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THE BRDICKA SERUM FILTRATE REACTION APPLIED TO NORMAL AND CANCEROUS MENSTRUAL FLUIDS.

A Pilot Study

by

Richard T. Rappolt, B.A.

1967

This paper is respectfully dedicated to A. Ross McIntyre, M.D., Ph.D., on this his 30th year as Chairman of the University of Nebraska Medical School's Physiology and Pharmacology Department.

THE BRDICKA SERUM FILTRATE REACTION APPLIED TO NORMAL AND CANCEROUS MENSTRUAL FLUIDS: A Pilot Study

by

Richard T. Rappolt, B. A.*

The utilization of the Brdicka serum filtrate reaction (BSFR) in the laboratory studies of normal and cancerous serum, the methodology, instrumentation, and serial tracings of a protracted therapeutic remission have been previously reported by the author.[1]

Recently the opportunity presented itself to obtain patients in various stages of gynecological cancer for the polarographic investigation of their sera. A review of the literature revealed that previous work[2] showed a high degree of correlation (89.1%) between cancer of the female organs and a positive BSFR, as was its relation to the clinical stage of same.[3] Failures, it was stressed, were encountered predominantly in what appeared to be stages 0 and early I in the international classifications[4] of carcinoma of the endometrium (uterus) and portio vaginalis (cervix). This is unfortunate in that 80-90% of the cases in these classifications have a better than 5 year survival rate after appropriate treatment.[5] This failure of the BSFR to detect increments of acid glycoproteins (MP-2 and 3) in the Alpha-1 Globulin fraction[6**] of peripheral venous serum is presumably due to the fact that by definition a stage 0 (in situ or intraepithelial) carcinoma of, say, the cervix, has not

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**Much valuable information concerning Plasma Proteins in Health and Disease may be gleaned from the Annals of the New York Academy of Sciences, Vol. 94, Art. 1, Pages 1-336, 31 Aug. 1961.

000938

penetrated the basal epithelium[7] and its proteolytic breakdown metabolites are extruded, and removed from the body along with its exfoliative products via the female introitus.

It occurred to this observer that perhaps the periodic flow of menstrual fluid, or frank bleeding from the vaginal orifice, which is so often the herald of a gynecologic neoplasia, might be utilized for the BSFR in that these naturally occurring biologic fluids perfuse those areas of the female genitalia where malignancies are most wont to initiate themselves.

The aesthetic problem of collection was satisfactorily solved with an internally worn, rubber menstrual cup,*** Tassette,[8] the capacity of which approaches 28 ml.; this represents 25-50% of the average menstrual flow. (See Figure 1).

For normal controls seven healthy female volunteers, from a non-clinical population were sampled on three successive periods, representing 21 normal BSFR. No conclusions were made as to the height of the wave relating to age (22-38), marital status (5 married, 1 divorced, 1 single), parity (1-4 children), or chronic disease state (1 unspecified vaginitis, 1 hypothyroid).

The first day of "spotting" was noted by the subjects and the Tassette was not inserted until the morning of the second day. The contents of the cup were emptied into a plastic, rubber stoppered, refrigerated, centrifuge tube until its 15 ml. capacity was half full; this represented two or three collections from the menstrual cup at 3-4 hour intervals.

***Supplied for this study by Tassette, Inc., 170 Atlantic Ave., Stamford, Connecticut, through the courtesy of Mr. Robert P. Orek.

The fluids as received by our service were unclotted, viscous and partially hemolysed. The lack of clots or accretia was attributed, in part, to the practice of initiating collection on the second day of the flow.

After centrifugation the port colored serum was denatured and deproteinated in the usual manner.[9] During the deproteination with the 20% Sulfosalisylic acid it was noted that the precipitated insoluble protein instead of forming white curds, as was to be expected with peripheral supernated, formed brownish-red curds, which carried along with them, apparently, the highly colored portion of the menstrual serum, as the physical appearance of the clear, aqueous filtrate was identical to that of the treated peripheral venous serum.

The Polarographic double wave of normal, fresh menstrual blood (See Figure 2) approaches sameness in configuration and height range of normal peripheral venous blood, and varies only slightly with consecutive periods. This correlates well with the report that the (polarographically active) muco-proteins in the peripheral blood of healthy females do not change appreciably during various days in their menstrual cycle.[10]

The wave heights reported are those of our particular laboratory techniques and although the polarographic constant B as suggested by Kalous and Pavlicek[11] might easily have been applied to the results of our controls it was felt that the publication of the heights per se would be more appropriate in the nascent stages of investigation, especially in recording the pathological waves.

Figure 3 is the tabulation of all the normal controls done to this date in groups of three. Previous work with normal peripheral blood gave catalytic double wave height spans of from 31 mm. to 49 mm. measured from the diffusion current of the cobaltic wave.

REPORT OF TWO CASES WITH GYNECOLOGICAL CANCER

(1) Cervical Carcinoma:**** A white, married, female, age 32, Para 0-0-0-0, noted heavy period with clots in November, 1962; examined by her physician December 21; appropriate diagnostic tests indicated a poorly differentiated squamous cell carcinoma of the cervix with extension into the endometrium (Stage II): 1st radium treatment December 26 and 2nd radium treatment January 4; total hysterectomy January 29.

The peripheral blood and menstrual blood from this patient were done one week prior to surgery, (See Figure 4), at the time it was felt there was no residual radiation inflammation. An additional peripheral blood tracing (68.4 mm. not illustrated) two days post-surgery delineated the inflammatory reaction of a surgical manipulation plus possibly the action of a recently administered cytotoxic agent, Thio-TEPA. The conclusion was made that the greater wave height in the genital blood as contrasted to the pre-operative peripheral blood tracing is due to its association with the cancerous lesion; furthermore prognostic conclusions can be more fruitful with concurrent tracings of both the genital and peripheral serums.

