University of Nebraska Medical Center
BREAKTHROUGHS FOR LIFE.

University of Nebraska Medical Center
DigitalCommons@UNMC

MD Theses
Special Collections

1952

A Possible new screening technique for cancer

John Richard VaVerka
University of Nebraska Medical Center

This manuscript is historical in nature and may not reflect current medical research and practice. Search PubMed for current research.

Follow this and additional works at: https://digitalcommons.unmc.edu/mdtheses

Recommended Citation
VaVerka, John Richard, "A Possible new screening technique for cancer" (1952). *MD Theses*. 1867.
https://digitalcommons.unmc.edu/mdtheses/1867

This Thesis is brought to you for free and open access by the Special Collections at DigitalCommons@UNMC. It has been accepted for inclusion in MD Theses by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

A POSSIBLE NEW SCREENING TECHNIQUE FOR CANCER

Use of a Fluorospectrophotometer

John Richard VaVerka

Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

December 15, 1951

Omaha, Nebraska

I. General Introduction

A. Necessary--reliable screening and diagnostic techniques:

Today, in a civilization such as ours, one hundred and thirty out of every 100,000 deaths each year, are due to cancer. At first glance this figure may seem low, but since the average duration of a case with a malignant tumor is about three years, the prevalence rate is about 400 per 100,000 individuals. This means that in our population today, one out of every two hundred and fifty people has cancer (1).

Since the advent of modern medicine attempts have been made to find a test diagnostic for cancer. Such a test would be specific, much as is the Wasserman test for syphilis. That there is at present, no one clinical or physical test not beset by an excessive percentage of false positives and/or false negatives should be agreed upon by all workers.

It may be pointed out here, that a working difference may be made between the terms "Screening" and "Diagnostic" as applied to cancer tests. A screening test should have a zero incidence of false negatives while it may be permitted to have a low incidence of false positives. That is it would overlook no cases

of malignancy, while it occasionally might falsely identify some other condition as being malignant. To be diagnostic, a test should have no false negatives, and no false positives.

In actual practice, most tests now called diagnostic, have a low error of false positives, while the chances for false negatives occuring are inherent in their nature. For example the Biopsy and the Papanicolaou tests may not pick up an early carcinoma of the cervix, tissue not being taken from the proper area or exfoliating cancer cells not being in the material obtained. This is not even considering the other form of human element involved, the pathologist and cytologist. The high degree of training and skill required in these men is well known, as is the fact that different pathologists may disagree as to the interpretation of the same tissue sections.

B. Basis of action:

There have been a multiplicity of tests published with the range of false positives extending from four percent to thirty percent. In general the tests showing the most usefulness may be divided into four modes of action:

1). Enzymic

These compare the protein-splitting or lipolytic qualities of the body fluids with normal body fluids, or measure in vitro enzyme-hydrolysis. An example of such a test is the measurement of Beta-Glucuronidase activity (2). These tests are difficult to carry out, due mostly to the problem of preparing the substrates, but seem to have a high degree of specificity. Opinions of these tests, however, vary considerably with different workers.

2). Antigen-antibody

These tests usually use water and fat solvent extracts of tumor tissues as antigens, but better results have recently been obtained using purified protein fractions of cancerous tissues (3).

3). Measurement of body fluid elements

Such methods compare the concentration of various elements of body fluids directly or indirectly with the normal (4), (5).

4). Pathologic and cytologic

Examples of these techniques are the before mentioned biopsy and Papanicolaou studies. As was already stated concerning the latter two

methods, the best of all tests to date require highly trained individuals to read them, and then instances will arise where there is great debate as to diagnosis. Measurements based upon altered metabolism of malignant tissues have shown varied results and, in general, fail to meet the definition of diagnostic. The ideal goal is a reliable, economical physical test capable of being read by relatively unskilled personnel. It should require no long series of complicated procedures, and would function best as a quick test the general practitioner could perform in his office. Should a test even meet the requirements of a rapid and economical screening technique that the doctor could use as part of a routine physical examination, it would fulfill a definite need.

II. Review of Body Fluorescence

A. Reaction of normal body tissues and fluids:

Fluorescence is the ability of some substances to respond with a visible light of longer wave lengths, when irradiated with ultraviolet light, generally of the shorter, invisible wave lengths. This is known as "primary" fluorescence. When a substance will fluoresce under ultraviolet light only after it has been previously sensitized by another compound (called a fluorochrome) it is said to exhibit "secondary" fluorescence. An example of the former is vitamin A; when exposed to ultraviolet this emits a quickly fading green fluorescence. An example of secondary fluorescence is that emitted by the tubercle bacillus under ultraviolet after being sensitized by the fluorochrome auramine.

Under ultraviolet light most tissues and complex organic substances derived from animal or vegetable sources display fluorescence. This general tissue flourescence is not a pure color but contains all of the visible spectrum of colors. (This may be verified by viewing this fluorescence through filters ranging from blue to red.) The observed color depends upon

the modification of this general "white" fluorescence by numerous factors applicable to any ultraviolet illuminated surface. In the main, these are the selective optical absorption properties of tissue and the physical surface characteristics. The former are modified in tissue by hormonal stimulation and the latter by such physical deformities as are produced by scarring and neoplasia. In addition to these truly altered colors there may be changes in hue due to factors producing "superficial" fluorescence; for example the presence of a surface fungus (6). One must also consider the ultraviolet source. If it is not filtered out, reflected ultraviolet light seen with the fluorescence may cause secondary fluorescence within the eye as well as producing an artifact due to reflected visible ultraviolet rays. There is also the difference introduced by the sensitivity of various eyes as a color receiver. Bearing these things in mind then, descriptions of normal localized body fluorescence will be given along with the changes seen with pathology.

