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The search for information in regard to cancer has lead into many and varied fields of research. A colossal detail of study in the fields of etiology, heridity, pathology, treatment, clinical and laboratory diagnosis has produced a wealth of material on the subject of cancer. It is the purpose of this paper to summarize the more important methods of serological diagnosis which have been proposed, tried, accepted or rejected in the search for accurate laboratory aids in the diagnosis of cancer.

The Abderhalden reaction was the first based upon specific antibodies of a fermentative nature (1). In this test the blood serum is dialyzed with substrate and tested for color reaction with ninhydrin. A violet color occurs if positive. Results range from 55 to 70 per cent in the hands of various observers, but the test does not differentiate carcinoma and sarcoma.

R. Weil was probably the first American investigator to study the hemolytic reactions in cases of human cancer (2,3).

Weil studied the blood of human beings suffering from new growths. The effects of the patients serum in regard to hemolysis of his own corpuscles and alien corpuscles in a large series of diseased conditions, both malignant and otherwise, were carefully worked out. Weil concluded the reaction of hemolysis is not pathognomonic of malignant tumors, early or late, that it occurs in a considerable proportion of other diseases, and that a large proportion of tumors present an altogether different type of reaction. On the other hand a much larger percentage of malignant new growths presented this type of reaction (hemolysis) than do cases of other diseases. Weil concluded that something of value may eventually come from the use of hemolytic reaction studies in human diseases but it would have to come with a refinement of method which will correspond to the complexity of

of the factors involved.

The importance of the method in its application to human diseases was given considerable prominence by Crile, with the following results (4):

(1) Normals - 107 tests. No hemolysis.
Test of Miscellaneous diseases, number 50; hemolyzed 4, these 4 included 1 hemoglobinuria, 1 eclampsia, 1 gastric case diagnosis not made, and 1 hematuria.

Carcinoma 50, hemolyzed 39.

Sarcoma 16, hemolyzed 13, making a total of 66 cases of malignancy in which 52 hemolyzed.

Carcinoma recurrence, or cured, to prove if cured-- 10 tests made, 9 no hemolysis, 1 questionable.

Papilloma, 2 tests -- hemolysis 1, no hemolysis 1.

Tuberculosis, 21 tests -- hemolyzed 9.

Chronic suppuration 10 cases with no hemolysis.

(2) Of the 13 cases not giving hemolytic reaction, one gastric case, diagnosis not proved; one sarcoma of spine, advanced case; one suspected sarcoma, advanced case; one carcinoma recurrence, advanced case; one cystic overy; five carcinomota breast, advanced cases; one lymphosarcoma, advanced case; two epithelimata, advanced case.

Suspected malignant cases not showing hemolysis, and positive diagnosis proving no malignancy: two gastric cases, four gall bladder cases, two tumors of thigh; two cirrhosis of liver; one tumor of chin; one tumor or clavicle; one cystic ovary; and one breast case.

Dr. Weil's own experience in a smaller number of human cases had failed to reveal any such specific corpuscular resistance in cancer cases (3). He believed that the serum in human cancer is sometimes hemolytic, often not so, and that other diseases such as tuberculosis, often show the same conditions. He did not believe that the reaction in human beings to be of any

value at that time and that the specific resistance of red cells to the hemolytic sera of the individuals from which they were derived was a phenomenon which needs much further study.

Elsberg (5) in 1909 reported that washed red blood cells taken from normal individuals and injected under the skin in the forearm of a patient, that in those patients in whom a reaction was obtained an elevated round red 2-4 cm. papule appeared within one to two hours and disappeared within twenty-four hours. In the patients who did not show this reaction there was nothing to be seen at the site of the injection excepting the needle puncture. Elsberg gave thirty-four injections to twenty patients with known carcinoma and every case had a positive reaction. Of four patients with known sarcoma, three gave a positive reaction. Injections were given to 100 normal individuals or to those suffering from diseases other than sarcoma or carcinoma and in all but three cases the reaction was negative.

Elsberg (6) in a later series of cases found that in 69 cases of positive or probable carcinoma 89.9 per cent gave a positive reaction while in a series of 325 cases not proven to be carcinoma, 94.3 per cent no characteristic skin reaction was found.

Elsberg concluded that the subcutaneous injection of human red blood cells prepared in his particular manner is in certain individuals followed by a characteristic and easily recognizable local skin reaction at site of the injection.

This reaction is of interest as it was probably the first attempt to secure a reaction visible in the skin by the injection of an activating substance in a cancerous patient. The later literature gives no mention of subsequent work, criticism or results of this method, however, it was proven accurate only in a realtively small number of Elsberg's own cases and the test apparently does not react differently in sarcoma or carcinoma.

E. Freund found that carcinoma extract with carcinoma serum and sarcoma extract and sarcoma serum respectively yield a specific turbidity or precipitation, whereas none occurs with non-carcinomatous serum and non-sarcomatous serum (7). His method is based on the observation that the isolated cells of carcinoma are dissolved by normal serum, whereas, the cells are resistent to carcinoma serum. In the former case the distinctive action of normal serum on the cancer cell is attributed to the fatty acids of the serum which are not present in carcinoma serum. The test consists in extracting undegenerated carcinoma tissue with sodium chloride and monobasic phosphate of sodium solution and pressing the isolated cells through a fine cloth. The emulsion is heated with dilute acetic acid, neutralized and filtered. If a realiable extract has been obtained this in suitable quantities with carcinoma serum will show distinct opaqueness, whereas, normal serum will be transparent.