(2) Endometrial carcinoma:***** A white, female, age 65, para 5-2-1-2, 2 years post-menopausal, 40 years a diabetic, with chronic pyelonephritis had bleeding and passage of clots for the past 1½ years, and lower abdominal pain for the past two years. Genital blood, which contained multiple clots and purulent material, and peripheral venous blood recorded

****Patient made available for this study through the courtesy of Irving Shapiro, M.D. and Colin Schack, M.D., Omaha, Nebraska.

*****Patient made available for this study through the courtesy of Warren Pearse, M.D., Chairman, Department of Obstetrics and Gynecology, University of Nebraska College of Medicine.

the BSFR on the 2nd hospital day. (See Figure 5). Serial sections of the operative specimen following x-ray therapy and surgery revealed a poorly differentiated adenocarcinoma of the endometrium without myometrial invasion, though there was present a diffuse inflammatory reaction. It was felt that despite the fact that no local extension was present, the peripheral BSFR was increased due to the secondary infection of the endometrium plus the chronic pyelonephritis; emphasizing again the total clinical picture of the patient that must be considered in interpreting the BSFR intelligently.

It is to be stressed that this study does not purport to make any hard and fast conclusions about the clinical practicality of the method herein described. It is intended to be a pilot study only in the hopes that more informed colleagues will be able to adopt it, possibly, as an investigational adjunct of the Papanicolaou smear, Schiller test, Colposcopic examination, punch or conization biopsy,[12] and other newer screening tests for gynecological cancers such as the interference microscopic exam of fresh cancer cells,[13] Tampon smears,[14] von Bartalanffy's acridine orange fluorochrome dye,[15] the Davis examination-by-mail irrigating solution pipette,[16] and the plastic cervical cap used in vivo radioautography of the cervix.[17]

The author wishes to acknowledge the valuable assistance of:

Mrs. Bernice Hetzner and her Staff of the University of Nebraska Medical School Library.

Mrs. Elizabeth Olmstead and her Staff of the Harvard Medical School Library.

Miss Clara Meckel of the University of Chicago Medical Library.

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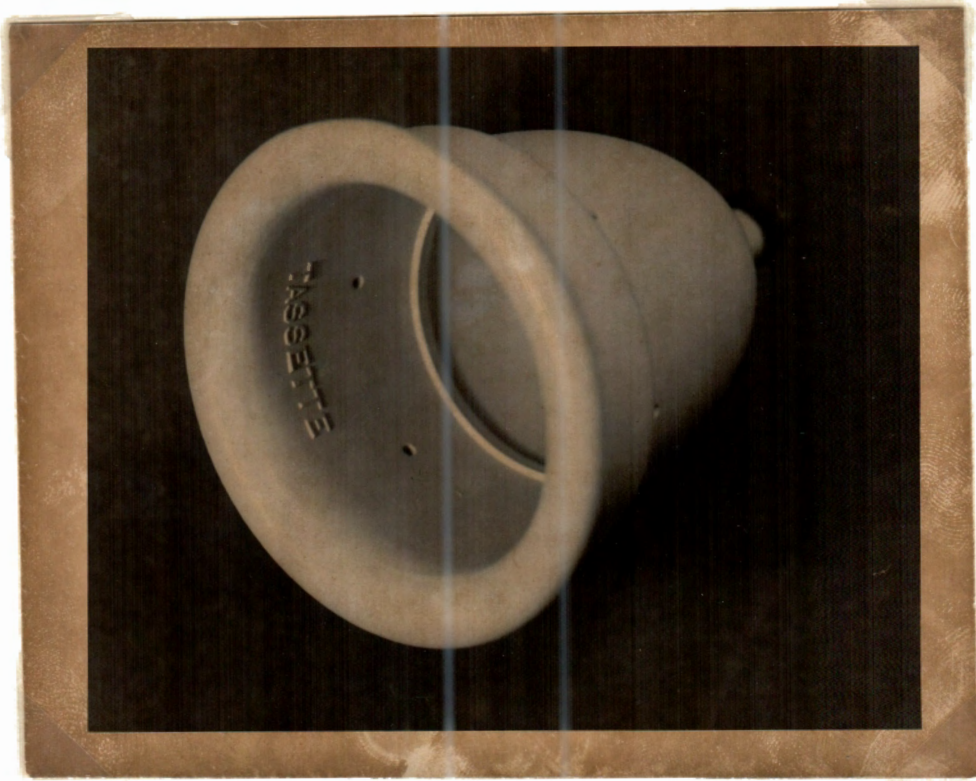

