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Albumin-s test in the diagnosis of malignancy

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THE ALBUMIN-S TEST IN THE DIAGNOSIS OF MALIGNANCY

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INTRODUCTION

The increased interest in the problem of malignancy on the part of both the layman and the scientist in recent years is apparent daily. The layman is as aware as the scientist that the largest single obstacle thwarting the scientist's attempt to defeat cancer is the problem of diagnosis, particularly early diagnosis. For many years simple and painless diagnostic methods have been developed and tested, but none has been all-encompassing nor foolproof. Quite accurate and usable tests have been developed for specific types of carcinoma, but no single test for cancer of all types has been found. The purpose of the work reported in this paper was to determine the value of a test which might possibly be of the latter type. The value of a test for cancer present in any organ of the body and of any histological type is apparent to anyone having any knowledge of the problem of cancer.

The test to be presented in this paper is a serological test. To furnish a background for this presentation a brief historical review of serological tests for malignancy may be considered. Serodiagnostic tests fall roughly into three categories, and, to keep some order, the tests will be divided into these categories, namely: (1) enzymic reactions, (2) immunological re-

actions, (3) comparative concentrations of serological constituents or physicochemical characteristics of these constituents.

(1) Enzymic reactions:

Probably the first test of this type was reported by Freund and Kaminer in 1910. They felt that serum from normal patients caused a lysis of carcinoma cells which was greater than the lysis produced by serum from carcinoma patients.

Shaw-McKenzie (1914) reported that normal serum augmented the action of pancreatic lipase and, incidentally, found that serum from carcinoma patients showed a lessened tendency in this direction.

Aberhalden (1922) postulated the existence in serum of specific proteolytic enzymes for specific carcinomas, as well as for other foreign proteins.

Fuchs (1926), using a different enzymatic approach, stated that serum from cancer patients hydrolyzed fibrin from normal blood but not fibrin from the blood of cancer victims. Conversely, he reported that serum from normal patients hydrolyzed fibrin of cancer blood but not that of normal blood.

Bernhard (1933) reported a finding similar to Shaw-McKenzie's, saying that he found an increase in a lipase

action of serum from cancer patients.

(2) Immunological reactions:

Brieger and Trebbing (1908) reported that their work indicated antitrypsin factors in carcinoma serum.

Hirszfield, et al, (1929) attempted to demonstrate a specific immune reaction using hydrolysates of tumor tissue as an antigen. Mann and Welker used the same type of antigen in 1940. Lehmann-Facius (1932) improved upon this type of reaction by using a phosphatide fraction as an antigen. The fraction was obtained by acetone precipitation of the benzene soluble lipids in extracts of cancer tissue. This antigen showed rather marked specificity for cancer serum.

Hirszfield, et al, Reichner (1933) and Witebsky and Morelli (1933), while not able to show specific immune reactions, could demonstrate some degree of specificity by absorbing out common tissue factors. Kidd and Friedewald (1942) used purified protein fractions of tumor tissue as an antigen.

(3) Serological constituents:

Kahn (1923) found that serum from cancer patients flocculated in glycerin at low dilution when sodium oleate was added.

Ascoli and Izar (1910) added a 10% solution of ricinoleic acid in methyl alcohol, plus normal saline to serum. They felt that cancer serum showed a heavy flocculation, while normal serum showed only a slight opalescence.

Roffo in 1925 developed a rather simple test in which he added 1% neutral red to sera, a red color being positive for malignancy, while a yellow hue was negative.

Starlinger and Winands (1928) demonstrated a rise in the fibrinogen level of serum in cancer.

Probably the most valuable of the tests of this class, and the most widely used, are based on the work of Kay (1929) in which he found an increase in serum alkaline phosphatase in hyperplastic bone disease and the work of Gutman and Gutman (1938) wherein they demonstrated the presence of serum acid phosphatase in metastatic carcinoma of the prostate.

Noel (1931) reported an increased saponification and acid number of the fats in cancer sera.