Shaw-Mackenzie drew attention to a new test of cancer by means of a turbidity reaction in the serum (7). (The test thus resembles Freund's test). It consists in adding an ether extract of cancer tissue, with or without saponification to cancer serum. This is followed by an appearance of turbidity after incubation which does not appear at all, or not to the same extent, with non-cancerous serum. Shaw-Mackenzie tested in this way 136 sera, including 58 normal sera. In 41 cases the previous positive or negative diagnosis has been confirmed by the test. In 35 cases in which no details of the cases have been given or the diagnosis was uncertain, a correct diagnosis was made as determined subsequently. In two further cases positive results were not confirmed in patients not suffering from cancer.

Murray in 1923 brought forward some evidence that a neoplastic reaction worked at one site in an animal, confers some resistance to such a change at another site and he suggests that there is some systemic

constitutional change (8).

From these findings Fry proposed that if such be the case, then it is not improbable that the medium of distribution of the locally produced change is the blood. On these grounds a serological reaction would appear a priori to be practicable.

In experiments carried out by Fry it was found that extracts of cancer tissue used as an antigen in compliment fixation experiments often showed marked anti-complimentary properties. From this it seemed possible that a method, in the nature of a colloidal flocculation reaction, which dispensed with the hemolytic system might prove of value.

Fry in 1925 published a new flocculation reaction for the sero-diagnosis of malignant disease using an extract of breast carcinomata (9). Over the whole series of 500 cases, he presented comprising malignant and non-malignant conditions, 75 per cent correct results were obtained. In 239 cases proven malignant disease, 170 positive flocculations were obtained (71 per cent correct). In 261 controls comprising healthy individual cases of non-malignant neoplasams, medical and surgical conditions, 200 gave a negative result (78 per cent correct). Healthy individuals gave a uniformly negative reaction except in one instance. Non-malignant tumors usually gave a negative reaction. In the absence of malignant disease, flocculations did not in the majority of cases occur with syphillitic conditions. With acute febrile conditions, for example tuberculoses or sepsis-positive flocculations may occur.

Ruffo found that by adding 5 drops of a 0.1 per cent neutral red solution to 2 cc. of pure non-hemolytically obtained serum of a carcinomatous patient, the serum becomes reddish, while the serum of a healthy

individual or of one with another disease maintains its yellow color (10). Otto Bajc examined the reaction in clinically observed material of 33 serums which were prepared according to the prescription. It proved to be non-specific and unreliable as of 10 serums of positively malignant tumors, only 5 that is, 50 per cent reacted positive. Of 23 serums, of positively not carcinomatous patients, 7, that is about 30 per cent, showed a positive reaction. The unequivocal failure of the reaction was also reported by other workers according to Bajc.

Landau started his studies on a precipitation test for malignancy in 1928 and in a series of 767 demonstrated carcinoma cases, 75.3 per cent gave a positive reaction, and in 826 proved cases of sarcoma, 75.5 per cent gave a positive result (11).

In 1929 Landau and German demonstrated a certain affinity of an alcoholic extract of carcinomatous tissue for the serum of patients having malignant tumors. (11). After modifications of their technique, a series of nearly 300 cases has given results which are sufficiently encouraging to warrant further intensive study. While complete accuracy has not been demonstrated, it must be borne in mind that none of the serological tests for syphillis, now thirty-two years old, is absolutely accurate.

Landau's method in brief, involves preparation of an alcoholic extract of malignant tissue for the antigen which when mixed with suspected cancerous human raw serum, and using known malignant and non-malignant human serum as controls, produces a sedimentation with malignant serum if positive and leaves the serum translucent without sedimentation, if negative.

Syphillitic serums gave 15 per cent positive reactions. Landsteiner showed (1902) that extracts from all normal tissues gave positive results with syphillitic serums.

In dissecting malignant tumors (to secure cancer tissue for the antigen) it is impossible to remove all traces of normal tissue since a certain amount of fairly normal stroma is produced in any tumor growth and also bits of normal tissue are found in any area which a malignant tumor is invading or replacing.

Tuberculoses gave 24 per cent positive reactions. At the present time no satisfactory explanation can be given for this fact. It can only be surmised that a similiar reactive substance is produced in the blood in both conditions.

In normal pregnancy, 6 per cent gave positive reactions, it is to be remembered, however, that in this condition a false positive syphillitic reaction is occasionally found.

The simplicity of Lederer's carcinoma reaction induced Zweig and Lauber to test its reliability in forty-four cases (12). The diagnosis of carcinoma had been established by surgical treatment and by histologic examination of thirty-three, carcinoma was absent in none and the diagnosis was doubtful in two. A detailed description of the technique of Lederer's reaction is not given, but the authors point out that it is based on the protective action of the blood against suspension colloids, which in carcinoma is supposedly reduced. On the basis of their observations they reject the method as unreliable.

In 1929 Vernes showed that there was flocculation of the blood serum of cancer patients after the addition of a 4.5 per cent solution of copper acetate (13). The flocculation was always more accentutated in patients with cancer than in normal individuals or those with other diseases. In making this test, repeated examinations are necessary. It has been found that there is a parallelism between the serologic curve of the reaction and the course of the cancer.

An elevation of the curve often precedes the clinical signs of recurrence. The height of the curve diminishes after operation or the application of radium or x-rays provided that beneficial results are obtained, while it remains at the same level when the treatment has not produced beneficial results.

Mondain, Douris, and Beck, in studying the Thomas-Binetti reaction, showed that the phenomenon of the reduction of methylene blue, attributed to the cancer serum, was really due to the action of bacteria in the extract used as a reagent and hence, was unreliable (14).

The Botelho reaction involving a nitric acid, iodine, potassium iodide solution with suspected serum offers about 75 per cent correct results (14). This is a precipitation reaction and is simply a precipitation of albiumnoid bodies in an acid medium by a non-specific reagent.

