Without doubt the greatest handicap to cancer research has been the small number of quantitative methods available for measuring the activity of tumors. Until the last decade, with a few exceptions, most notably in investigations with Warburg techniques, the science of cancer research was attempting to operate without precise measurements, a feat commendable in the attempt but clearly inefficient if not impossible. This accounts in large part, I think, for the slow rate of development of important facts about this difficult problem of growth.

Since 1929 it has been possible to recognize the presence, and more recently the activity of several neoplasms by chemical means. With the exception of myeloma, in all cases the component measured has represented an abnormally large concentration of a normal constituent of bodily cells. No specific neoplastic product as yet has been recognized quantitatively. Mostly the devices have concerned the tumors of man largely because chemical analysis of liquids is somewhat easier than solids and men are juicier than mice. The following components are available for measurement of cancer activity:

1. Bence-Jones proteinuria in myeloma.
2. Melanin precursors in the urine in melanoma; these colorless compounds after excretion can be oxidized (45) to melanin.
3. Chorionic gonadotrophin in the blood and urine (64, 16) in trophoblastic tumors of the testis and uterus.

4. Ketosteroids in the blood and urine of patients with tumors of the adrenal cortex (51, 18) and of the interstitial cells (57) of the testis. The androgenic hormones, trans-dehydroandrostosterone (10) and androsterone (57) have been isolated in these cases in large amounts.

The most highly developed diagnostic agents in cancer are found among the proteins of plasma, the principal subject of this paper which, it is hoped, will supplement the recent reviews of Toennies (56) and Gutman (24).

There are fashions in science as in ladies apparel. After the work on proteins by Emil Fischer and his contemporaries early in this century, protein investigations were greatly influenced by the methods of the Swedish physical chemists which have yielded results of high importance. Recently there has been a return to the techniques of pure organic chemistry in the elucidation of the reactive groups of proteins.

ENZYMES

The overall activity of enzymes must be interpreted not only in terms of what amounts of these catalysts are present but also in the light of associated promoters and inhibitors of enzymic action.

(a) Alkaline phosphatase.—Kay (33, 34) made the important discovery that in hyperplastic disturbances of bone there are abnormally large amounts of alkaline phosphatase in the serum, the increases being correlated in a general way with the severity of the disease. In this connection increased osteoblastic activity leads to abnormally great fabrication of the enzyme which finds its way into the blood. Two effects occur in connection with skeletal tumors. Primary osteogenic tumors as such cause an elevation. Also, in connection with osteoblastic carcinomatous metastasis the reaction is due to the proliferation of non-malignant
osteoblasts which is evoked by the presence of certain epithelial tumors (e.g. prostate) in the skeleton; most metastatic tumors in bone however do not stimulate osteoblast growth. Control of the neoplasm is reflected inter alia in the movements of the osteoblastic mass set in motion by the regression of neoplasms and demonstrable by estimations of this enzyme. In most patients with skeletal metastasis from prostatic cancer, the induced remission (28) is followed by a temporary regression of neoplasms and demonstrable by estimation of the enzyme level to normal. Clearly the reaction of the host tissue to the activity of a neoplasm may be followed closely by determining systematically the alkaline phosphatase of serum in patients with metastatic cancer resident in the skeleton.

(b) Acid phosphatase.—The discovery of Gutman and Gutman (22) that this enzyme is increased in the serum in prostatic cancer has added to the elegance of diagnosis and prognosis in this disease and also has provided a useful measuring device for investigative purposes. The nature of proof is sometimes of greater interest than that which is proven; by means of the acid and alkaline phosphatases of serum it was first demonstrated (28) that widespread carcinomatosis in man frequently is susceptible to more or less control through the use of chemotherapeutic agents. The control in a small percentage (20 per cent) of cases in the author's series of patients with prostatic cancer has lasted more than nine years and appears to be of indefinite duration.

Prostatic cancer no matter how large does not effect elevation of the phosphatases of serum, so long as the disease is confined to the locale of the pelvis.

The acid phosphatase content of serum rises only when the tumor has metastasized to bone marrow, lymph node, or liver. Apparently no mechanism exists for the entrance of molecules of so large a size as acid phosphatase except in regions where production of the plasma proteins normally takes place. While slight elevations of acid phosphatase in the serum occur in several non-malignant diseases, such as osteopetrosis, considerable elevations (e.g. values greater than 10 King and Armstrong units per 100 cubic centimeters) indicate metastatic carcinomatosis of the prostatic gland.