B. Changes seen with pathology other than neoplasm:

The normal bluish-white skin fluorescence of the scalp is usually not well visualized due to the overlying hair. However clinical usage has long been made of the fact that a ringworm infection of the scalp (Tinea capitis) may be readily diagnosed by the use of a Wood's light. This is a filtered light source, passing only invisible ultraviolet light. Under this radiation the fungus infected areas of hair and scalp glow with a bright blueish-green fluorescence. It has likewise been long known that when the mouth is irradiated with far ultraviolet (short wave length, invisible ultraviolet rays) the dorsum of the tongue and sometimes a coating on the teeth display a reddish-orange fluorescence, while the teeth themselves appear blueish-green (7). This reddish color was traced to a natural pyrrole pigment, porphyrin, coating the tongue and teeth. However, only recently was this observation put to clinical usage (8). About 82% of healthy persons show fluorescence on the tongue, usually on the posterior dorsal surface. There is considerable individual variance in intensity but no apparent sex variance. Also the incidence of fluorescence declines with age, only 47% of people over eighty years of age showing its presence. Pernicious anemia, iron-deficiency anemia and sprue syndrome patients lost this sign in

two-thirds to three-fourths of the cases, while all cases (seven) with B-complex deficiency (especially riboflavin) showed no fluorescence. Almost all these patients when placed on corrective therapy showed restored tongue fluorescence. Treatment of patients with penicillin, streptomycin, chloromycetin and aureomycin destroyed fluorescence by their action upon microorganisms, the probable porphyrin producers. Whether or not the oral microorganisms synthesize the porphyrin is unknown, as its chemical structure is identical to iron-free decomposition products of hematin and to magnesium-free decomposition products of chlorophyll.

Porphyrins, combined with iron (heme), form the active part of such molecules as hemoglobin, myoglobin, peroxidase, catalase and cytochrome. Hence they may be found in all cells from bacteria and yeasts to those of the higher animals (9). The most common porphyrin in man is protoporphyrin-9, which is the basic nucelus of hemoglobin after iron and globin have been removed. Normal hemoglobin degradation by the liver probably does not result in the formation of protoporphyrin, but the iron porphyrin ring is opened before the iron is removed. As has been stated, free porphyrins

fluoresce red under ultraviolet light.

It has been frequently demonstrated that porphyrins are usually present on the female genitalia in amounts sufficient to cause a red glow when examined under ultraviolet illumination (10), (11), (12). Actually a correlation was made between six colors of vulvar fluorescence and the patient's functional status.

Prior to adolescence, the perineal and vulvar surfaces exhibit a yellow color. After puberty, the colors gradually change to green and following menarche they maintain a light purple fluorescence (13). During, and immediately following menstruation, the vulva demonstrate a bright red color under ultraviolet. Some of this color appears to be within the tissues, while the bulk of it seems to be on the tissues and can be partially wiped away. (It should be born in mind that fresh blood does not fluoresce and is black under ultraviolet light, while blood undergoing bacterial decomposition demonstrates red porphyrin fluorescence.) Soon after the cessation of menstruation the vulva re-assume a light purple fluorescence. This color remains until the next menses.

With an existing pregnancy, a very deep purple color appears within the first month. In a series of

thirty-six patients, using this as a presumptive sign of pregnancy, there was one false positive and no false negatives (11). This color persists until term and, on the first postpartum day, is replaced by a vivid red, an expression of rubra lochia. Should the patient receive a therapeutic abortion or a cesarean section, this deep purple color will also rapidly disappear. it is interesting to note that tubal pregnancies and hydatid mole did not develop the deep purple of normal pregnancy and that in cases of threatened abortion where the deep purple color disappeared the likelihood of salvage was very remote (11).

Incomplete abortion and other causes of abnormal uterine bleeding cause a mixture of light purple and deep red areas to appear on the vulva.

As women go through the menopause, the light purple color is replaced by a yellow or light green. Post menopausal patients show these same colors or a deep brown general skin hue. Women showing clinical signs of hypoestrinism also demonstrate this light yellow color. Both these patients and post menopausal women had a return of light purple fluorescence after one or two weeks treatment with the natural female sex hormones or with diethylstilbestrol.

Contrasted to the vulva, the cervix and vagina do not display the range of colors described. A light purple or a gray-purple is seen throughout active menstrual life and with pregnancy. Post menopausal patients show a dead-white or a grayish brown vaginal and cervical surface. As with the vulva, this fluorescence will change to a light purple following a one to two week treatment with sex hormones.

Fungus infections contrast sharply from this color scheme. These appear, usually in patches, as yellow-orange or silver fluorescence. An occasional orange fluorescence, presumably due to smegma, occasionally is seen beneath the clitoris.

This color cycle is regular enough to warrant the statement that whenever intensely red fluorescence is seen on the vulva or on vaginal swabs of a post-menopausal woman or woman in mid cycle, it should be regarded as a sign of abnormal uterine drainage and should require study to rule out malignancy (10).

C. Changes seen with neoplasm:

Before going on to review changes in fluorescence seen with neoplasia, it would be well to review factors known to be involved in carcinogenesis. An outline

may be made of three basic factors required to produce neoplasia (Table I), (14). Factor I is any "carcinogenic" substance, such as methylcholanthrene, benzpyrene and estrogens. Should these substances not be oxidized nor metabolized, whether in a normal or faulty manner, they probably would exert no effect. Factor II is the catalyst or enzyme necessary for this breakdown. Thus the two of these would produce a carcinogenic reaction. This would explain why a carcinogenic substance may not produce a cancer at the site of injection, but at a location where the necessary enzyme system is present. To enable a normal, non-sensitized cell to respond to these carcinogenic reactions there would have to exist a third or sensitizing factor (Factor III). It has been shown that several porphyrins may act as sensitizing agents to carcinogenic stimuli, in that animals with high rates of porphyrin metabolism and animals receiving injections of porphyrins show markedly increased response, and rate of response, to carcinogenic substances (14). It is also known that abnormal porphyrins are produced in excess in certain bacterial infections, trauma and under the influence of many substances like coal tar (15). In a similar fashion the virus and milk factor may serve to form these