Witebsky in studying the serological specificity of carcinomatous tissue believes from his investigations that it is undoubtedly possible to show specific, serologically demonstrable structures in carcinomatous tissue.(15). They may appear either in the alcoholic extracts or on the isolated tissue globulues.

Kotrnertz and Weber state that the Freund-Kaminer test mentioned previously is based on the difference in the behavior of normal serum and that obtained from cancer patients toward cancer cells (16). Normal serum dissolves cancer cells, whereas serum of patients having carcinoma does not and, when mixed in equal amounts with normal serum, the serum of cancer patients will protect cancer cells against destruction. It was further established that the intestinal contents acted in the same way as the serum and that the addition of animal fatty acids enhanced the destructive power against cancer cells. It was likewise shown that Bacillus coli has the power of destroying cancer cells, but if grown on

an acid medium it develops a protective power for the same cells. According to Freund and Kaminer, the cell-destroying substance is contained in normal fatty acid and can be extracted from it by ether. The "destructive" substance is presumably bound up in some other combination with the fatty acids. This last acid, designated as the "carcinoma acid", is utilized in the test. The technic consists in injection of 0.05 or 0.03 cc. of the acid intracutaneously into the flexor surface of the forearm. Below this a 0.03 cc. control injection of physiologic solution of sodium chloride or of tricresol is made. A positive reaction manifests itself in from twenty-four to forty-eight hours by the appearance of a nodule surrounded by an area of redness. Inf lammatory redness alone is regarded as a negative reaction. Observations were made on four groups of patients: (1) proved cases of cancer; (2) a control group of patients suffering from other than malignant disease; (3) clinically uncertain conditions; (4) tumors other than cancer. In the first group the test was correct in 86.4 per cent. There were fourteen negative and ten doubtful reactions. In the control group of 118 patients there were 58 negative, 36 uncertain and 24 positive reactions. In the fourth group of 7 mesenchymal tumors there were one negative, four doubtful and two positive reactions. The authors conclude that a positive reaction is of value when signs suggesting a malignant condition are present. A positive reaction alone does not establish the diagnosis of a malignant condition in the absence of other evidence. The negative reaction is of a greater value than the positive one. The reaction is of value in differential diagnosis.

The reaction introduced by Freund and Kaminer was tested on 85 patients by Orator and Arens (17). They found a strictly intracutaneous injection should be made into the upper arm of 0.15 cc. of a 1 per cent emulsion of carcinoma fatty acid (prepared in a pure crystallized form by Freund and Kaminer)

in a 0.3 per cent solution of tricresol so that a taut, pale pomphus about 1 cm. in diameter results (the technique should be strictly observed). In subjects of carcinoma a hard, sharply outlined infiltration the size of from a lentil to a cherry-stone forms in the depth of the skin in twenty-four to forty-eight hours. Redness or swelling without definite demarcation is not typical. The test is perfectly harmless. It has proved a good plan to postpone the final judgment on the result of the test until six to eight days has alapsed. If by that time there is still a definite nodule it speaks for carcinoma.

In 38 patients the ready prepared inoculation serum placed at their disposal was used. Out of the 19 subjects of carcinoma, 10 showed a positive, 3 a doubtful, and 3 an apparently negative reaction; of the 19 patients free from carcinoma there was only 1 positive reaction. In 45 cases, the pure white crystals of carcinoma fatty acid were emulsified in 0.3 per cent tricresol. The reactions were a little stronger so that only 0.1 - 0.15 cc. was injected intracutaneously: of the 26 certain cases of carcinoma all reacted positively, 12 who were free from carcinoma (among them 2 with sarcoma) negatively, 7 feverish cases showed uncertain "fever reactions." The positive nodules were accompanied by redness of the skin which became livid in a week while at the same time the nodules began to decrease in size. They were still definitely palpable at the end of two weeks, after three weeks, remains of them could still be felt.

The test should be subjected to further clinical examination.

Weber and Schule report their results with Links' serum diagnosis of carcinoma in 100 clinical cases (18). Blood is drawn from the cubital vein into a receptacle so arranged that each stage of coagulation can be separated, yielding three serum fractions instead of one.

The three specimens are subjected to a chemical analysis consisting of the determination of the number of cations utilizing the potassium and magnesium content of the serum. The three fractions are placed in receptacles and are treated by heat so as to rid them of all organic particles. The resultant clear fluid is examined for its potassium content by titration and for magnesium by a colorimetric micromethod. It is not the total of the six figures obtained but the relationship of the total to the erythrocyte found that determines whether the test is positive or negative. The authors do not furnish further details except to state that figures above 100 are considered a positive test, while those below are considered negative. The test was positive in the forty-one cases of definitely established carcinoma. In a control group of forty-two cases that were definitely not carcinoma, five gave a figure above 100, in other words were positive. This gives 88.1 per cent of correct results and 11.9 of errors. In a third group of eleven suspected cases of malignancy, the test proved to be correct in seven, probably correct in two, and undetermined in two. The authors feel that the small number of cases tested does not allow any conclusions regarding its specificity. It appeared significant, however, that all the sarcoma cases gave a negative result suggesting a specific response on the part of the epithelial tumors.

Benjamin Gruskin, a Philadelphia clinical pathologist, in 1929 brought forth his original test for cancer based on serological reactions. As it appears to be the most promising of recent work in serological diagnosis his observations are given in detail.