Figure 1: Tassette, rubber menstrual cup

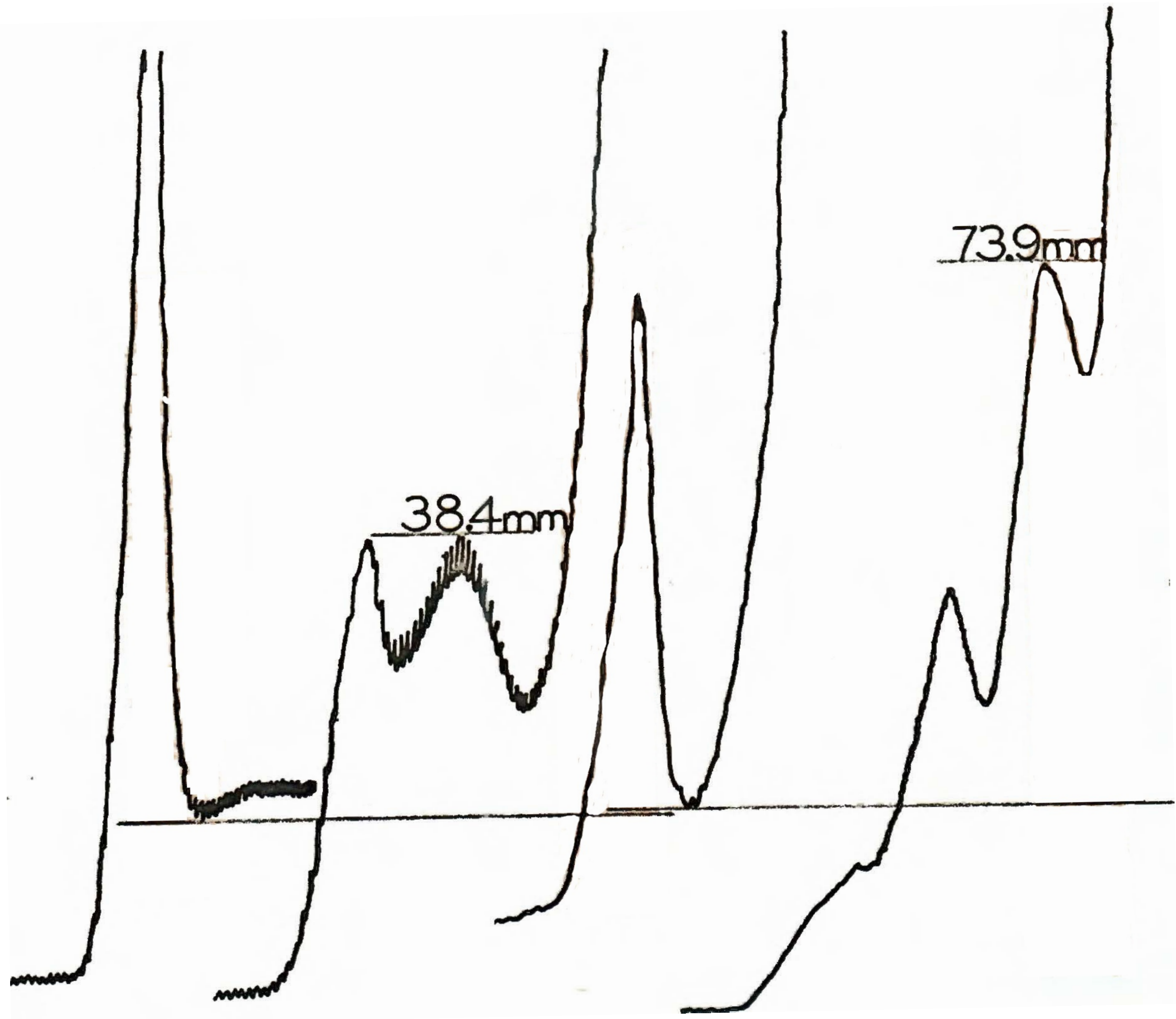

Figure 2: Left to right--Cobaltic reference wave, normal menstrual wave, Cobaltic reference wave, flowing mercury electrode tracing of same normal menstrual serum.