(c) Aldolase (Zymohexase), and Isomerase.—Aldolase is an enzyme widely distributed in animal tissue which catalyzes the reversible cleavage of fructose-1-6-diphosphate into the phosphates of glyceraldehyde and dihydroxyacetone, the equilibrium between the two products being established by a second enzyme triose-phosphate isomerase. Warburg and Christian (60) found that these enzymes were very much increased in the serum of rats bearing large Jensen sarcomas, the rise being roughly parallel to the size of the tumors; the enzymes in the serum of normal rats were not elevated. Sibley and Lehninger (50) using a simplified method of their own devising confirmed these findings with other tumors of rats: surgical excision of the tumor or the administration of ethyl carbamate caused a decline of aldolase to normal levels. Further, they found a significant elevation of aldolase in 20 per cent of 104 cases of cancer in man although there was no obvious correlation with the clinical findings.

**ENZYME INHIBITORS**

(a) Anti-proteolytic factors.—Brieger and Trebing (9) demonstrated that serum from cancer patients could inhibit the proteolytic effects of pancreatic extracts. Using more refined methods Clark et al. (11) have reinvestigated this problem finding that the anti-trypsin factor of serum is greatly increased in cancer. They incubated dilutions of serum with trypsin, then added fibrinogen and later thrombin; the presence of a clot indicates undigested fibrin, hence the presence of an anti-trypsin factor and the greatest dilution of serum which permits coagulation is the end-point. Clark reported that 75 per cent of cancer patients gave positive reactions, 7 per cent were doubtful and 18 per cent negative; there were 9 per cent of false positive reactions in patients with advanced tuberculosis or active infections.

(b) Tyrosinase inhibitor.—Duboff and Hirshfeld (14) reported that a factor in the blood serum of cancer patients inhibited the aerobic oxidation of tyrosine by crude potato tyrosinase, suggesting that this inhibition might form the basis of a serum test for cancer. Marx (39) found that there was no close correlation between malignancy and tyrosinase inhibition although there was a small but statistically significant difference between normal and cancer sera.

(c) Hyaluronidase inhibitor.—Hakanson and Glick (25) reported that the inhibitor of hyaluronidase present in human serum was some 52 to 140 per cent increased in cancer; significant increases also occur in infections.

**NON-ENZYMIC PLASMA PROTEINS**

In this field the greatest volume of work has been concerned with separation of proteins by electro-chemical or centrifugal methods. In the Tiselius (55) technique of electrophoresis a current
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is passed under standard conditions through a solution containing a mixture of proteins such as plasma. Many proteins migrate at varying rates producing a series of boundaries which may be identified by optical and photographic means. The simplicity of the method is also its weakness in that the separation of proteins depends on the single property of differential migration in an electric field and certain proteins very different with respect to size and chemical composition have similar mobilities causing them to appear in a homogeneous peak.

The Svedberg method of ultra-centrifugation actually has had a more restricted usefulness than electrophoresis in the study of whole plasma because of more limited resolution unless many photographs are taken during examination of this complex mixture of proteins; its chief value lies in estimating the molecular size of purified proteins and in determining the homogeneity of prepared fractions. In these situations it is indispensable.

Total protein.—In most cases of significant cancer there is hypoproteinemia (48, 53) and all investigators report moderate to pronounced decreases in late cancer; the only known exception is myeloma where the total protein content of plasma is usually considerably increased. In this discussion myeloma will be considered in a separate category. Excluding myeloma, electrophoretic analysis gives evidence of wasting disease (41, 48) but is not specific for cancer.

Hypoproteinemia largely signifies hypoaalbuminemia. In the classical consideration, hypoproteinemia implies deficiencies of intake, absorption or synthesis of protein or, on the other hand, increases of wear-and-tear (catabolism) or excretion. Any or all of these may be operative in cancer.