As a working hypothesis Gruskin formulated the assumption that in the ordinary course of normal cell growth each type of cell produces a lysin against the other type of cell(19). That is to say, connective tissue cells normally have the property of generating certain lysins

which are antagoniatic to excessive growth of epithelial cells, so that although an inciting factor such as chronic irritation may be present, carcinoma does not develop. If, on the other hand, the epithelial tissue fails to produce such a lytic agent, a carcinoma results when the proper extrinsic factors are present. The opposite cycle follows in the production of sarcoma, that is, a deficiency or lack of lytic agent normally produced by connective tissue results in the presence of proper extrinsic factors in the production of sarcoma. In other words, the maintenance of an equilibrium between the connective and epithelial tissues is considered as dependent upon the presence of antagonistic lytic agents. The hypotheses of Waldeyer and Thiersch of an equilibrium between the connective tissue and epithelial elements and the tissue tension hypothesis of Ribbert (20),(19), are in direct accord with this theory. The ability of normal serum and the failure of cancer serum to autolyze cancer cells in vitro has been demonstrated (Freund and Kaminer,(17), and points to the presence of lytic substance in normal blood serum.

The theory is also in accord with the general nature of opposing physiologic forces found in normal biologic activities, where the opposing physiologic forces found in normal biologic activities, and the counteracting principles tend to maintain an equilibrium in normal cell growth, that is, the maintenance of the endocrine balance, the vascular functions of dilation and constriction and the thermogenetic and thermotactic apparatus.

Based on this conception of the possible causes of malignant disease, it was believed that a lytic agent might be produced by the inoculation of animals with purely embryonic epithelial cells in the case of carcinoma and connective-tissue cells in the case of sarcoma.

In this way, amboceptors would be developed in such treated animals and there sera might have the property when injected into a patient suffering from malignant disease to destroy, in case of carcinoma, embryonic epithelial cells and in the case of sarcoma, connective tissue cells, based upon the accepted serologic principles of the production of lytic agents.

The second consideration was to demonstrate the correctness of the relationship between malignant new growths and the lytic agent produced by immunizing animals. Experiments were performed to prove the existence of such amboceptors on the basis of production of precipitins, recognizing that if precipitins were formed between the amboceptor and the serum of a cancer patient, a homologus relationship would be established and it would be demonstrated that no difference exists between embryonic cells of one species of animals and another. In order to determine whether the precipitin reaction is due purely to the immunizing properties of the embryonic cells, control animals were inocculated with pancreatic tissue obtained from mature animals and it was found that the sera of these animals failed to produce precipitins when brought in contact with the serum of cancer patients. According to Ewing this may be due to the presence in embryonic tissue of specific growth-stimulating substances. Having demonstrated the specificity of amboceptor produced by immunizing animals with embryonic cells, it was logical to attempt to utilize this immunologic principle as diagnostic test.

It is important to point out that the principle upon which the present test is based, differs fundamentally from that of any other test which has been presented in the following ways:

(1) because the test is purely biological in character, and (2) because the amboceptor is produced by means of purely embryonic cells. It should be noted that most of the sero-diagnostic tests in which tissue cells have been used have been performed with an antigen composed of both epithelial and connective-tissue cells. Fry (9), for instance, used breast cancer as an antigen, but adds that cellular carcinoma is preferable to scirrhous cancer. When the theory upon which this test is based is considered, it is readily seen that the presence of adult connective-tissue detracts from the specificity of the antigen and it is suggested that this factor may be responsible for the better results obtained with the more cellular growths. It is believed that the compliment-fixation test for cancer could be rendered more specific if pure embryonic cells such as are employed in the Gruskin test were used in the preparation of antigen. Attempts to utilize this principle in a compliment-fixation test are under way.

In an attempt to simplify the test and make it more useful for practical purposes, it seemed plausable to attempt to utilize the embryonic tissues directly in the form of an antigen instead of the amboceptor produced by animal immunization. In this way the difficulties encountered in the production of the amboceptor would be eliminated. It was found for instance, that very few rabbits were capable of producing a satisfactory amboceptor. A further difficulty was encountered in the turbidity of the serum in some animals. The following technique was employed in the preparation of the antigen.

Antigen for Carcinoma: Mammalian embryos are used (calves, sheep, pigs). They must not be in a later stage than the second month of pregnancy.

Using a sterile technique the pancreas and submaxillary glands are dissected out. The capsule and ducts are removed and the tissue is macerated in saline solution and the mixture is rubbed up until the fluid becomes milky or opalescent in appearance. At this point, the cells suspended in salt solution are centrif uged until the supernatant salt solution is perfectly clear, the salt solution is then discarded and the epithlial cells are placed in a porcelain dish and dried at a temperature of 75 degrees C° to a doughy consistency. During this period, it is essential that the tissue be stirred thoroughly at fifteen minute intervals in order to permit uniform drying. The tissue is then placed in a glass-stoppered bottle to which is added three times its volume of acetone. The mixture is permitted to stand in the ice chest for twenty-four hours with frequent shaking. The acetone is then poured off and replaced by one and a half volumes of fresh acetone for another twenty-four hours. The acetone is again decanted and the tissue is placed in a mortar to which five times its volume of absolute alcohol is added. This is macerated for about fifteen minutes until the alcohol becomes somewhat milky. The mixture is then kept in the ice box for five days during which time it is forcibly shaken at two-hour intervals. The alcohol extract is then decanted and is ready for use.

Antigen for Sarcoma: A mixture of Wharton's jelly and red bone marrow of calf embryo is macerated in a mortar, to which two volumes of salt solution are added. This is placed in a porcelain dish at a temperature of 75 degrees C until the mixture becomes grayish in color. The salt solution is then decanted and the tissue is placed in three times its volume of acetone for twenty-four hours. The acetone is then

decanted and replaced by one and a half volumes of acetone for twenty-four hours. The acetone is again decanted and the tissue placed in a mortar and macerated with five times its volume of alcohol for fifteen minutes until the alcohol extract assumes a light brown color. The mixture is then placed in a glass-stoppered bottle in the ice chest for five days, during which time it is shaken at two-hour intervals. The alcohol extract is then ready for use.