Several tests have been developed which depend upon a precipitation reaction by various reagents. Bendien (1931) used sodium vanadate and acetic acid, and the degree of precipitation was important; Wigand (1926) used tannic acid and carbol fuchsin as precipitants, obtaining precipitation in twenty-four hours in certain

dilutions of carcinoma serum; Lowe (1933) used sodium vanadate on one specimen of serum, sodium vanadate plus 56° c on another, and ether extracted still another specimen before reaction, thereby obtaining a ratio of precipitation as a basis for diagnosis; Vernes (1932) precipitated serum proteins by increasing amounts of calcium acetate, establishing a turbidometric curve with specific variations found in serum taken from carcinoma patients. Botelho (1926) observed that citric acid enabled the iodine in potassium iodide to precipitate proteins more readily in cancer serum than in normal serum. Mondain, et al, (1926) modified Botelho's test somewhat. Duris and Giquel (1924) employed simple distilled water as a precipitant, while Suranyi (1927) used both hydrochloric acid and a combination of acetic and sulfosalicylic acids. Sachs (1932) developed a precipitation reaction using alcoholic lecithin as a reagent.

Kopaczewski (1934) reported that gelation of serum by lactic acid was more rapid in cancer sera than in normal sera.

Luiks (1934) found that the potassium-magnesium ratio of cancer serum is altered during clotting.

Brdicka (1933) measured serum albumin fractions by polarographic wave studies and found an alteration in carcinoma.

Winzler and Burk in 1944, Petermann and Hogness, and Petermann and Karnofsky in 1948 reported a mucoprotein in the serum of patients with carcinoma of the lung or stomach, which is not normally present.

Black (1947) reported that the reducing power of serum is diminished in the presence of malignancy and used methylene blue as a reagent to show this characteristic.

Shetlar, et al, (1949) found that a certain serum polysaccharide which is associated with serum protein shows a higher level in the serum of patients with carcinoma than in normal individuals.

In the same year Huggins, et al, aroused considerable interest when they reported that an alteration in serum albumin where cancer is present causes a lessened thermal coagulation of serum and, moreover, that the amount of iodo-acetate necessary to inhibit this coagulation is lessened. This fact was assumed to be due to a diminution in serum albumin.

Herly (1950) reported that cancer sera transmit more light and fluorescence than normal sera when exposed to ultraviolet light.

Waldvogel and Schmitt (1950) found that the anti-trypsin titer of rat serum was increased in malignancy.

THE ALBUMIN-S TEST

The test presented in this paper was reported originally by Kahn in 1924. A portion of the serum albumin referred to as the hydrophile fraction, or albumin-A, was extracted with ammonium sulphate and measured. He found this portion to be much reduced in cancer. He also discovered that a high concentration of this albumin was present in tumor tissue. Hanke, et al, referred to the albumin-S fraction of serum in 1944. This substance corresponded to the previously mentioned albumin-A. Albumin-S was defined in this report as "that fraction of serum protein which remains dissolved at 41.5 gm%. Ammonium sulphate, at pH 6.8, in a 1:250 dilution of serum. In human cancer albumin-S values are decreased to less than half, even when total albumin is normal or three-fourths normal." In this same paper, furthermore, it was stated that bile acids possess the ability to decrease this soluble albumin fraction alone. A substance extracted from urine showed this same power, and since this urinary constituent is elevated in the presence of malignancy, it was postulated that this substance

(a non-saponifiable benzene soluble extract) was responsible for the finding of a lowered albumin-S fraction in cancer.

Since the first scattered reports on the albumin-S test some changes in technique and material have come about, however the principle of the test remains the same. Albumin-S is the most soluble fraction of serum albumin and is now known to constitute three to fifteen per cent of the total albumin of normal serum. It is now felt that in cancer the albumin-S value ranges from one-half to two-thirds of normal.