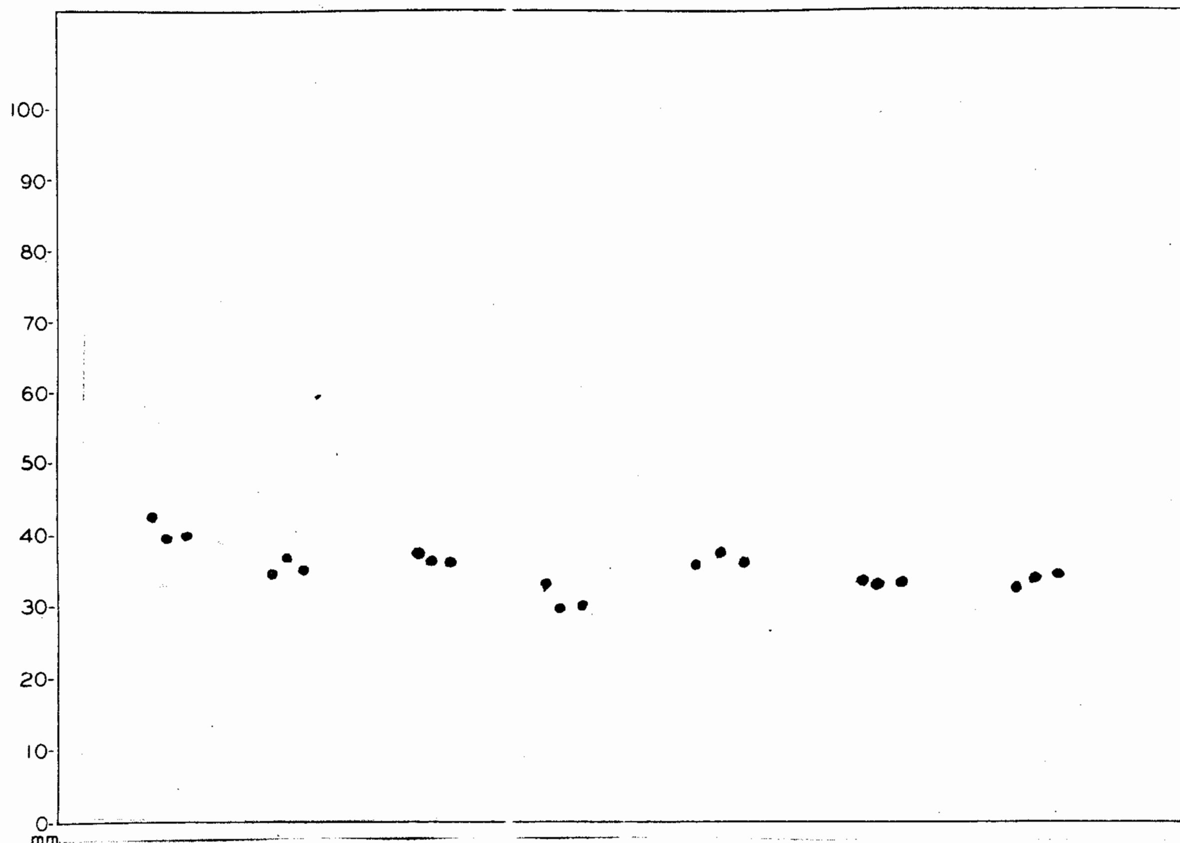

Figure 3: 21 normal BSFR or menstrual controls.

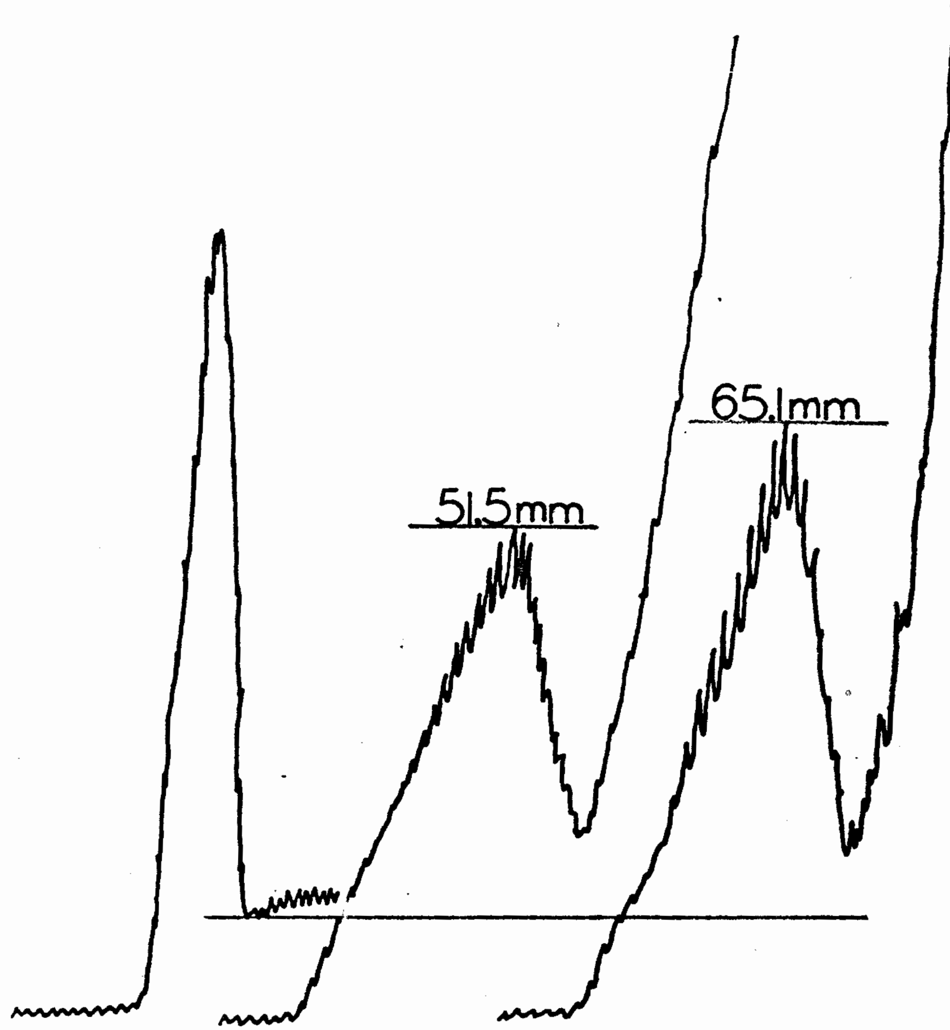

Figure 4: Left to right--Cobaltic reference wave, peripheral blood from cervical carcinoma, genital blood from cervical carcinoma.