TECHNIQUE OF TEST: Five to ten minims (depending upon titration) of antigen is placed in a test tube to which 30 minims of suspected serum (which has been previously diluted 50 per cent with normal saline solution) is added slowly drop by drop. This is allowed to stand for two minutes. It is then agitated until floculation takes place. Ten minims of salt solution is added and is allowed to stand for two minutes. It is agitated again and read against a frosted electric light. A positive reaction is indicated by floculation. A negative reaction produces no floculation. It is important that the drops be added slowly and at regular intervals. Adding the drops too rapidly might give a negative reaction in positive cases. It is also important to distinguish between air bubbles and true floccula. The former always rise to the surface, whereas, the latter settle to the bottom.

If the suspected serum has a very low specific gravity and looks watery it is best not to mix serum with salt solution, but use it in its original state, as adding salt solution is liable to render a positive serum negative.

Titration: A known positive and negative serum are employed in order to determine the least amount of antigen necessary to produce a positive reaction. When this endpoint is reached, the same amount is

used on the known negative and should give a negative reaction. It is essential to determine this endpoint accurately and utilize the least amount of antigen necessary to produce a floculation. If the known positive of this amount is exceeded by even a few minims, a floculation might take place in the negative serum.

PRECAUTIONS: The following precautions should be observed in the preparation of the material: It is essential that the capillary pipettes used in the test should be of equal caliber. It is evident that any difference in the caliber of the pipettes would render the test inaccurate. It is also very important that the cells for the antigen and amboceptor be prepared of pure embryonic cells of the type desired - - for example, the capsules and ducts of the pancreas and submaxillary gland must be removed thoroughly so that no remnants of fibrous tissue will be left. In the preparation of the connective-tissue amboceptor as well as the antigen, Wharton's jelly must be free from blood. The antigen must be dried until it assumes a doughy character -- not more nor less than that. Special care should be taken to avoid scorching the tissues while drying. It is essential that the drying process be continuous, preceeding to the finish without stopping.

Gruskin's summary and conclusion follow: (1) The theory has been proposed that normal tissue equilbrium is dependent upon a balance maintained by the opposing action of lytic agents reproduced respectively by connective tissue and epithelial cells and that neoplasia is the result of a lack of such lytic agents on the part of the tissue invaded. (2) Based upon this theory, a diagnostic test for malignant disease has been developed. (3) The principle of this test is based upon the homologous relationship between embryonic cells and the sera of patients suffering from malignant disease.

(4) The homologous nature of embryonic cells of different mammalians has been demonstrated by the ability to develop amboceptors and antigens with embryonic cells of different mammals.
(5) A new source for obtaining embryonic cells has been developed and described. (6) This test has been employed in a group of cases of malignant and non-malignant disease and results obtained which tend to substantiate the proposed theory and validity of the test.
(7) The results obtained are of theoretical interest in substantiating the proposed hypothesis and are believed to be of practical importance in the serodiagnosis of carcinoma and sarcoma.

TABLE I —RESULTS OF TEST IN KNOWN MALIGNANT TUMORS

No. of Cases	Pathologic Report	Serologic test
138	Carcinoma of various types	Positive 130 Negative 3 Doubtful 5

TABLE II — RESULTS OF TEST IN KNOWN BENIGN TUMORS

No. of Cases	Pathologic Report	Serologic test
20	Various benign growths	Positive 2 Negative 17

TABLE III - RESULTS OF TEST IN NON-MALIGNANT DISEASE

Disease	No.of cases	Negative	Positive
Endometritis	12	12	0
Diabetes	72	70	2
Lues	61	60	1- Leupoplakia of tongue
Pernicious anemia	3	3	0
Nephritis	27	27	0
Thyroid disease	156	152	4
Tuberculosis	5	5	0
Osteomyelitis	4	3	1 - faintly positive
Mastitis	5	5	0
Amebic dysentery	1	0	1 -serum contained Chyle
Polycythemia	2	2	0

TABLE IV — RESULT OF TEST IN NORMAL ADULTS

No. of Cases	Negative	Positive
146	143	3 - Faintly positive

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In 1932 Gruskin published his results with a new method whereby malignancy could be determined by means of an intradermal test. This reaction is based on the theoretical consideration that malignant cells are born embryonic and remain embryonic in contra-distinction to normal cells which are born embryonic but later mature (21). The protein of normal cells differs from that of these embryonic malignant cells, which, as a chemical proof may be shown by the fact that malignant growths and embryonic cells when incinerated leave a heavy deposit of organic salts, whereas, normal tissue and benign growths, upon incineration, permit only a very faint deposit of inorganic salts. Biologically, in syphillis in utero during the first six weeks, the spirochete does not attack the fetus but rather is to be found in the placenta or maternal site. Thus it is clearly evident that when the fetal cells are truly embryonic the spirochete does not attack them except when the cells become mature. By virtue of the fact that the malignant cell is embyonic in character and that its protein is different from that of the normal cell, the specificity of these proteins is mainfested by their production of a precipitation with malignant sera and pseudopod formation when injected intradermally. Since malignant cells are embryonic in character, antigens have been prepared from pure embryonic cells. For carcinoma are used the epithehal cells obtained from the pancreas or liver of embryonic calves; and for sarcoma is used Wharton's jelly for the embryonic stellate connective tissue cells which it contains. Saline extracts of these tissues are used for the intradermal test. Because of the homologous nature of the embryonic and malignant proteins, the extracts produce a precipitation with the sera of malignant patients, and pseudopod formation when injected intradermally into malignant individuals.

"Preparation of Antigen, Preliminary Steps"

It is essential that in the preparation of the antigen, both for carcinoma and sarcoma, all glassware and other utensils used be chemically clean, serile, and of neutral reaction (22).