THE ESTIMATION OF ALBUMIN-S IN SERUM

A great variety of procedures have been used for the measurement of albumin-S, which differ chiefly in three respects: (1) the nature of the protein-precipitating or extracting solution, (2) the experimental conditions of time, temperature and humidity, under which the fractional precipitation or extraction takes place, and (3) the manner of separating solid and liquid phases, that is by filtration or by paper extraction. These methods all have three things in common: (1) they measure a small fraction (3 to 15%) of the albumin of normal serum, (2) they usually distinguish normal from cancer

sera, and (3) in the conditions under which albumin-S is measured, there is a step that is a range in the concentration of the precipitating (or extracting) solution through which there is no significant change in the measured amount of albumin-S. At all other concentrations of the precipitating (or extracting) solution, a small change in its concentration causes a marked change in the amount of protein which is precipitated (or extracted); but at a certain concentration of precipitating (or extracting) solution, the one used in the analytical procedure, a significant change in the concentration of the precipitating (or extracting) solution causes no change in the distribution between solid and dissolved protein.

In the method here described, o.1 ml serum is placed on a strip of filter paper, and after a certain time, during which the serum proteins become fixed onto the paper, the paper is immersed for one hour in 38% ammonium sulfate solution at pH 6.8. The concentration of ammonium sulfate and the duration of extraction are so chosen that a small fraction of the albumin, called albumin-S, passes into solution, while the rest of the albumin and the globulin remain on the paper. After removal of the paper, the concentration of protein in the ammonium sul-

fate solution is determined by photoelectric measurement of the turbidity which is formed on heating in a boiling water bath.

There are two methods of handling the filter paper in the procedure. The simpler method consists merely of a five minute drying in air after the serum has been applied to the filter paper and before immersion in the reagent. The second method involves a humidification of the filter paper since humidity appears to have some effect. The series presented in this work was done by a mixture of the two methods, with the first method giving a better percentage of usable results. The second method could not be used on humid days. The description following is concerned with the humidifying method of handling the paper, since it is the only one of the two that requires explanation. In all other respects, exclusive of the handling of the filter paper before immersion, the two methods are identical.

Preparation of filter paper: Schleicher and Schull #595 filter paper is cut into strips 12 x 100 mm. A hole 6 mm. in diameter is punched with its center about 6 mm. from one end. If the method utilizing humidifying conditions is used, the following further treatment is followed. A group of 50 or more such papers is suspended by this hole from a glass or rust-proof metal hook, at-

tached to the lower surface of a rubber stopper, which in turn fits into a storage bottle, or flask, containing a 1 to 2 cm. layer of water, so that the lower end of the paper hangs about 2 cm. above the surface of the water. It is important that no liquid ever touch the paper. Since water usually condenses on the walls of the container, it is important that the papers do not come in contact with these walls. The papers should not be packed too tightly in order that water vapor may circulate among the papers. The papers are thus stored over water for at least twelve hours. As described later, in order to insure complete saturation with water vapor, immediately before the serum is measured onto the paper, each paper is suspended singly for at least five minutes in a container saturated with water vapor. The humidification of the paper thus involves two steps: a preliminary exposure to saturated water vapor of a group of papers for at least twelve hours and a final exposure singly for at least five minutes.

Preparation of 38.0g% $(NH_4)_2SO_4$, 0.01 M ammonium phosphate pH 6.8: Weigh out 760 gm. ammonium sulfate in a liter beaker and transfer with the aid of about 1600 ml water to a 2 liter volumetric flask. Add 9 ml 85% H_3PO_4, 16.3 M, (commercial concentrated orthophosphoric acid)

measured accurately with a pipette, rinse any concentrated H_3PO_4 from the neck of the flask with a little water and add 20 ml concentrated NH_4OH (about 18 M). Direct contact of the concentrated H_3PO_4 and concentrated NH_4OH should be avoided because of the violent spattering and evolution of heat. Dilute to the mark, mix, and note that all the ammonium sulfate has passed into solution. Adjust to 20°, dilute to the mark again if necessary, and mix again. A slight turbidity, if present, may be removed by filtration.

Measure the pH with a glass electrode. If it is not between 6.6 and 7.0, add minute amounts of concentrated NH_4OH or concentrated H_2SO_4 until it is 6.8 ± 0.2. Since the buffer index is about 0.04, 0.2 ml concentrated H_2SO_4 (36 M) or 0.4 ml concentrated NH_4OH added to 2 liters will cause about 0.1 pH change. On exposure to air the solution gradually loses ammonia and thus becomes more acid, therefore it should be stored in a bottle with a tightly fitting rubber stopper. A variation of 0.1 pH causes a change of about 0.2 scale divisions in the photoelectric reading.