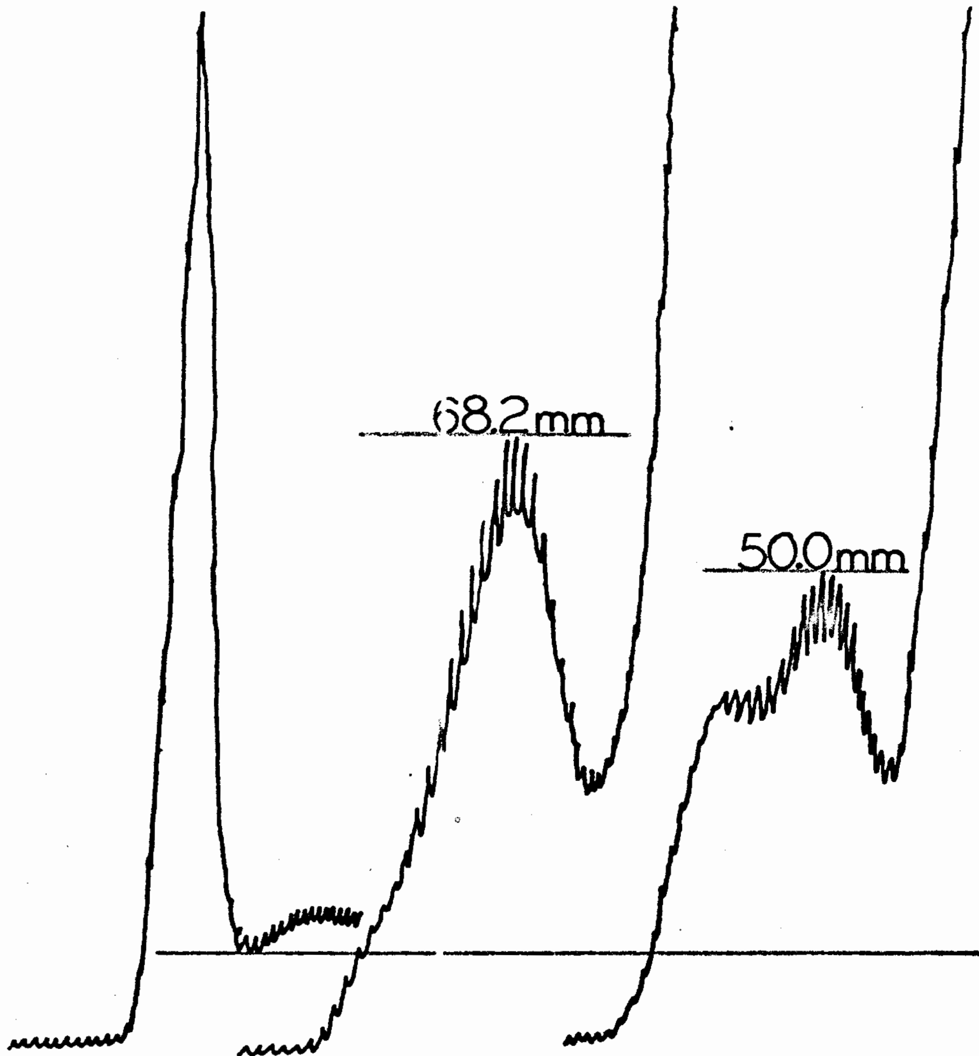

Figure 5: Left to right--Cobaltic reference wave, genital blood from endometrial carcinoma, peripheral blood from endometrial carcinoma.

The Brdicka Filtrate Reaction: A Neglected Polarographic Aid for the Detection, Diagnosis, and Prognosis of Neoplastic Processes

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This article is in press and will appear in the July issue of the Nebraska State Medical Journal.

The

Brdicka Filtrate Reaction:

A Neglected Polarographic Aid *for the* DETECTION, DIAGNOSIS, *and* PROGNOSIS *of* NEOPLASTIC PROCESSES*

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Omaha, Nebraska

THE application of diverse techniques and instrumentation from the fields of electronics, physics, and chemistry to medical problems is one of the important developments in modern medicine. It is the purpose of this paper to point out the utility of one such technique — polarography — as an aid to laboratory studies of human cancer. Also included is the research in this field currently being carried out at our institution.

Theory of Polarography

In the year 1922, Professor Jaroslav Heyrovsky (Nobel Prize, 1959) and his collaborators at the Charles University, Prague, Czechoslovakia, introduced polarographic analysis. This technique is an outgrowth of the singular properties exhibited by an electrolytic cell consisting of a large, difficultly polarizable reference electrode; a small, readily polarizable electrode in the form of a mercury drop growing at the end of a glass capillary tube, and a solution containing trace concentrations of electro-reducible, or electro-oxidizable materials. An electrolyte possessing a strongly electropositive ion, called the supporting electrolyte, is also necessary.

When an electromotive force, changing in value, is impressed across such a cell and the resultant current is plotted as a function of the applied voltage, a curve such as is shown in figure 1 is obtained.

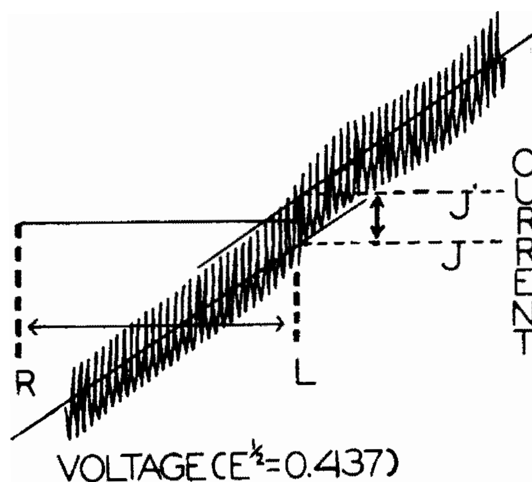

Figure 1. 6.53 micrograms of lead in 80 ml. of urine. Note the characteristic $E_{½}$ for lead. The concentration is arrived at by comparison with previously run standards.

The wave height, as measured from the current axis, is proportional to the concentration of the trace ionic material, while the location of the inflection point along the voltage axis is characteristic of that particular trace ion.[1, 2, 3, 4]

History and Theory of the Brdicka Filtrate Reaction

Dr. Rudolf Brdicka and his associates, while investigating by means of polarography the electrolytic deposition of cobalt from an ammoniacal cobaltammine solution (figure 2), sought a suitable suppressing agent to depress the sharp current maximum resulting from this reduction. Blood serum being available, and already having been used to suppress other similar current maxima, was used. Unexpectedly, not only was the reduction maximum suppressed, but the presence of serum proteins gave rise to a polarographic double wave occurring at a slightly higher voltage, as seen in figure 3.