"Preparation of the Antigen for Carcinoma" Mammalian embryos are used (calves, sheep, pigs). They must not be in a later stage than the second month of fetal life. This is readily recognized by their relative smallness, and by the smoothness of the skin (for instance, there is no formation of hair), In securing these embryos, the abdomen must be opened under sterile conditions. The pancreas and submaxillary glands are dissected out, and placed in a sterile dish. A hypotonic salt solution is poured over these, and if possible, allowed to freeze, the object being to permit an easier removal of the fibrous capsule. The capsule and the ducts, etc., are then removed by careful manipulation with small tissue forceps. The epithelial tissue is picked out. It is placed in a mortar in which sterile copper gauze is inserted (to facilitate the maceration) and macerated in physiologic salt solution. At this point, the cells suspended in salt solution are centrif uged and the supernatant salt solution is discarded. The epithelial cells are placed in a porcelain dish and dried at a temperature of 75 degrees C. for a few minutes until the water separates, which should then be poured off, leaving the cells in a state of doughy consistency. These are then covered with ether, shaken up, the ether decanted and the cells placed at room temperature for a few minutes to allow the remaining ether to evaporate. It is then rubbed up with twenty-five times its volume of one-tenth normal sodium hydroxide, by adding the sodium hydroxide slowly, one cubic centimeter at a time, and macerating it vigorously until the cells are

thoroughly rubbed up, so that on standing for awhile the least amount of the remaining cells fall to the bottom. It is placed at room temperature for twenty-four hours, after which it is centrifuged at a low speed for ten minutes, the supernatant fluid is then pipetted off and neutralized with one-tenth normal hydrochloric acid, drop by drop, until it is brought to neutrality, at a P_h of seven.

Recent experiments have shown that embryonic liver may be used to advantage in the preparation of the antigen for carcinoma, since a larger amount of material may be obtained with greater ease. From the embryonic liver the cells are obtained in the following manner: the whole liver is cleaned of the capsule and washed with water to remove as much blood as possible. It is then placed in an Erlenmeyer flask with water and shaken vigorously until all the cells are separated from the fibrous tissue. The cells and water are then centrifuged at high speed for five minutes, the water poured off and the cells washed repeatedly until all traces of blood are removed. For the intradermal antigen the wet cells are rubbed up with twenty volumes of one-tenth normal sodium hydroxide, allowed to stand for twenty-four hours, and then neutralized and prepared as in the case of the embryonic pancreas. For the serum antigen, the cells are placed in five volumes of acetone for twenty-four hours, then centrifuged, the acetone poured off and the cells dried in a desiccator. They are then rubbed to an impalpable powder and mixed with alcohol as described in the case of the embryonic pancreas.

Antigen for Carcoma -- A mixture of Wharton's jelly and red bone marrow of calf embryo is macerated in a mortar, to which two volumes of salt solution are added. This is placed in a procelain dish at a temperature of 75 degrees C until the water separates, which should then be poured off, leaving the cells in a state of doughy consistency. These are then covered with ether, shaken up, the ether decanted and the cells

are placed at room temperature for a few minutes to allow the remaining ether to evaporate. It is then rubbed up and with one-tenth normal sodium hydroxide, in the proportion of one gram of cells to 25 cc. of sodium hydroxide. The sodium hydroxide is added slowly, one cubic centimeter at a time, macerating the cells vigorously until they are thoroughly rubbed up, so that on standing for awhile the least amount of the remaining cells fall to the bottom of the tube. The tube containing the solution is placed in the ice box for twenty-four hours after which the supernatant fluid is pipetted off and neutralized with one-tenth normal hydrochloric acid, added drop by drop, until it is brought to neutrality, at a P_h of seven.

After being neutralized, the antigens are then placed in small vaccine bottles and kept in a cool, dark room, and are ready for use.

The Test Proper -- Two-tenths of a cubic centimeter of the antigen is injected intradermally with a very fine needle. Care must be taken that the injection should not be forced, so that no false pseudopods will be formed. In positive cases, a slight area of inflammation with pseudopod formation appears within fifteen minutes. In negative cases, no such reaction takes place. It is advisable to use a control of plain physiologic salt solution with each test. The control must always be negative, showing no inflammation and no pseudopods.

Precautions -- In patients who are emaciated and where the skin assumes a paper-like thinness so that a correct intradermal test is impossible to be performed, it is advisable to resort to the serologic test described by the author in the American Journal of the Medical Sciences, April 1929 (19). For in these cases on account of the irregularity in the contour of the skin, one might mistake the natural appearance for pseudopods, and, vice versa, the pseudopods might not be easily distinguished.

It is also advisable not to perform the intradermal test on patients with septic temperatures or jaundice, nor soon after x-ray, radium treatment, or anesthesia, as false reactions might take place in such cases.

The theory is advanced that the characteristic embryonic protein is carried not only in the blood stream but also finds response in the fixed cells, as expressed by the allergic reaction. The correct results obtained in a great number of positive and negative cases have been demonstrated, so that we feel justified in publishing this preliminary report. It is of interest that in 116 cases of intradermal tests done on students under the auspices of Professor Fanz, head of the Department of Pathology of Temple University School of Medicine, the following results were obtained:

One hundred and seven students gave no reaction.

Eight students gave a slight reaction to carcinoma, and of these, the following history was obtained: 1 had a maternal history of malignancy for three generations; 1 had a maternal history of malignancy for two generations; 6 had a family history of malignancy for one generation.

One student gave a slight reaction to sarcoma and no reaction to carcinoma. He had a paternal history of sarcoma (23).