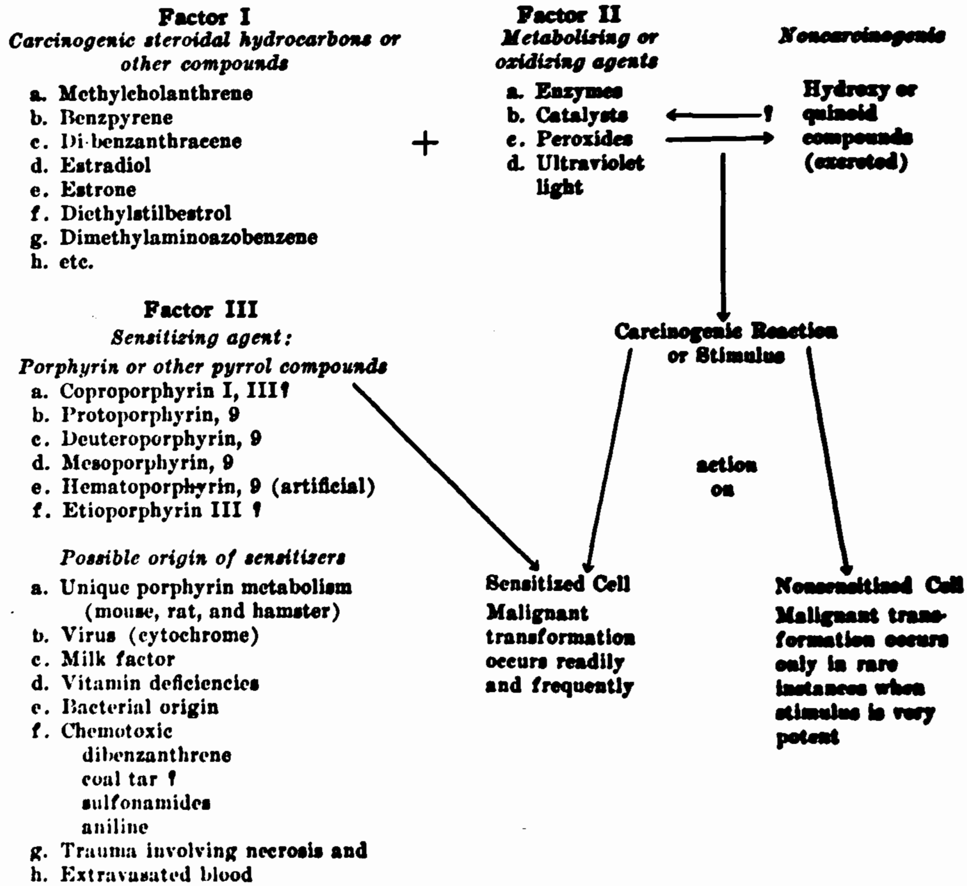


Table I. Three hypothetical factors necessary for carcinogenesis (14).

pyrrol compounds. Experiments have shown that several porphyrins, both injected and naturally present, accumulate in neoplastic, embryonic and regenerating tissues (16).

The above forms the basis of an hypothesis that a diagnostic or screening test for malignant growth might be constructed, utilizing the spectral identification of specific fluorescing compounds. Table II shows the structural formulae of the more common animal porphyrins.

Work published to date upon the utilization of ultraviolet light as a cancer diagnostic tool has been directed down two main channels. The first utilizes the altered transmission or absorption of ultraviolet rays directed through the suspicious tissue sections or body fluids. Gross and microscopic techniques have been devised, usually both methods utilizing a spectrophotometer as a means of measurement (17), (18). The second method of approach has been to observe and to measure intensity of fluorescence seen with the tissues or fluids. Only this latter approach will be considered here.

Soon after the widespread acceptance of the Papanicolaou technique (19), methods were sought to

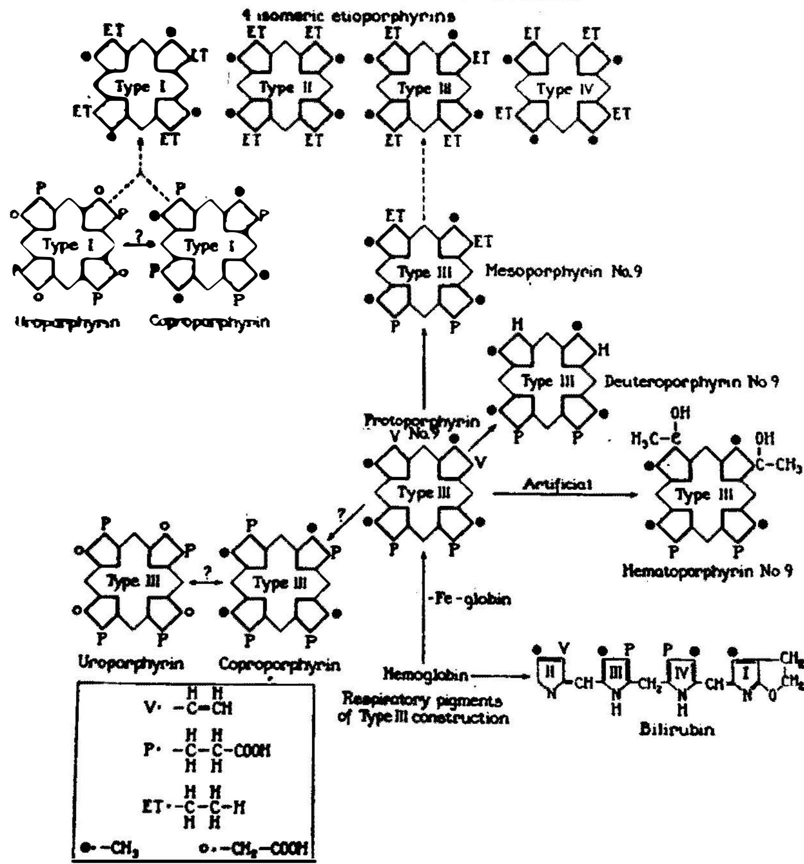


Table II. The structural formulae of the porphyrine (15).

avoid the occasional debated interpretation and to facilitate readings of the slides in general. One such technique replaces the polychromatic stains normally used with neutral, basic and acidic fluorochromes in sutiably buffered solutions (20). Smears are previously made in the normal fashion. When the stained slides are viewed in ultraviolet light through the microscope, the individual cells are seen to fluoresce in various nuclear and cytoplasmic colors against a black background. Malignant cells have a similar fluorescent staining pattern as the type cell from which they are derived, but differ from the normal cell, aside from morphology, in two ways: 1) The malignant cells are "hyperchromatic", that is, they fluoresce more brightly than do the normal cells. 2) The cytoplasm and nucleus of cancer cells demonstrate altered colors of fluorescence. In addition to this, bacteria in general, Trichomonas vaginalis, Monilia, red blood cells and individual types of white blood cells may be identified. The author rates this test as a screening method, and states statistics will be published in later articles.

Other investigators have discovered that human urine normally contains two fluorescent factors, one blue and the other red (21), (22). These were re-

spectively named the B and R values, and their ratio to each other called the B:R ratio. Using a Phaltz and Bauer Fluorophotometer, (which measures the intensity of observed fluorescence) twenty-four hour urine specimens of 400 patients were examined under an ultraviolet light with an emission range of 310 to 420 millimicrons (mμ). The numbers obtained as values are expressions of the relative intensities. The control group comprised 150 cases of which 100 enjoyed good health and were free of benign or malignant growth and 50 cases were free of benign or malignant growth with associated common ailments. Maximal R factors were 2 while B factors covered a wider range. The <u>minimal</u> B:R ratio was 6.0:1.0. In the 50 cases with miscellaneous ailments (none had markedly impaired kidney function, liver damage or jaundice) the R values were below 1.5 and the B values correspondingly low; the B:R ratio remained 6.0:1.0.