*A preliminary report was presented at the May 1961 meeting of the Omaha Research Club, an affiliate of the American Federation for Clinical Research.

†The author wishes to express his appreciation to Dr. H. R. Wetherell for helpful suggestions during the preparation of this manuscript.

The height of this wave varied with the concentration of the serum.[5]

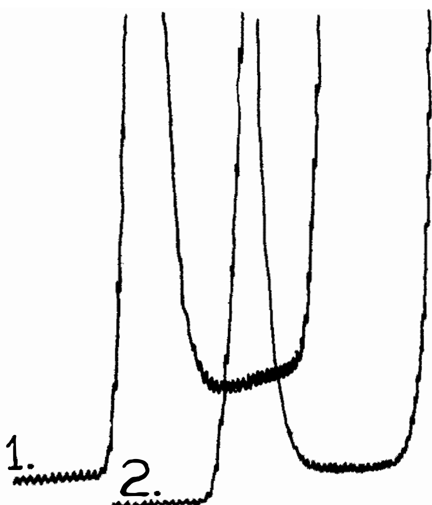

Figure 2. The reduction of the Brdicka cobaltic solution used as a base line for all filtrate reactions. The first run had a shunt ratio of 1:200, while the second is 1:500. Note the maximum on each curve.

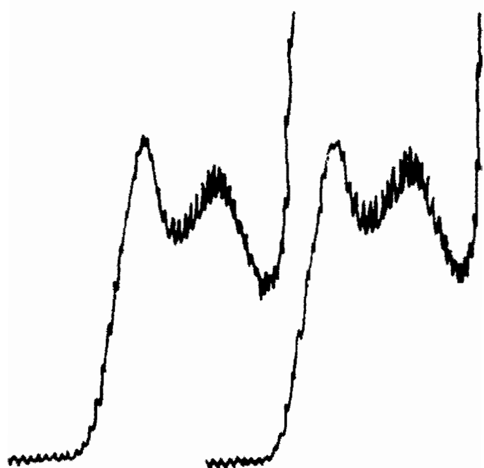

Figure 3. Two different negative filtrate reactions run during the early studies in order to determine normal distributions of wave height. Note the suppression of the maxima and the additional wave (as compared to figure 2) due to the serum protein degradation products.

Dr. Brdicka was intrigued by this serendipitous observation and by the enzymatic studies of Waldschmidt-Leitz on normal and cancerous sera.[6] To prepare the serum before electrolysis, he devised a denaturation, deproteination procedure employing dilute alkali and sulphosalicylic acid. Additional experimental work disclosed that the polarographic serum filtrate wave was caused by degradation products of serum proteins being detoxified by sulfhydril groups. This reflected itself in cancerous sera by a complete impoverishment of the serum constituents containing the cystine groupings.[5, 7, 8, 9] Briefly, if there are more degradation products of proteins, then there is less cystine, and hence a larger protein wave, as seen in figure 4.

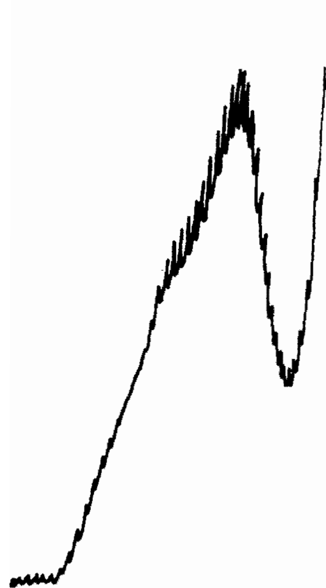

Figure 4. A positive serum filtrate reaction from a patient with terminal disseminated cancer. Note the obliteration of the cobalt maximum and the increased wave height as compared to figure 3.

As of 1947, approximately 15,000 samples had been examined at the Radiotherapeutic Institute, Bulovka Hospital, Prague,[9] and the following general conclusions, which have been confirmed by many independent investigators, were stated:

1. When the height of the filtrate wave lies within statistically normal limits the test is called negative.

2. The height of the filtrate wave increases according to the development of the malignant process. The more rapid the increase of the serum filtrate wave with time the poorer the prognosis.

3. When a tumor is surgically removed or treated successfully by irradiation or chemotherapy, or both, the Brdicka filtrate wave gradually becomes less pronounced. Accordingly, metastases are manifested through a return and a

periodic increase of this serum filtrate wave.

4. In order for the test to have diagnostic significance all other diseases of a general inflammatory nature must be ruled out. (Figure 5).

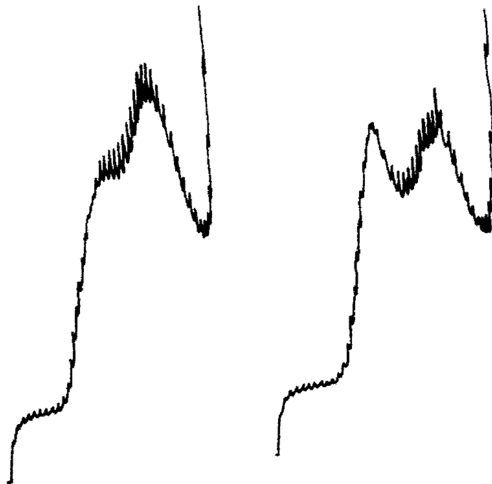

Figure 5. The initial filtrate reaction, taken the 2nd hospital day from a patient admitted for bloody stools, is strongly positive. The second tracing was taken two weeks later following conservative ulcer therapy. The patient eventually was sent home and has remained asymptomatic on a strict dietary regimen.