Fibrinogen.—Frequently but not invariably (17, 18, 53) the plasma fibrinogen is increased in cancer—at times doubled or quadrupled. Also, in cancer there is usually an increased sedimentation rate of the erythrocytes due at least in part to the increased fibrinogen. Gray and Mitchell (19) first added electrophoretically pure proteins to heparinized whole blood studying the rate of sedimentation thereafter; fibrinogen was more efficient in increasing the rate than any of the globulins of which y-globulin was least effective while on the contrary albumin slowed the rate of settling. Following the excision of gastric cancers the fibrinogen and a-globulin (41) levels returned to normal in a few weeks.

Albumins.—The plasma albumin content is always decreased in the presence of a considerable cancer. Petermann and Hogness (41) found that in gastric cancer the level is far below the lowest normal; following gastrectomy, increases (41) in albumin are extremely slow even when the patient is in strongly positive nitrogen balance. It is the last component to return to normal after recovery (48).

Luetscher (37) showed that purified equine or human serum albumin although forming a single boundary at pH 7.4, separated into two components at pH 4 in acetate buffer; the faster moving component constituting two-thirds of the normal albumin, in common with most of the plasma proteins, carried a positive charge while the slower fragment moved to the anode. In cirrhosis and certain other diseases the faster moving component was diminished in amount leaving the negatively charged albumin preponderant. Petermann and Hogness (42) found that in gastric and pulmonary cancers and in some lymphatic leukemias the amount of the negatively charged protein component of plasma was significantly increased.

Globulins.—The a and / globulins are associated with much lipid and carbohydrate components (12) and the y-globulins are, at least in part, immune bodies. Isolated a-y globulin has a higher polysaccharide content (48) than any other plasma protein.

The a-fractions are often increased in chronic infection (49) and in conditions of increased tissue destruction irrespective of its cause. In cancer both of the a-globulins are commonly increased but the a-y fraction to a greater extent than a-y, often conspicuously so. Seibert et al. (47) discovered an increased polysaccharide level in the serum always associated with increased a-y in both cancer and advanced pulmonary tuberculosis. These elevations which may amount to as much as two-fold increases above the normal values are interpreted by them as concomitant upon tissue destruction; the polysaccharide level is not dependent on the state of metabolism (48).

The / and y globulins are usually not very abnormal in cancer serum, although in certain cases with metastasis to the liver and jaundice (48) and also in Hodgkin’s disease (49) the y-fraction is slightly increased.

Multiple Myeloma.—In common with other neoplasms this disease when full blown is associated with decreased albumin (35, 23) and increased fibrinogen in the plasma but the most striking characteristic is an increased total plasma protein content. While the plasma proteins may be normal in amount and pattern (24, 36) in 62 per cent of 282 cases (23) the values were greater than 8 grams per cent; the total protein has been found as high as 13.8 grams per cent, three-quarters of the increment being “globulin” (40).
This rare disease has been much studied since Bence-Jones described the occurrence and striking thermal coagulative characteristics of the albuminuria in myeloma. These proteins are not globulins and have a molecular weight (34) of about 37,000. Using neutral salt precipitation techniques the increased protein of plasma has been salted-out in different cases with various sodium sulfate concentrations (23) between 13.5 and 21.5 per cent thus accompanying any of the globulin or albumin fractions; usually it is precipitated (23) with the 17.4 per cent fraction—the Pseudoglobulin I in the Howe terminology.

Bayne-Jones and Wilson (1) could demonstrate at least two antigenically different groups of urinary Bence-Jones proteins. Certain patients excrete a single protein of these types while others have both kinds in the urine (27).

The electrophoretic patterns of myeloma serum may be normal (24). More commonly there are increases of either the β (36) or γ (35) peaks, or an intermediary (M) spike (23) between them representing an abnormal pattern. These patterns are of great diagnostic importance provided other supporting data are present. By adding Bence-Jones proteins obtained from the urine of different patients to normal serum (23) it was possible to reproduce the several electrophoretic patterns found in clinical patients. Because of the lack of good methods for estimating Bence-Jones protein quantitatively in plasma it is difficult at the present time to differentiate between the increased protein fragments in myeloma as being globulins or Bence-Jones proteins migrating with the same electromobility.

REACTIVE GROUPS OF SERUM PROTEINS

Many rather quaint, if not weird, but apparently related and suggestive observations have been made which seem to indicate a systemic defect in cancer. Most of the techniques have one thing in common—they are rather unattractive to chemists, but many a stout heart beats under a rude cloak.