A group of 91 cases with proven benign growths (fibroid uteri, adenomas and polyps of the gastrointestinal tract, uterine polyps, hypertrophied prostate, fibroadenomata of the breast, cystic mastitis, benign ovarian tumors, endometriosis, bone tumors, pituitary adenoma and thyroid adenoma) and a group of 21 normal

pregnancy cases all showed consistently significantly raised R values above 2.0. B values were proportionately raised with resultant higher B:R ratios. It is interesting that the amniotic fluid showed this same elevated B:R ratio with B and R values markedly increased beyond the controls. It is known that urinary constituents of amniotic fluid are shared by fetal kidney and placenta and that the placenta acts as a barrier to most foreign substances.

In a group of 101 cases with proven malignant tumors (gastrointestinal, pulmonary, thyroid, uterine, prostatic, ovarian, bone, breast tumors and acute lymphatic leukemia and lymphosorcoma) the R values were strikingly elevated and the B values correspondingly depressed. The resulting B:R ratios were all below 5.0:1.0. In fourteen instances, there were six false positives and eight false negatives. The latter were all cases of known low grade of magnitude (four cases of Hodgkin's disease, two chronic lymphatic leukemias, and one case each of reticulum cell tumor and giant cell sarcoma). The six false positives were cases of cirrhosis of the liver with liver damage confirmed by liver function tests and blood chemistry. In all six cases the B:R ratios returned to normal following treatment resulting

in normal function tests and chemistry.

Twenty-two cases of malignancy were followed for one to two years following radical surgery. Every case demonstrating no remission of tumor growth evidenced a change to normal (greater than 6.0:1.0) B:R ratios, while those demonstrating metastasis retained their abnormal B:R ratios (less than 5.0:1.0). As a brief summary, the normal B:R ratios were never <u>less than</u> 6.0:1.0; cases with benign growths and pregnancy showed elevated B:R ratios, while cases with malignancy exhibited significantly elevated B values and depressed R values with a B:R ratio never <u>above</u> 5.0:1.0.

It is interesting to correlate these findings with the former discussion on pyrrol compounds and the three hypothetical factors in the hypothesis of the sensitization of the cell to malignant transformation. There the stress was placed upon the role of the red fluorescent porphyrins.

In studying the centrifuged fasting blood serum of 500 patients an ultraviolet light source of 320 my was used and results were recorded as to relative colors seen visually, and relative intensity values as measured with a fluorophotometer (23), (24). Normal serum was seen to fluoresce throughout the entire fluid column with

a yellow or olive-green color and has a very turbid appearance. Cancer serum loses fluorescence or appears turquoise blue or purple (especially along the edges of the tube) and loses its turbidity. In this series there were thirty positive reactions, twenty-nine of which were proven by tissue examination. The thirtieth case was a patient with mycosis fungoides with no evidence of malignancy. Pregnant patients gave a reaction midway between normal and malignancy. Medical and surgical diseases such as tuberculosis, syphilis, leprosy, anemia, liver and kidney disease, intestinal obstruction, bleeding peptic ulcer, prostatitis and cholecystitis, as well as others gave the same reaction as normal sera. All cases receiving radical tumor excision demonstrated normal serum reactions within twenty-one days. The sera of patients treated with deep X-ray, radium and nitrogen mustard gave a reaction different from both normal and cancer serum. (This difference was not explained).

III. Fluorospectrophotometric Technique

A. Theory:

To date then, all of these color changes reported with tissue and body fluids have utilized the human eye as a color perceptor and, at best, a fluorophotometer with or without a nephelometer to record intensity and turbidity. No one has attempted to identify the spectral composition of the colors observed. As shall be pointed out, two colors may appear identical to the eye and yet be made up of entirely different wave length combinations. It would seem that a study is definitely warranted to ascertain whether or not the various conditions mentioned, including malignancy, produce one or more characteristic bands or even sharp wave length peaks of fluorescence.

Such a program was outlined and, following approval of the department, work was begun in the laboratory of the department of Obstetrics and Gynecology. This program consists of the following:

1). Female patients to be studied with a Beckman Model DU Quartz Spectrophotometer, modified to measure both the spectrum and intensity of fluorescence of:

(a) twenty-four hour urine specimens
(b) blood serum
(c) vaginal fluid
(d) extracts of vaginal pads or tampons during menstruation
(e) amniotic fluid (of pueperal patients)

These studies to be done in a series of "normal" women and a series of women with malignancy, hormonal and vitamin disturbances, as well as medical and surgical disorders, in an attempt to "cover" all known main causes of fluorescent changes. These women to be followed in the menstrual cycle by daily temperature charts.

2). Male patients to receive similar studies of blood and urine.

With both sexes, various tissue specimens and body fluids, such as gastric washings, would be studied when indicated.

3). If possible, to do visual and photographic studies with cutting filters of fluorescence of cervix and vulva.

4). Whenever possible the results should be compared with standard tests now in use (biopsy, etc.).

The source of ultraviolet is a General Electric BH4 lamp. For visual and photographic work this is mounted in a modified Bausch and Lomb Spherical Lamp Housing (25),

while the lamp for the fluorospectrophotometer is made an intrinsic part of the instrument. The wavelength of ultraviolet utilized is 365 mμ and is isolated by various filters (Corning #3389, #5840, #5860).

It should be brought out here that there is no "magic" involved in the 365 mμ line. Various workers have reported it as exciting maximum biological fluorescence (26). Since the study proposed is a new approach it would be well to study the characteristics of the entire ultraviolet band; however a start must be made at some specific point.

B. Modification of the Beckman DU Spectrophotometer:

Since there is no commercial spectrophotometer available to measure fluorescent spectra (27), it would be necessary to modify the Beckman Model DU which is available.

The spectrophotometer as it exists is diagramed in Fig. I (28). Radiant energy from an incandescent lamp, A, is focused on the slit, D, by means of the concave mirror, B, and the plane mirror, C. The beam entering the slit, D, is collimated by the mirror, E, and passes through the quartz prism to the reflecting surface, F. After reflection at F the beam returns along nearly the

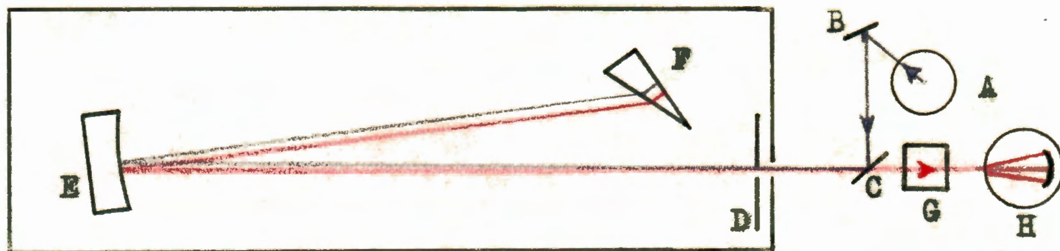


Figure I. Optics of the Beckman quartz spectrophotometer.