5. Negative findings for patients suffering from a demonstrable destructive tumor are rare, and occur mostly for nonmetastatic skin tumors and less often for the beginning tumors of the female organs. In cancer of the liver and biliary system the test has been proven less reliable.[5, 9]

6. Our investigations indicate that the degree of efficacy of therapy can be judged by following changes in the polarographic filtrate wave. In our experience the test indicates the cytotoxic effect of a chemotherapeutic alkylating agent* whose dosages thus far have been regulated by the platelet count or other indices of bone marrow damage. We beliepe that a safe level of effective therapy can be judged and damage to nonmalignant cells can be anticipated by several weeks by keeping the serum filtrate wave at the lower limits of a positive (pathological) test. These conclusions can be inferred by following the series of tracings from one patient as seen in figure 6.

*CYTOXAN, brand of Cyclophosphamide, Mead Johnson & Co., Evansville, Ind.

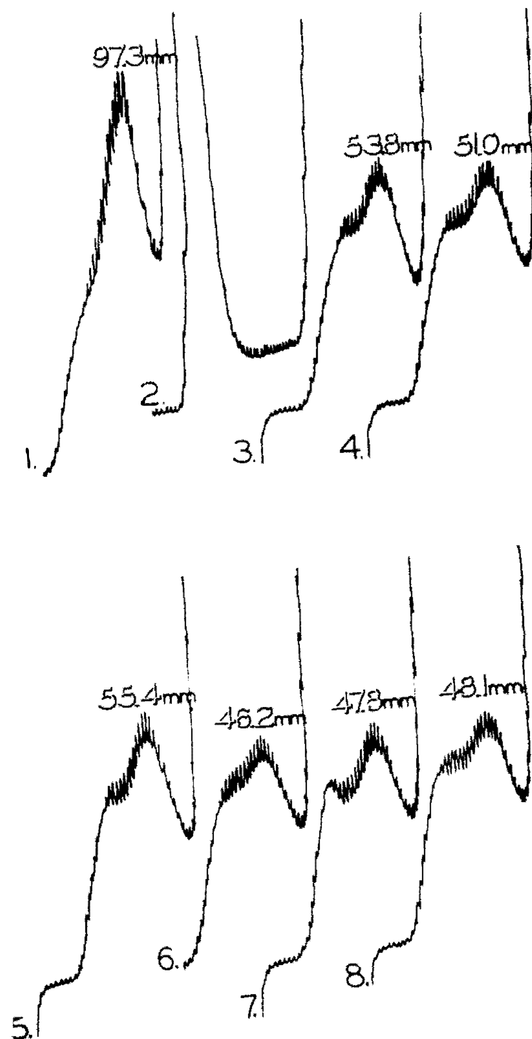

Figure 6. A series of tracings from one patient diagnosed as bronchogenic carcinoma metastasized from a caecal focus.

1st Tracing — 22 October, 1960. Strongly positive, suggestive of an advanced malignancy.
2nd Tracing — Cobalt solution electrolysis used as a base line for measuring protein wave height.
3rd Tracing — 8 May, 1961. Platelet count, 110,000; received X-ray therapy from November, 1960 to March, 1961 for a total of 4,200 R.
4th Tracing — 11 May, 1961. Platelet count, 183,-000; received 400 mg. of cyclophosphamide parenterally from 8 May to 11 May.
5th Tracing — 17 May, 1961. Platelet count, 251,-000; received 600 mg. cyclophosphamide from 11 May to 17 May.
6th Tracing — 12 June, 1961. Platelet count, 91,-000; receiced 1500 mg. cyclophosphamide orally from 17 May to 9 June; drug therapy was discontinued on this date.
7th Tracing — 19 June, 1961. Platelet count, 106,-000.
8th Tracing — 25 July, 1961. Platelet count, 94.-000.

Laboratory Method

Following is the detailed laboratory procedure that our service has adopted for rou-

tine clinical evaluations by means of the Brdicka serum filtrate reaction:

1. A fasting blood sample, approximateyl 4-5 ml., is withdrawn from the patient's antecubital vein, with a needle large enough so that hemolysis does not occur (20 gauge or larger).

   n.b. We have found that nonfasting as well as fasting runs have fallen within the experimental error. We have not obtained false negative or positives with nonfasting blood.

2. Remove needle and gently express contents of syringe into a 15 ml. conical centrifuge tube.

3. Allow blood to clot, and if necessary clotted blood may be kept in the refrigeratory for several hours.

4. After loosening the edge of the clot from the centrifuge tube with a glass rod, centrifuge at 2500 r.p.m. for 15 minutes; then at 4500 r.p.m. for 15 minutes.

   n.b. A refrigerated centrifuge is recommended.

5. After centrifugation, withdraw 0.4 ml. serum with a calibrated 1.0 ml. pipette, or preferably a micropipette, and discharge contents into a microbeaker of 3-5 ml. capacity.

6. Transfer the beaker to a refrigerator and blow in 1.0 ml. of 0.1 N KOH (previously refrigerated) and allow to denature at a temperature between 0-10°C. for 45 minutes.

   n.b. Denaturing time is critical, as we have recorded false positives with longer denaturations.

7. After removing the beaker from the refrigerator, immediately add 1.0 ml. of 20% sulfosalicylic acid; allow protein fractions to precipitate out for 10 minutes, stirring with a small glass rod 50x at beginning and 50x just before 10 minutes have elapsed.