Kahn (32) observed that the serum of cancer patients is deficient in “Albumin A,” the most soluble albumin fraction precipitated only with the stronger concentrations of neutral salts. In the original test, later refined (26), three drops of ear blood were collected on paper, dried and then soaked in 37.5 per cent ammonium sulfate, a concentration which, by definition, did not cause the precipitation of “Albumin A.” The paper was then placed in boiling water where cloud formation in the hot water comprised a negative test indicating the presence of protein not salted-out, while opalescence-to-clear was positive. In addition to cancer the test was positive in pregnancy, starvation and cirrhosis.

The enzymes papain and methyl glyoxalase require -SH groups for activation (59). Purr and Russel (44) dialyzed papain to remove the -SH activator and then added blood from patients or rats with cancer; the whole blood in cancer had less activating power than normal blood. These findings were confirmed by Waldschmidt-Leitz et al. (58) and extended to partly inactivated glyoxalase; they observed that the activating groups of blood were resident in the serum, that of cancer being about one-half as effective in this regard as normal serum.

As shown by polarographic analysis also, sulfhydryl groups are deficient in cancer serum. Brdička (6) added an ammoniacal cobalt solution to serum and found that the catalytic polarographic waves were smaller in cancer than in normal serum. Using sera denatured in several ways the differences were more pronounced (7) being 3 to 50 per cent lower in cancer than the height obtained with normal serum; acute inflammation yielded results similar to cancer. On hydrolysis of sera with boiling 5N HCl, the hydrolysates (8) of cancer serum were found to contain less cystine than normal serum. It has long been known that there is in serum a small quantity of non-dialyzable proteose which is soluble in sulfosalicylic or trichloroacetic acids but is precipitated by more efficient protein precipitants such as phosphotungstic acid; these proteins are not heat coagulable. Brdička (8) made the important observation that after sulfosalicylic acid treatment with removal of the precipitated proteins the catalytic wave is higher (more proteoses) in cancer than in normal serum. Brdička’s interpretation of these findings was that in cancer there is a pathologic abbau of the serum albumins. Neither of these findings is specific for cancer; they also occur in the serum in inflammatory states.

These indications of increased proteoses in cancer serum were confirmed by Winzler and Burk (61) using the blood of rats and rabbits with malignant tumors and inflammatory processes; the increase of proteoses above normal may be 12-fold and small quantities of the proteins added to culture media stop the growth of yeasts. Further studies by Winzler (62, 63) and associates revealed that the proteoses are mucoproteins; the carbohydrate/tyrosine ratio was the same in cancer as in normal serum demonstrating that the mucoproteins are similar in both states. The mucoprotein has an isoelectric point below that of serum.
albumin and it contributes to the a-globulin fraction when electrophoresis is done at pH 8.4. The reducing power of serum in cancer is decreased. Savignac, Gant and Sizer (46) added serum and alkali to methylene blue and boiled the mixture under standard conditions, observing the time required for decolorization. The reducing time of the dye is prolonged in cancer, uremia, and cirrhosis and they suggested that the methylene blue reduction test depended on the actual or potential reducing groups in the albumin fraction. Considering the mechanisms of the reduction of methylene blue by serum under the alkaline conditions (pH 11) employed by Savignac and co-workers, Stadie (52) demonstrated that heat converted a large part of the bound sulfur to S^-2, which was the sole agent responsible for the reduction; he could find no correlation between the reduction time and the presence or absence of malignancy in 315 experiments.

Black (2) simplified the methylene blue reduction test and also omitted alkali from the system and confirming Savignac found a tendency towards deficient reduction in cancer serum which he ascribed to the albumin fraction. In the Black test methylene blue is added to serum and the tubes are placed in a bath of boiling water, the time of decolorization being determined. The plasma of patients (2) with malignant diseases “could be differentiated with a high degree of accuracy”; false positives occurred when the serum proteins were less than 5 grams per cent, and in cirrhosis. Black (3, 4) postulated that the decreased reduction of methylene blue by cancer serum might be due to changes of spatial configuration of the albumin molecule accounting for a delayed appearance (unmasking) of the reducing groups, there being no significance in the total number potentially present. In a small series of cases we also found in this laboratory decreased reducing properties of the blood in cancer. Jensen et al. (31) examined the reducing power of serum by measuring colorimetrically the amount of triphenylformazan (TPF) formed when serum was heated with triphenyltetrazolium chloride at 80° C. Sera from 108 individuals were compared on the basis of their reducing power per milligram of serum protein. The results were expressed in the form of an arbitrary index,

\[
\text{micrograms TPF in 0.5 ml.} \quad \frac{\text{gm. of protein per cent}}{} 
\]