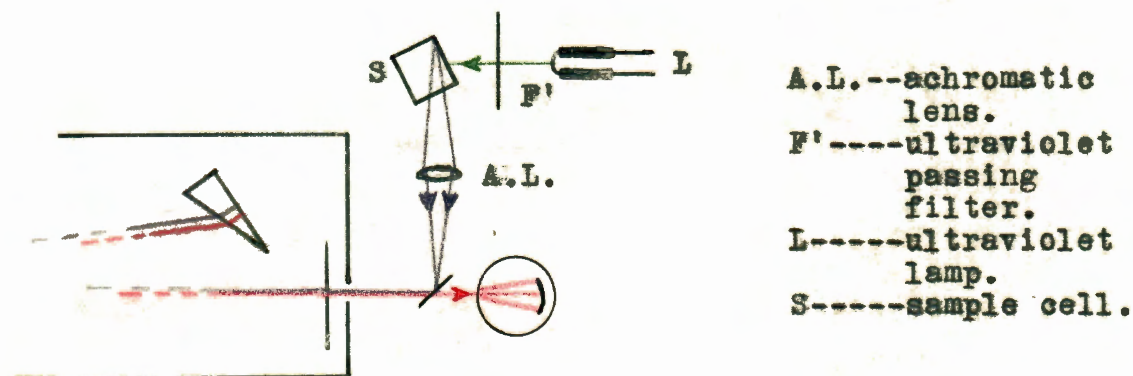


Figure IIa. Changes to measure fluorescence spectra, Burdett and Jones technique (31).

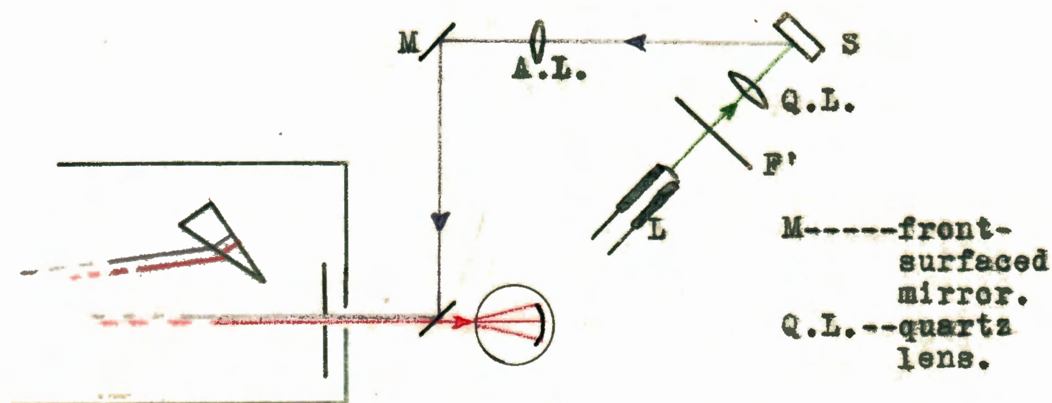


Figure IIb. Modification of Burdett and Jones technique.

same path to the same slit, D, where it emerges slightly above the entrance beam and the mirror, C. After passage through the sample or sample compartment, G, the beam is incident on the phototube, H.

Thus, in terms of transmission or directional reflectance, it makes a relative measurement of radiant energy (or radiant flux) as a function of any given wavelength isolated by the quartz prism.

There is not much literature available concerning the modification of this instrument to measure fluorescence spectra (29), (30), (31), (32). After reviewing the works published it was decided to modify the technique of Burdett and Jones (31), (Fig. II). They use a high pressure quartz lamp, filtered to furnish 365 my excitation. This ultraviolet light is beamed into a quartz absorption cell and the resulting fluorescence is taken off at an angle greater than 90° and beamed with a coated achromatic lens onto the slit mirror of the monochromator.

In doing this they are utilizing the observation that intrinsic intensity of surface fluorescence increases rapidly with an increasing angle of observation. While they are measuring emission occuring in the front layer of the cell, it is felt that there is one major fault

with their technique.

In using a great angle of reflectance, they tilt the sample cell in such a way as to force the fluorescent rays through the test solution before reaching the monochromator. This introduces the error of absorption by the solution. It may be seen then, that this could not only affect the intensity of the wave length measured, but even shift the peaks of fluorescence near or in the absorption bands of the solute.

This could be avoided by focussing the ultraviolet beam just inside the window of the quartz cell and then measuring fluorescence emerging from the same side of the cell. Care will be needed to avoid reflection, and perhaps a front surface mirror will be needed.

This technique will decrease the overall light intensity given off but it is hoped this factor will be overcome by the sensitivity of the instrument. Should this not be the case, a circuit utilizing photomultiplier type tubes with current amplification factors of 200,000 and 2,000,000 has been devised, and tested. This would replace the phototube circuit of the present instrument.

Using this method the worker will be insured correct relative intensities and positions of all peaks measured. It is felt this is absolutely essential for at least the

initial or purely research phase of the investigation.

Whichever circuit is used it will be imperative to correct for the non-uniform spectral response of the phototubes (or photomultiplier tubes). This can be done by calibrating them against a standard 3200°K tungsten lamp, using Planck's equation for radiation of a black-body (33-36). The absorption spectra of the various parts of the optical system must also be corrected for; other considerations will not be discussed here (37-41).

C. Design of two office instruments:

We all know the important factor in cancer--early recognition. Whatever be the treatment, its results are determined by how early it is utilized. Therefore any test, to be truly successful, must be done in the doctor's office, and it must be one that is quickly and inexpensively performed.

Reasizing that the very rational, much less the results, of this proposed test is unproven, it was nevertheless felt that work should be carried on simultaneously upon the design of some instrument that could be used in the office of the practitioner. There is only one type of spectrophotometer that can be produced cheaply enough to enjoy general usage. This would use filters

instead of a prism to isolate the wavelengths at which measurements are to be taken. Of course it is not as accurate, but it can be constructed to operate within very narrow bands.