A summary of cases subjected to the intradermal test is given below: (21). The following chart is a resume of 75 cases from Bacon's service giving a positive or negative Gruskin test verified by operation, necropsy or both.

No. of cases	Gruskin Test	Histopathology
59	Positive 59	Carcinoma 58 Chronic inflammation 1
16	Negative 16	non-malignant tissue 16

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Cases of Dr. Wayne Babcock

No. of Cases	Gruskin Test	Histopathology
42	Positive 42	Malignant tissue 41 Non-malignant tissue 1

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Cases of Dr. Chevalier Jackson

No. of Cases	Gruskin Test	Histopathology
39	Positive 37	Carcinoma 37
	Doubtful 2	Non-malignant 1 Doubtful carcinoma 1
8	Negative 8	Non-malignant 6 Malignant 2

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Cases of Dr. Hammond

No. of Cases	Gruskin Test	Histopathology
4	Positive 3	Carcinoma 3
	Negative 1	Menengitis 1

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Cases of Dr. Hersey Thomas

No. of Cases	Gruskin Test	Histopathology
15	Positive 10	Carcinoma 10
	Faint positive 1	Carcinoma 1
	Negative 4	Non-malignant tissue 4

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At the Philadelphia General Hospital a group composed of specialists in the various fields of medicine (internists, surgeons, gynecologists, pathologists, readiologists, etc) cares for all cases of known or suspected malignance. A committee of three chosen from this group was appointed to determine whether the Gruskin skin test was sufficiently reliable to be adopted as a routine diagnostic procedure. The committee secured the antigen from Dr. Gruskin and was familiarized in the technique and interpretation of the test by its originator.

McFarland, Clark and Friedman's study was carried out to determine whether the Gruskin skin test for the diagnosis of cancer was sufficiently reliable to be adopted as a routine procedure (24). They state the technic resembles that employed in allergic work, although the resulting reactions are not at all comparable in intensity. A 3 plus Gruskin, for example, would be no more than a plus reaction to ragweed pollen. The technic demonstrated by Gruskin was carefully followed: 0.2 cc. of the first antigen, and 0.1 cc. of the later antigens, said to be stronger and better because of the lessened bulk, were injected intradermally into the skin of the arm of the suspect with a 27 gauge hypodermic needle. From fifteen to twenty seconds were required for the injection and, in those cases regarded as positive, the blanched wheal resulting from the injection soon became reddened and very delicate pseudopods could be observed at its periphery in from five to ten minutes. The results were expressed aa zero or plus, two plus, three plus and four plus, according to the intensity of the reaction. A total of 199 tests upon 174 individuals was made, each test being checked by two observers.

Control tests were made upon 71 patients (85 tests) suffering from a variety of chronic, non-malignant diseases and 14 doctors and nurses in supposedly good health: 74.1 per cent of these tests proved negative.

Of 22 positive reactions (including seven two plus and one three plus) among the controls, 8 were in presumably healthy nurses. Two patients with parotid tumors gave negative reactions, while a third case of parotid tumor gave a negative reaction upon the first test and a two plus upon a later one. Negative tests were also obtained in one case of giant cell tumor, one of Hodgkin's disease and two of lymphatic leukemia.

The malignant cases consisted of 103 known cases of cancer in which the diagnosis had been confirmed by a biopsy, or in which it was clinically evident through the occurrence of metastases, by observation at laparotomy or through x-ray examinations.

In 32 cancer cases confirmed by biopsy and already under x-ray or radium treatment, 34 tests gave 82.3 per cent positive and 17.7 per cent negative reactions. One case yielded an ambiguous result, and another differed in the strength of the reaction according to the antigen used. In a group of 27 known cancer cases that had never received any form of irradiation, 32 tests gave 68.7 per cent positive and 31.3 per cent negative reactions. In a group of 20 cases, in which the diagnosis of cancer was clinical only, and in which there had been no irradiation, of 22 tests, 81.8 per cent were positive and 18.2 per cent negative. In 24 patients who had been irradiated and in whom the diagnosis of cancer was clinically outspoken, 26 tests gave 69.2 per cent positive and 30.8 per cent negative results. A total of 114 tests in 103 cancer cases gave 86 positive and 28 negative reactions. In the 86 positive tests there were only 21 two plus and three (three plus) reactions.

The average percentage of accurace for the entire series of 199 tests, including both positive and control series, was 74.8 per cent.

However, duplicate determinations were made in 10 individuals and in 3 of them the result changed from positive to negative to vice versa.

Difficulty is encountered in the application of the test in cachetic and dehydrated individuals. Many times a reaction suggesting malignancy appeared but disappeared so rapidly that it had to be read 'plus-minus' and regarded as negative in the calculation for percentage accuracy. It was suggested..... that if wet dressings were continuously applied to the skin for 24 hours before the test, the local dehydration might be overcome, and the measure did seem to be of some value in the few cases in which it was tried.

The occurrence of an unusually marked reaction to the test in a patient who had just received an intravenous injection of 'Synadol,' a proprietary remedy containing emetine and various lipoids, suggested that it might be used as a means of intensifying the reaction. The Gruskin antigen itself was tried as an intensifying agent by immediately following the endodermal injection of the antigen by a subcutaneous injection of 0.5 cc. of the same antigen. Within 24 hours the patient experienced itching of the skin, and repetition of the intradermal test performed at that time produced more distinct reactions in several cases. In one case a typical urticaria, localized to one arm, followed the second test. Control cases did not react similarly.

Unquestionably any test which will aid in the early diagnosis of cancer should be a distinct adjunct in the institution of early treatment, and so assist in the care of the disease. Such a test, however, should be so simple as to be easily performed, and so reliable as to be superior to all other diagnostic measures. From McFarland, Clark and Friedman's experience the "Gruskin skin test for cancer" does not fulfill these specifications.