In order to check the concentration of ammonium sulfate measure accurately 5 ml portions of the solution into 30 ml beakers weighed to 0.1 mg. Evaporate gently

at 90 to 100° to avoid spattering and, finally, heat in an oven at 110° for two days. The net weight should be 1.940 g ≠ 5 mg. (1.900 g $(NH_4)_2SO_4$ and 0.040 g $NH_4H_2PO_4$). If the concentration is not correct to 0.1%, the solution should be appropriately diluted by addition of water, or concentrated by the addition of solid $(NH_4)_2SO_4$, to make it correct.

Arrangement of bottles saturated with water vapor: Twelve 500 ml wide-mouth bottles(or Erlenmeyer flasks) are fitted with rubber stoppers into the middle of the underside of which is inserted a glass or rust-proof metal hook, from which one of the filter paper strips can be suspended. About 50 ml water are added to give a layer 1 or 2 cm. deep. The bottles are arranged in three rows of four each.

Procedure: Measure accurately 20 ml of the 38.0 g% $(NH_4)_2SO_4$ solution into each of a series of numbered test tubes, of 16 mm. internal diameter. Since duplicates are run on all sera, the number of tubes of $(NH_4)_2SO_4$ solution will be twice the number of sera to be analyzed.

Transfer single strips of filter paper from the storage bottle into each of the twelve containers saturated with water vapor. Fill a 0.1 ml pipette (with a fine tip to insure precise volumetric measurement) and adjust

the meniscus to the upper mark. Take one of the papers which has previously been suspended singly for at least five minutes in a humid container and at once measure the 0.1 ml. serum onto the paper in the course of twenty to forty seconds while spreading the serum over a length of 70 mm. of the paper, starting about 15 mm. from the end with the hole (where the paper is held) and ending about 15 mm. from the lower end. Transfer the paper back to the humid container, taking care to avoid contact of the paper and the walls of the container. Continue with another paper and serum sample until two rows (a total of 8) are measured. Remove each of the first four papers from the humid bottles and immerse at once in the appropriately numbered tube of ammonium sulfate solution. Note the time so that the duration of immersion can be controlled to be 60 $\pm$ 2 minutes.

Replace the single papers in the first row of four humid containers by transfer from the stock bottle and continue with the measurement of serum onto the papers in the third row. Next, transfer the papers from the second row to the appropriate ammonium sulfate tubes and replace with new papers from the stock bottle. Measure serum onto the papers in the first row, followed by transfer of the papers in the third row into their appropriate tubes containing ammonium sulfate solution. The manip-

ulation of each row requires about five or six minutes, four minutes for the serum measurements and one or two minutes for the two kinds of paper transfers from the four humid containers to the corresponding ammonium sulfate tubes and later replacement of the paper in these humid containers from the stock bottle which holds the large supply of papers. This schedule automatically insures that each paper will be exposed singly to the humid atmosphere at least five minutes before the serum is measured and at least another five minutes after the serum is measured. Prolonging these times of exposure to ten or fifteen minutes, or even to an hour, causes no significant difference in the result.

When the time of immersion of any paper is 60 ± 2 minutes, remove the paper with the hooked end of a small glass rod. According to the schedule previously outlined for the measurement of the serum four papers will be transferred to the $(NH_4)_2SO_4$ solution tubes in the course of one or two minutes once every five or six minutes, and just an hour later these paperswill be removed. If this schedule is adhered to, the timing of the removal of the papers will be facilitated, but any longer time schedule of serum measurement or exposure in the humid containers may be adopted, so long as the duration of extraction in the ammonium sulfate solution is kept at

sixty minutes. Collect a group of tubes containing the clear ammonium sulfate solution extracts in a wire rack and place in a boiling water bath for three to five minutes. If the water is not actively boiling, the apparent turbidity as measured in the photoelectric colorimeter will be too great. Since the immersion of the cold tubes usually stops the boiling, it is important, in order to insure uniformity, that the water be actively boiling with the tubes immersed for one-half to three minutes.