Since the exact color range to be measured is unknown, a special set of eleven filters, constructed on 35mm film, was prepared by Technicolor Laboratories of California. These are so designed that combinations of them can isolate very narrow spectral bands, with a total range extending from near ultraviolet to near infra-red; about 320 my to 900 or 1,000 my. (In any finished instrument fewer filters made of glass would probably be used.) Twelve holes were bored in a circular aluminum disc, one to mount each filter and one a blank. Three such discs or "filter wheels" were mounted in tandem to allow various filter combinations.

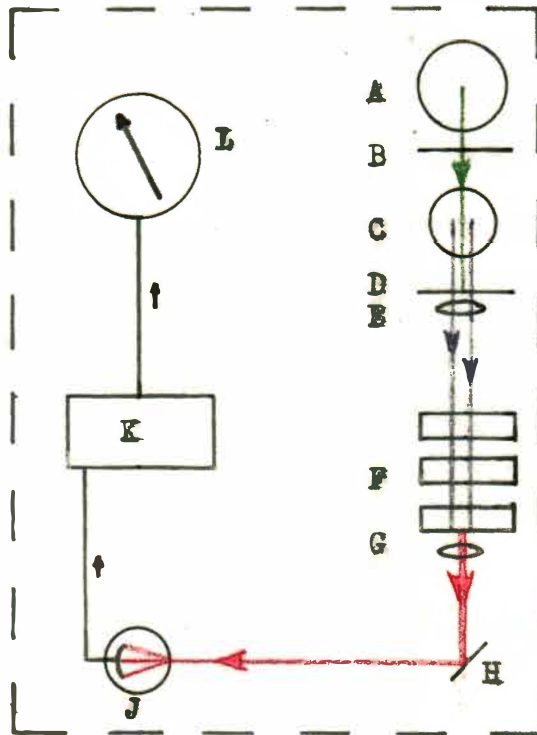
In the search for a suitable ultraviolet source, a large number of war surplus Sylvania H-35-A blacklights were discovered. These are high-intensity fluorescent lamps with a readily available commercial counterpart. A spectral analysis of their output was performed and it was discovered that their radiation extended from 3400-3800 Angstrom units (340-380 my) with a very sharp, high-amplitude peak at 365 my. These were deemed almost

ideal and, after expermenting with various phototube circuits, a working model was completed (Fig. III). The instrument was then calibrated against the Beckman DU spectrophotometer (42).

Radiation from the ultraviolet tube, A, passes through a filter, B, to remove all visible wave lengths, and strikes the quartz test tube containing the specimen, C. The fluorescence obtained passes through a second filter, D, to remove all ultraviolet rays and is collected by lens system, E. It then passes through the filter tandem, F, and is re-focused by lens, G, upon a front-surfaced mirror, H. This directs the light path to the phototube, J. These impulses are amplified in a conventional type of Wynn-Williams bridge circuit, K, which can measure illumination levels corresponding to a phototube current of only 10^{-10} ampere. Scale deflections are measured on the microammeter, L.

To make this instrument more versatile, it has been so designed that the light source can be replaced with an incandescent lamp and the instrument may then be used as a standard colorimeter or spectrophotometer. Thus the doctor may use it in many common laboratory determinations.

There is a second type of machine of value at



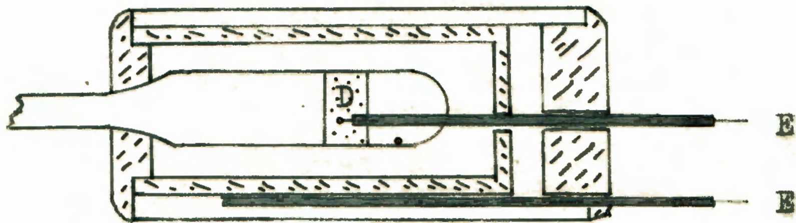
A--ultraviolet lamp.
B--ultraviolet-passing filter.
C--quartz specimen test tube.
D--fluorescence-passing filter.
E--achromatic lens.
F--filter tandem.
G--achromatic lens.
H--front-surfaced mirror.
J--phototube.
K--amplifier.
L--microammeter.

Figure III. Design of office filter-flurospectrophotometer. (Note: "A" may be removed and an incandescent lamp substituted to use as a colorimeter.)

least in research on the project (43), (44), (Fig. IV). This is an ultraviolet probe consisting of a long thin quartz rod or tube with an elongated bubble, containing a small mercury globule, at one end. This red is one-fourth inch in diameter, seven inches long, and has about a 30° bend in its distal one-third. The elongated bubble at its proximal end is five-eighths inches in diameter, two inches long, and is surrounded by a bakelite cylinder three inches long by one and three-fourths inches wide. The bakelite cylinder contains two electrodes placed in external contact with the quartz bubble and which are connected with a source of radio frequency energy by means of a suitable cable.

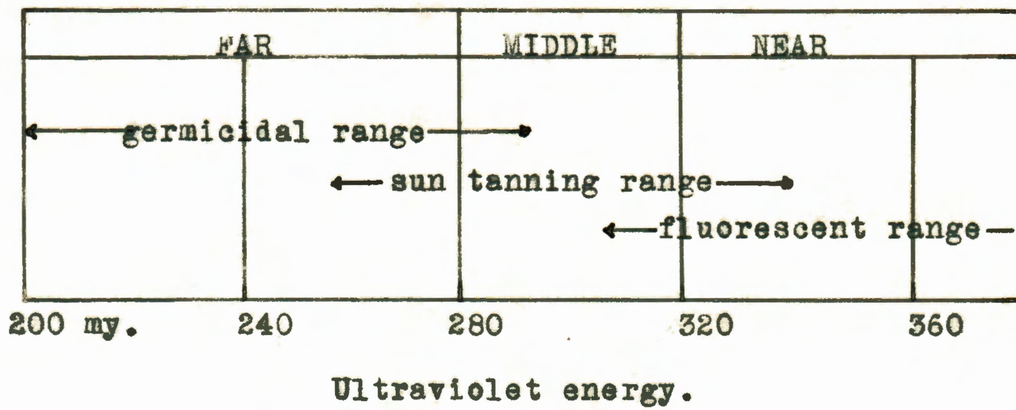
This probe makes an excellent means of reaching into body cavities, such as the vagina, with a small, powerful source of ultraviolet. It could also be well used for examining any small surface area. In this writers opinion, its usefulness would be extended by coating all but the tip of the probe with a layer of reflecting metal, such as aluminum. (This would probably be vacuum-deposited).

In Fig. V is shown the wave length spectrum extending from ultraviolet to infrared with brief descriptions as to uses.