Gruskin declares this to be an allergic reaction. He also observed that various types of embryonal cells emit such differently chemical sensitizing products as to permit of the identification of various types of tumor by the use of antigens made from different types of embyonal tissue.

McFarland cannot agree that there are essential differences between embryonal and adult cells other than can be accounted for by their difference in activites, growth and multiplication preponderating in the former and secretion, contraction, nerve impulse transmission, etc. in the latter it is also entirely in error to suppose that cell multiplication is indicative of embryonal character in the sense used by Gruskin. Noting that patients in the best of health occassionally give positive reactions -- Gruskin concludes that the test not only reveals the presence of existing cancer, but also may foretell the future probability of cancer. In other words, the test that is to be depended upon for the immediate diagnosis of the disease may only be indicating its future and remote possibility. Such an unfortunate admission destroys the value of the test. We want to know whether the patient has cancer now, not that he may get it twenty years hence.

-- But this is not all, the reactions of immunity and allergy are among the most specific known, so it becomes necessary to consider carefully the so-called antigens employed in the presentation of the test— from the experience of our friends, ourselves and the originator, this antigen is extremely difficult to make— there is no method of titration by which its usefullness can be measured or the quantity necessary to produce the reaction determined -- the antigen is, therefore, too variable a reagent to depend upon -- this concept seems to be so contrary to all of the known facts of blood relationship, specific precipitation and allergy as to be unimaginable. If the extracted protein, constituting the "antigen" is an

embryonal extract, if cancer cells are also embryonal, and if the latter can be conceived to have sensitized the body to embryonal substance, it would not follow that the former would react with the latter. The embryonal extract used in the "antigen" is more than an embryonal extract; it is an embryo sheep extract, or calf extract or pig extract. The phylogenetic divergence of these animals is from the very beginning, etc. -- but behind all this lies a more profound biologic defect, for the whole scheme is founded upon certain assumptions that are oncologically unsound. We have become accustomed to employ the words "carcinoma" and "sarcoma" so familiarly that they have come to imply more knowledge than is justified by the facts, Carcinomas and sarcomas are clearly separated in theory but not in fact. The sarcoma group is extremely vague and puzzling and has been subject to many subtractions in recent years and will be subject to many more, no doubt. Almost the whole oncological classification and nomenclature is in a confused state that affords such evidence of present ignorance of the origin and nature of the tumors themselves as to make it impossible of such specificity as the Gruskin test presupposes can obtain.

Gruskin himself thinks that worthy of consideration is the possibility that innoculation of a patient with an antigen made of cancer tissue, enough to produce an allergic reaction, might exert antigenic influences in encouraging malignancy in an inherently susceptible individual (27). The fact that implantation of cancer tissue does not develop malignancy does not prove contrary to the above possibility for the simple reason that when cancer is implanted, degeneration of the implant rapidly takes place on account of the lack of blood supply, and therefore, the antigenic influence does not assert itself; while when an

extract of purely epithelial cells is introduced, their characteristic protein might act immediately, as demonstrated by the allergic reaction.

S U M M A R Y

The more important serological reactions for diagnosis of cancer are given below in summary:

Test	Correct Results	Comment
ABDERHALDEN	55% - 70%	Does not differentiate sarcoma from carcinoma
WEIL	Variable in hands of different workers	Action of hemolysis does not differentiate carcinoma from sarcoma
ELSBERG	89% - 100%	The first intradermal test does not differentiate carcinoma from sarcoma. Insufficient data to draw definite conclusions
FREUND	Results not tabulated	A precipitation serum reaction which differentiates sarcoma and carcinoma. Insufficient data to draw any conclusions.
SHAW-MACKENZIE	98% -100%	A turbidity test on sera, only 136 cases mentioned. Insufficient data to draw definite conclusions.
FRY	71% -78%	A flocculation reaction tested in 500 cases. Errors obtained in sepsis and tuberculosis.
ROFFO	30% -50%	A non-specific test, negative reaction does not rule out cancer. Test little value.

S U M M A R Y (Continued)

Test	Correct Results	Comment
LANDAU	75%	Precipitation test worked out on over 1,000 patients. False reactions occur in tuberculosis, syphillis and pregnancy.
VERNES	Results not reported.	Flocculation of cancer blood serum with copper acetate.
BOTELHO	75%	Precipitation reaction with suspected serum.
FREUND-KAMINER	About 85%	Intradermal test using carcinoma fatty acid. The reaction is of value in differential diagnosis in conjunction with the clinical findings. The test should be subjected to further clinical examination.
LINK	About 80% or better	Chemical test of serum content. Sarcomas do not respond. Small number of cases tested does not allow conclusions as to its specificity.
GRUSKIN	Above 90%	Serum flocculation test to differentiate carcinoma and sarcoma. Test based on the homologous relationship between embryonic cells and the sera of patients suffering from malignant disease.
GRUSKIN	Well above 90% in hands of some observors. McFarland (24) find percentage of accuracy to be about 74.8%	Intradermal test based on the theory that the characteristic cancer embryonic protein is carried not only in the blood stream but also finds response in the fixed cells, as expressed by the allergic reaction.

CONCLUSIONS: (See next sheet)

S U M M A R Y (Continued)

CONCLUSION:

1. A brief summary of some of the more important methods of serologic diagnosis has been presented.

2. The Gruskin tests have been presented in some detail.

3. This writer is not in a position to judge the relative merits and disadvantages of the tests propounded by Gruskin.

4. Serological methods for diagnosis of cancer offer possibilities in developing valuable adjuncts to the diagnosis of malignancy, and as such are worthy of more general attention and research.

W. L. Mays
4-15-36

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