Remove the tubes from the boiling bath and allow to stand until cool (one-half to two hours at room temperature). Then read the turbidity in a photoelectric colorimeter as follows: With a 420 blue filter set the clear 38.0 g % $(NH_4)_2SO_4$ solution at 100, note and record the center setting. Invert each tube to insure uniform mixing and transfer the contents of each tube in succession to this same colorimeter tube, after rinsing each time with about two 1 ml. portions of each suspension.

Dr. Hanke, by communication, states that normal serum shows a G reading of from 88, or a net turbidity of 100-88 (12 scale divisions) to 91.5 (8.5 scale divisions). By reference to the resultson normal sera in this series one sees that the highest normal reading obtained was 11.5

scale divisions, and only 25% read 8.5 or over. It is his feeling that a reading of less than 7.5 scale divisions is positive for cancer and 7.5 to 9 is listed as doubtful. However, in this series the majority of the normal readings (80%) ranged from 6 upwards, so our range of readings showed some variation from those he uses. From these differences one might wonder if the normal and malignant ranges of readings must be worked out for each locality before the test can be applied.

PATIENT TESTING AND RESULTS

Ninety-five patients were tested in this series. It must be admitted that this series is too small to present any conclusive evidence about the test, however, limitations of time and technical difficulties prevented the series from being larger. Work on this project began early in the spring of 1951 and ran through the summer. For the ninety-five usable determinations approximately three hundred testswere run, the remainder being discarded due to faulty technique, control reading veering too far from a reasonable range, all results in any one mixed group being very similar, etc.

The patients tested were divided into three classes: (1) suspect, or ill patients subsequently found to have no demonstrable carcinoma, (2) controls or known normal

persons, (3) known cancer patients, including those afflicted with lymphoma. It soon became apparent that obstetrical patients tested frequently showed readings in the malignant range, and so for purposes of reporting they are classed separately. Twenty-six suspects having such various pathology as hernia, appendicitis, myoma, chronic cervicitis, uterine prolapse, urinary infection, etc., were tested. Twenty-eight normal (or as nearly normal as possible to tell) sera were tested. Twenty-seven known malignancies were checked, and these are divided as shown in the following tables:

Skin	2
Lung	2
Breast	3
Brain	1
Stomach	1
Colon	3
Rectum	1
Ovary	1
Uterus	1
Cervix	7
Vagina	1
Testis	1
Hodgkins	2
Leukemia	2
Total	28

Number of Malignancies of Each Type Studied

Table I

Genital system	11
Gastro-intestinal tract	5
Respiratory tract	2
Lymph	4
Breast	2
Skin	2
Central nervous system	1

Malignancies Tested Divided as to System Affected

Table II

Ranges of reading in the three gross categories were quite broad, and, consequently, the value of the test is reduced. However, the grouping of readings in each category is a little better. The ranges are shown in Table III.