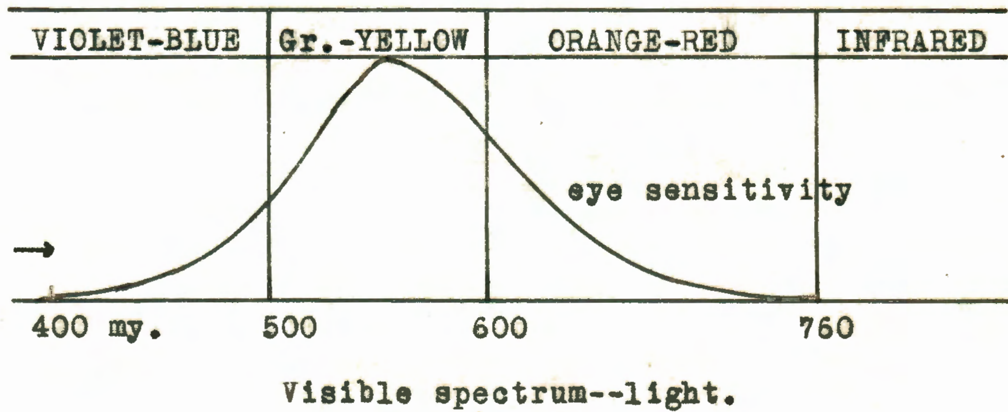


A--quartz tube.
B--inert gases.
C--mercury globule.
D--soldering band.
E--to radio frequency oscillator.

Figure IV. Ultraviolet probe (43), (44).



Ultraviolet energy.



Visible spectrum--light.

Figure V. Ultraviolet and visible wavelength spectrum.

IV. Isolation and Purification of Human Fluorescent Compounds:

It is merely suggested here that it might prove of value to attempt isolation and identification of these various fluorescing body compounds. Perhaps abnormal curves may be produced in the laboratory animal using these compounds alone. Such work is not included in the scope of this paper. Reference is made to excellent results in isolation of urinary porphyrins (45).

V. Summary

1. An explanation of the terms "Screening" and "Diagnostic" as applied to cancer tests was made. The basis of action of tests showing the most usefulness were divided into four categories:

   a) enzymic,
   b) antigen-antibody,
   c) altered body fluid elements,
   d) pathologic or cytologic.

2. The ideal test fulfilling the term diagnostic, should be rapid, economical, and capable of being read as part of a routine physical examination by the average doctor without additional special training.

3. In a review of normal body fluorescence it has been shown that women go through a monthly "fluorescence cycle", and that fluorescence techniques may be used as a diagnostic aid in the following:

   a) fungus infections,
   b) certain infectuous diseases (ex. Tuberculosis)
   c) certain anemias,
   d) certain vitamin deficiencies,
   e) pregnancy,
   f) evaluation of threatened abortion,
   g) evaluation of certain hormonal imbalances.

4. In a discussion of elements known to be involved in carcinogenesis an outline of three hypothetical basic factors required to produce neoplasia was made:

a) a carcinogenic substance (ex. methylcholanthrene)
b) a catalyst or enzyme necessary for metabolism of the former,
c) a sensitizing factor.

An effort was made to show that porphyrins might act as this sensitizing factor.

5. Fluorescent examinations in malignancy were reviewed including usage of primary and secondary fluorescence techniques. In techniques involving the latter it has been discovered:

a) That normal urine contains two fluorescent fractions, a blue and red. These were named the B and R factors respectively, and their ratio to each other, the B:R ratio.

b) That in a series of 400 patients it was determined the maximum R factor was 2, while B factors covered a wider range; the normal B:R ratio is a minumum of 6.0:1.0. Patients with a pregnancy or a proven benign growth have a consistently higher B:R ratio while those with proven malignancy have strikingly elevated R values and depressed B values with a resulting B:R ratio always below 5.0:1.0. This test was 86% accurate, all false positives being low grade Hodgkin's disease, leukemia, reticulum cell tumor and giant cell sarcoma. The false positives were proven cases of liver cirrhosis which showed normal

results after treatment.

c) That in all cases following surgery the test returned to normal and whether or not it remained as such correlated exactly with the incidence of metastasis.

d) Normal blood serum fluoresced a turbid yellow or olive-green while malignant serum is clear and shows no fluorescence or appears turquoise blue or purple. In a series of 500 cases the test was 97% accurate as to false positives and no false negatives were noted.

6. Note has been made of the fact that no worker has attempted to study the spectrum of these fluorescent phenomenon in an attempt to discover bands or wave length peaks characteristic of malignancy. (Several colors appearing identical to the eye may have a vastly different spectrum composition).

7. Bearing this in mind the Beckman DU spectrophotometer has been modified to analyse fluorescent spectra and the prototype was constructed of an office instrument that may be used both for this test and for several routine procedures.

VI. Conclusion

In conclusion, it is felt that malignancy anywhere in the body produces definite changes measurable, as altered fluorescence in both blood and urine. The few reports published thus far have shown an excellent degree of accuracy, however it is felt work to date, having used only visual and photometric methods may be greatly improved upon with spectral analysis techniques. These have been outlined and partially carried out.

Realizing successful treatment of cancer depends upon early recognition by the practitioner, an instrument has been designed that can be economicaly purchased and operated, both as a cancer test and as a general office laboratory instrument.

BIBLIOGRAPHY

1. Anderson, W.A. Pathology. 1st ed. St. Louis, Mosby, 1948. Chap. 19.

2. Lorincz, A., Novelli, J., McGoogan, L.S. and Odell, L.D. Beta-Glucuronidase Activity in Human Female Genital Cancer. Am. J. Ob. and Gyn., v.61, #3, p.527, 1951.

3. Kidd, J.G., and Friedewald, W.F. Purified Protein Fractions of Cancerous Tissue. J. Exper. Med., v.76, p.557, 1942.

4. Maver, M.M. Review of Serodiagnostic Tests for Cancer. J. Nat. Cancer Inst., v.4, p.571, 1943-1944.

5. Davidsohn, D.I. Serodiagnosis of Malignant Tumors. Am. J. Clin. Path., v.6, p.172, 1936.

6. Benson, R.C., Strait, L.A., and Chappell, C.C. The Clinical Specificity of Vulvar Fluorescence. Surg. Gyn. and Ob., v.92, #1, p.14, 1951.