In a group of 60 patients with various types of cancer 85 per cent showed a low reducing index of 9.9 or below; of the 15 per cent whose index lay above this figure two-thirds were cases of prostatic carcinoma which appeared clinically to be under good control by anti-androgenic measures. In a group of 48 individuals having no disease or non-malignant pathology, 80 per cent showed a reducing index above 10.0.

Abnormalities of reactive groups are also demonstrable by the heat coagulation of cancer serum. Black et al. (5) found that the plasma of cancer patients coagulated to a greater extent than normal plasma, the finding apparently being due to the increased level of fibrinogen since it was not operative when serum was used. In this test the density of diluted serum was determined in a colorimeter before and after heating for 10 seconds.

In contrast to plasma, cancer serum is deficient in its thermal coagulation properties. Some qualitative observations (15) demonstrated that with respect to normal serum, less flocculation occurred in heated serum from cancer patients. These observations have been recently confirmed (30) and placed on a quantitative basis.

Boiling undiluted serum for 30 minutes always causes it to coagulate while on the other hand any serum can be diluted to a point where it will not solidify when heated; normal serum may be diluted to a greater extent than cancer serum and still retain its capacity to gel. The serum from 84 normal persons coagulated when diluted so that the total protein was less than 1.5 grams per cent; only 8 per cent of cancer sera coagulated in so great dilution. Determination of the least coagulable protein concentration is a simple though rough screen for cancer.

The deficient coagulative ability of cancer serum may be demonstrated in a more refined manner by measuring the inhibition of thermal coagulation by iodoacetate. Gel formation in protein solutions is believed to depend on the formation of a 3-dimensional network of polypeptide chains. Sulphydryl compounds (e.g. cysteine, dimercapto-propanol) greatly enhance thermal coagulation while iodoacetate (29) in appropriate and small amounts blocks it. This effect of iodoacetate is unique among a class of iodinated compounds since iodoacetamide, methyl iodoacetate and iodoacetone do not inhibit coagulation. It has been shown by a number of investigators that iodoacetate and iodoacetamide react with -SH groups and to a less extent with amino groups of proteins; no appreciable difference exists at 100° C. in the rate of reaction of the model compound cysteine with iodoacetate or iodoacetamide. Both ends of the iodoacetate ion therefore are clearly involved in its peculiar inhibition of the coagulation of proteins by heat; iodoacetate combining with reactive...
groups of proteins increases the net negative charge on the protein molecule by adding mutually repelling electro-negative groups which prevent the close apposition of adjacent molecules.

Smaller quantities of iodoacetate are required to block the thermal coagulation of cancer serum (30) than normal serum. By determining the smallest quantity of iodoacetate required to prevent coagulation of serum by heat and relating this to the protein content of serum it was found that all of 85 consecutive cancers fell in a group with a low iodoacetate index (less than 9). The serum of all patients with active pulmonary tuberculosis resembled cancerous serum while that of pregnant women and newborn infants reacted like normal adult serum.

Madden and Whipple (38) have recently reviewed the evidence that serum albumin is made in large part in the liver. Griffin and Baumann (21) demonstrated that homogenates of livers of rats fed m'-methyl-p-dimethylaminoazobenzene failed to coagulate at 100° C. whereas similar homogenates from normal rats coagulated completely in the presence of a tumor even in one of the extremities of the host depressed the synthesis of an enzyme in the liver.

In summary the following evidence implies a defect in the serum albumin in cancer: a quantitative deficiency in one of the albumin fractions ("Albumin A," positively charged albumin); decreased cystine as indicated by decreased sulfur catalytic waves; decreased reducing groups; deficient thermal coagulation. The defect is not specific for cancer, occurring also in infection. The mechanism of the production of the albumin abnormality is unknown but presumably is due to the effect of some chemical agent on hepatic synthesis.

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