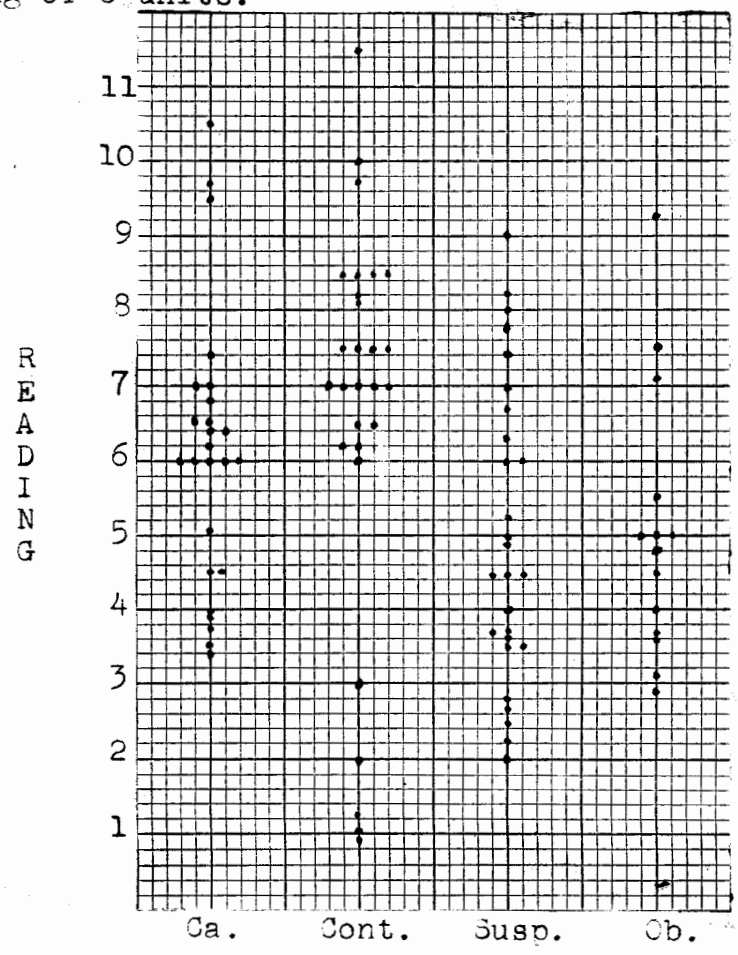
Controls	0.9 - 11.5
Suspects	2 - 9
Cancer	2.4 - 9.5
Obstetrical patients	2.9 - 9.25

Range of Readings in Each Group of Patients

Table III

The individual readings for each serum tested are shown in Graph I. Again, the range of results among the cancer group as compared to the suspect group appear disheartening but with a larger series might prove more valuable. While from this series the test shows little evidence of being valuable as a definitive diagnostic

test, it might very well be useful in the future as a screening test for supposedly well individuals, since a comparison of results in the cancer group with those of the control group show a sharper difference in grouping. Eighty per cent of the readings in the cancer group are below a scale reading of 6 units, while eighty per cent of the readings in the control group are above a scale reading of 6 units.



Individual Readings

Graph I

If one examines the results in each type of carcinoma, it is seen that the least satisfactory results are obtained in carcinoma of the cervix. Three out of the seven cases of proven carcinoma of the cervix showed G readings of over 6. This might have been true of some of the other types of cancer had this number of each of them been tested. However, it is well known that carcinoma of the cervix is a difficult lesion to diagnose in other tests for malignancy. One case was very heartening among the suspects. This case was suspicious for carcinoma of the cervix, including a positive Papanicolauv smear, but biopsy was negative, and the clinical evaluation after all tests were completed was that there was no carcinoma. The albumin-S reading in this case was 8.2. Other cervical lesions were troublesome in this series, for among the patients listed as suspects there were four diagnosed as chronic cervicitis and three as cervical erosions, all of whom showed G readings below 6.

Of the other individual types of cancer present in patients tested, only one case showed a G reading of over 6 (6.4), and this was in a carcinoma of the rectum. Since this is the only case of its kind in the series, no conclusion should be drawn.

Another suspect which showed a reading in what might be called the cancer range was a patient with a neoplasm

of the breast. Biopsy of this lesion was reported as adenofibroma, but the diagnosis was very guarded. The albumin-S reading in this case was 2.25. This patient was also suspected of having cervical carcinoma, but definite proof had not been obtained. Several patients of this type were included in the suspect series and might account for some of the false positives.

SUMMARY

1. A brief review of serological tests for carcinoma is presented.

2. A description of the albumin-S test for malignancy is given entailing principle, equipment and reagents and method of performance.

3. A series of 95 patients tested by this method is described. Patients are divided into controls (normals), suspects (non-malignant disease), known malignancy, and obstetrical patients.

4. The range of readings in each category are given in chart form, and the individual reading of each patient is presented in graph form.

5. An analysis of the data evolved is presented.

CONCLUSION

The series described in this paper is too small to formulate any definite conclusion concerning the value of the albumin-S test. However, the series indicates that the test may be of value as a screening test for supposedly well persons, since 80% of the normals read above a certain figure and 80% of the known carcinomas read below this figure.

The test is relatively simple to perform and would require no great amount of training to equip an operator.

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