that cancer tissues yield active extracts suggests that the test is not without good foundation. It remains to devise further control tests to eliminate the confusing reactions which occur in some other pathological conditions.

**NOTE:** The investigations described in this paper have been carried out under the auspices of the British Empire Cancer Campaign, Birmingham Branch. I am indebted to Miss Levi, Follow-up Officer for Cancer Patients in the Birmingham United Hospital, for data concerning the diagnosis of the cases examined and to the Honorary Staffs of the Institutions for facilities to pursue these investigations.

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5. **COKE, H.**; Charterhouse Rheumatism Clinic, Orig. Papers: 1, 43, 1937.