8. Pour the contents of the beaker down the rod onto Whatman No. 44, 7.0 cm. filter paper (W&R Balston, Ltd.) and filter for 12 minutes.

   n.b. The choice of time for a particular filter paper is critical and should be standardized for all subsequent runs. It may be longer or shorter than 12 minutes for a slower or faster paper, but the time must be kept the same for each succeeding run.

9. With a calibrated 1.0 ml. or micropipette withdraw 0.4 ml. of filtrate and transfer to a Heyrovsky flask.

10. Add 4.0 ml. of cobaltic solution of the following composition:

    0.001 M Co $(NH_3)_6Cl_3$*
    0.1 N NH_4Cl
    1.0 N NH_3

    Prepared as follows:

    0.2675 g. $Co(NH_3)_6Cl_3$
    5.35 g. NH_4Cl
    17.0 g. NH_3 (67.5 ml. of 28% NH_4OH)
    q.s. to 200 ml. with distilled water.

11. Drop enough mercury down the side arm of the Heyrovsky flask so that the pool covers the platinum contact.

    n.b. The platinum wire from the side arm may be depressed to touch the bottom of the Heyrovsky flask so that the amount of mercury in the anode pool can be held to a minimum.

12. The toxic properties of mercury are well known, and with this in mind several precautions should be adhered to while using this volatile metal.

    a. The used mercury should be stored under water and tightly stoppered.

    b. The dropping mercury electrode apparatus constructed according to the E. H. Sargent & Company's instruction manual should be kept in a large unbreakable pan in order to retain any spilled mercury.

    c. Powdered sulfur should be dusted around the work bench from time to time and allowed to remain for several days. Mercury as the sulfide, rendered less toxic, can then easily be swept up. The room should be well ventilated.

    d. The mercury is cleaned with the apparatus pictured on page 372 of the Annals of the New York Academy of Science, Volume 65, Art. 5, with multiple washes of

*Hexamminocobalti chloride, C. P., Amend Drug & Chemical Co., 117-119 East 24th St., New York 10, N. Y.

10% potassium hydroxide, distilled water, 15-20% nitric acid, distilled water, and finally filtered through a needle hole in the apex of a large piece of filter paper.

13. Polarography:

Room temperature: 20-25°C.
Adjust Span E.M.F.: 4.0 volts.
Shunt Ratio: 1:200.
D.M.E. Potential: Negative (reduction).

Photographic paper: Kodak Royal Bromide or Kodabromide F-1, 6x10 in.

Recording track: Commence initial run on track 10 of the Sargent-Heyrovsky Model x11 (10). Subsequent increments of 1.5 should allow one to record 5 or 6 well separated tracings on the same film.

Zero point: Galvanometer light at commencement of run should be on 5 of the visible current scale.

Run: Crank the film drum scale to 0.20; turn on motor, and open camera shutter simultaneously. This maneuver allows the reaction to be recorded from —0.8 volts. An alternate method is to turn on the motor with the film drum scale on 0.00 and then, just as the film drum scale reaches 0.20, open the camera shutter and make contact with the anode pool. This latter method would presumably give the analyst 2½ minutes of grace to correct any last minute errors of omission or commission. The run should be finished (that is, the galvanometer light has moved off the visible current scale) when the film drum scale reads approximately 0.45.

A summary of the appropriate filtrate reactions showing the high degree of accuracy achieved by this technique can be seen in figure 7.

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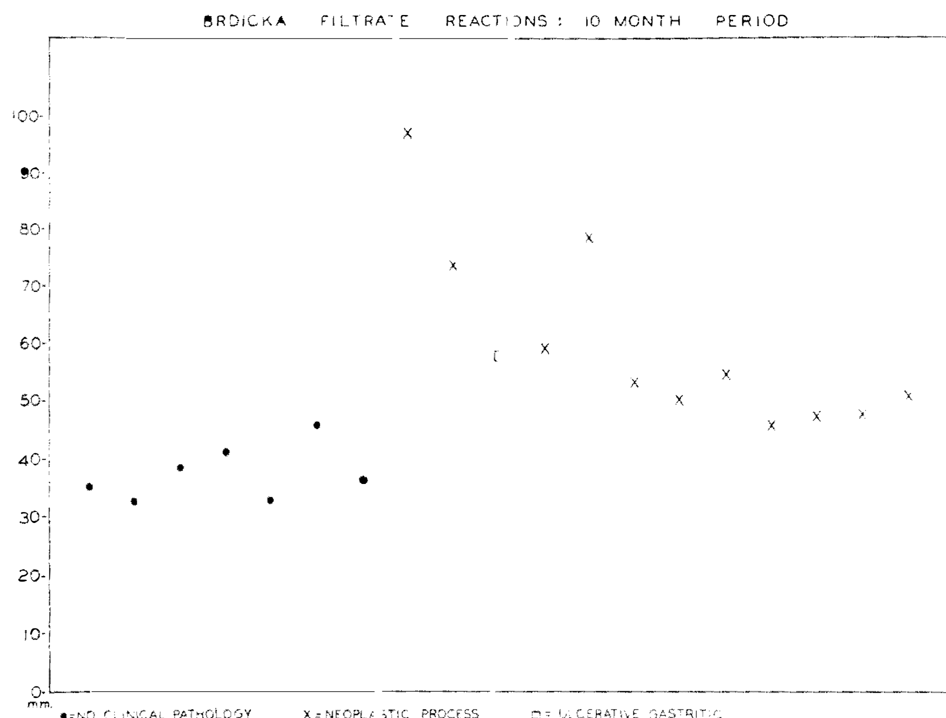
Figure 7



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