7. Hymans von den Bergh, A.A. On Porphyrin in the Mouth. Lancet, v.1, p.281, 1928.

8. Tomaszewski, W. Fluorescence of the Tongue. What's New. #158. N. Chicago, Ill., Abbott Laboratories, p.6, 1951.

9. Watson, C.J. Downey's Handbook of Hematology. 1st ed. New York, Paul B. Hoeber, Inc., v.4, p.2445, 1938.

10. Jones, E.G., Shaw, H.N., and Figge, F.H. Studies on the Red Fluorescent Porphyrin Deposits on Vagina and Cervix. Am. J. Ob. and Gyn., v.51, #4, p.467, 1946.

11. Benson, R.C., Strait, L.A., and Chappell, C.C. The Clinical Specificity of Vulvar Fluorescence. Surg. Gyn. and Ob., v.92, #1, p.14, 1951.

12. Margolese, M.S. Fluorescent Phenomena of the Vulva Associated with Sex Hormone Metabolism. J. Clin. Endocrinol, v.1, p.26, 1950.

13. Jones, E.G., Figge, F.H., and Hundley, J.M. The Red Fluorescence of the Genitalia of Women. Cancer Res., v.4, p.472, 1944.

14. Figge, F.H. The Relationship of Pyrrol Compounds to Carcinogenesis. A.A.A.S. Research Conf. on Cancer, p.117, 1945.

15. Dobriner, K., and Rhoads, C.P. The Porphyrins in Health and Disease. Physiol. Revs., v.20, p.416, 1940.

16. Figge, F.H., Weiland, G.S., and Manganiello, L.O. Cancer Detection and Therapy. Affinity of Neoplastic, Embryonic, and Traumatized Tissues for Porphyrins and Metalloporphyrins. Proceedings of the Soc. for Exp. Biol. and Med., v.68, p.640, 1948.

17. Sano, M.E., and Adolf, M.S. Ultraspectrophotometric Studies in Extracts of Normal and Tumor Tissue of Human Origin. Science, v.106, #2754, p.346, 1947.

18. Stowell, R.E. Spectrophotometric Studies of Malignancy. Cancer Res., v.5, p.788, 1945.

19. Papanicolaou, G.N., and Traut, H.F. Diagnosis of Uterine Cancer by the Vaginal Smear. New York, The Commonwealth Fund, 1943.

20. Friedman, H.P. The Use of Ultraviolet Light and Fluorescent Dyes in the Detection of Uterine Cancer by Vaginal Smear. Am. J. Ob. and Gyn., v.59, #4, p.852, 1950.

21. Jaffe, M. Zur Lehre von den Eigenschaften und der Abstammung der Harnpigmente. Virchows Arch. f. Path. Anat., v.47, p.405, 1869.

22. Rabinowitz, H.R. A Correlation of Fluorescence of Human Urine with Benign and Malignant Growth. Cancer Res., v.9, #11, p.672, 1949.

23. Herly, L. A Simple Diagnostic Blood Test for Cancer. Fourth International Cancer Res. Congress, v.6, p.1042, 1950.

24. Herly, L. Selective Differentiation of Blood Sera by Means of Transmitted, Filtered Ultraviolet Light. N.Y. J. of Med., v.50, #11, p.1373, 1950.

25. Pisha, B.V. Practical Method for the Illumination of Biological Material with Ultraviolet Rays. Science, v.108, p.142, 1948.

26. Urbach, E.A. Brightness of Apparent Fluorescence as a Function of the Exciting Intensities. J. Opt. Soc. Am., v.37, p.122, 1947.

27. Private Correspondence. Beckman Instruments, Inc. South Pasadena, Calif.

28. Cary, H.H. and Beckman, A.O. A Quartz Photoelectric Spectrophotometer. J. Opt. Soc. Am., v.31, p.682, 1941.

29. Beckman Instruments, Inc. Modification of Spectrophotometer to Measure Fluorescence Spectra. Anal. Ed. Ind. and Eng. Chem., v.15, p.275, 1943.

30. Fletcher, M., White, C.E., and Sheftel, M.S. Fluorometric Attachments for the Beckman Spectrophotometer. Anal. Ed. Ind. and Eng. Chem., v.18, p.204, 1946.

31. Burdett, R.A., and Jones, L.C. The Photoelectric Measurement of Fluorescence Spectra. J. Opt. Soc. Am., v.37, #7, p.554, 1947.

32. Sambursky, S.S., and Wolfsohn, G. Measurement of Fluorescence Spectra. J. Opt. Soc. Am., v.38, p.739, 1948.

33. Moon, P. A Table of Planckian Radiation. J. Opt. Soc. Am., v.38, p.291, 1948.

34. Forsythe, W.E., and Adams, E.Q. Radiating Characteristics of Tungsten and Tungsten Lamps. J. Opt. Soc. Am., v.35, p.108, 1945.

35. De Vore, J.R. Calibration of Photo-Multiplier Photometer. J. Opt. Soc. Am., v.38, p.692, 1948.

36. Harrison, T.R. Industrial Use of Radiation Pyrometers under Non-blackbody Conditions. J. Opt. Soc. Am., v.35, p.708, 1945.

37. Dieke, G.H. and Crosswhite, H.M. Direct Intensity Measurements of Spectrum Lines with Photo-Multiplier Tubes. J. Opt. Soc. Am., v.35, p.471, 1945.

38. Nahstall, G.A., and Bryan, F.R. An Application of Multiplier Phototubes to the Spectrochemical Analysis of Magnesium Alloy. J. Opt. Soc. Am., v.35, p.646, 1945.

39. Kessler, K.G., and Wolfe, R.A. The Measurement of the Intensity Ratios of Spectral Lines with Electron Multiplier Phototubes. J. Opt. Soc. Am., v.37, p.133, 1947.

40. Studer, F.J. A Method for Measuring the Spectral Energy Distribution of Low Brightness Light Sources. J. Opt. Soc. Am., v.37, p.288, 1947.

41. Engstrom, R.W. Multiplier Phototube Characteristics: Application to Low Light Levels. J. Opt. Soc. Am., v.37, p.420, 1947.

42. Comar, C.L. Filter Photometer Calibrated by Spectrophotometer. Anal. Ed. Ind. and Eng. Chem., v.18, p.626, 1946.

43. Atkins, C.E. An Ultraviolet Probe. Radio and Television News. v.45, #3, p.36, 1951.

44. Edwards, S.W. Efficient Ultraviolet Generator. Radio News. v.30, p.44, 1943.

45. Schwartz, S., Zieve, L., and Watson, C.J. An Improved Method for the Determination of Urinary Coproporphyrin and an Evaluation of Factors Influencing the Analysis. Dept. of Med. U. of Minn., private correspondence.