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William Kendall Mueller
Yale University

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William Kendall Mueller

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Department of Obstetrics and Gynecology
Yale University School of Medicine
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INTRODUCTION

Discovery of Haptoglobins - Haptoglobins are serum glyco-proteins capable of binding free hemoglobin in vivo and in vitro. The term haptoglobin is derived from the Greek word haptein, "to fix or to seize," because it unites with hemoglobin to form a stable complex.[1]

The original investigation of haptoglobinsin the 1940's was performed by Jayle and Abdellatif in France. They showed conclusively that a particular serum protein was capable of binding with hemoglobin and characterized it as a glycoprotein.[2] In 1950, Jayle and Badin showed that both haptoglobin and the haptoglobin-hemoglobin complex migrated electrophoretically as $alpha_2$ proteins.[3] In 1952 they demonstrated that haptoglobin was responsible for most, if not all, of the increase in $alpha_2$ globulins seen in "pathologic sera." The nature of the pathology was not specified.[4]

The initial studies of haptoglobin in the United States were performed by Tuttle, who, as a member of the Department of Pediatrics at the University of Tennessee, was unaware of Jayle's work.[5] Using filter paper electrophoresis with a veronal buffer, pH 8.6, he noted that hemoglobin by itself migrated at the same rate as the beta globulin of a serum sample migrating in a separate column. When the hemoglobin was mixed with fetal sera in consecutively large amounts, successively darker bands appeared in the beta globulin area. However, the mixture of hemoglobin with the sera of older children and adults yielded quite different electrophoretic results:

now the hemoglobin appeared in the alpha$_2$ globulins as one band. As successively larger amounts of hemoglobin were added to a given amount of serum, the alpha$_2$ band increased in intensity until a second band appeared, representing hemoglobin in the beta range. From these observations, he concluded that older children and adults had a serum protein capable of binding finite amounts of hemoglobin not present in infants, and that the complex of serum protein and hemoglobin migrated in the alpha$_2$ range.

Chemistry of Haptoglobin - Although the haptoglobins migrate with the alpha$_2$ fraction of serum protein, they have distinctive chemical properties oftheir own. They are 20% carbohydrate - specifically sialic acid, fucose, hexoses, and glucosamines - and 80% protein.[6,7] The protein portion consists of two different polypeptide chains termed alpha and beta. Every haptoglobin molecule has the same amino acid sequence in its beta chain. This beta chain binds to the carbohydrate moiety, and also free hemoglobin, when it is available, in a ratio of 1 to 1.[8,9] The carbohydrate itself, however, is not essential to the binding process.[10]

The alpha chain, in contrast to the beta chain, is not identical in every individual, but is subject to genetic variation.[11]

Genetics of Haptoglobins - In 1955, Smithies examined the electrophoretic distribution of serum proteins of normal adults and noted that the sera could easily be classified into three groups on the basis of the gel patterns of the proteins in the alpha$_2$ range.[12] One pattern consisted of a band which had migrated faster

than transferrin; a second pattern consisted of a number of bands which had migrated more slowly than transferrin; while the third pattern consisted of a band migrating more quickly than transferrin identical to the solitary band of the first type plus a number of more slowly migrating bands. It was further demonstrated that the proteins of these bands were capable of binding hemoglobin.

That same year Smithies and Walker postulated from extensive family studies that the three serum protein groups were determined by a single pair of allelic genes with incomplete dominance.[13] The persons having the serum protein pattern first described above were thought to be homozygous for one allele; the individuals with the second pattern were thought to be homozygous for the second allele, and the individuals with the third pattern were considered to be heterozygotes containing both alleles. In 1956, these same two scientists proposed the present nomenclature for phenotypes and genotypes[14]:

Old Phenotype	New Phenotype	New Genotype
Serum Protein Pattern 1	Haptoglobin 1-1	Hp^1 / Hp^1
Serum Protein Pattern 2	Haptoglobin 2-2	Hp^2 / Hp^2
Serum Protein Pattern 3	Haptoglobin 2-1	Hp^1 / Hp^2

The incidence of the various phenotypes has been carefully studied:

Study	# of Persons in Study	Haptoglobin Type (1-1)	(2-2)	(2-1)
Study 1 (U.S. military personnel)[15]	182	16.5%	37.9%	45.6%
Study 2 (Prague citizens)[16]	-	17.1%	35.1%	47.7%
Study 3 (Maryland citizens)[17]	192	12.5%	43.2%	44.2%

With the exception of a few rare subtypes of haptoglobins[18,19] and familial ahaptoglobinemia[1,20,21], every one can easily be classified into one of the three above basic phenotypes. The genetic properties of haptoglobins are now widely accepted and have been used in establishing paternity.[22]

Synthesis of Haptoglobins - All available evidence points to the liver as the site of haptoglobin synthesis. Rat liver perfused *in vitro* incorporates C^{14} labeled amino acids into haptoglobin.[23] *In vivo* studies in the dog show that amino acid incorporation into haptoglobin is abolished by hepatectomy.[24] A rise in haptoglobin levels of parenchymal liver cells produced by turpentine injections is abolished if the animal is previously damaged by the administration of carbon tetrachloride.[25] In man parenchymal liver damage is accompanied by low serum haptoglobin levels.[26] A most conclusive bit of evidence is a recent observation of a change in serum haptoglobin phenotype following human liver transplantation.[27]

The role of the adrenals in the production of haptoglobin is uncertain. They appear to have a permissive effect on the rise in serum haptoglobins seen in inflammation: adrenalectomy impairs the rise in haptoglobin levels.[28] Haptoglobin levels are significantly higher in men than in women, possibly due to the higher androgen production in man.

Clearance, Degradation and Turnover Rate of Haptoglobins - Both haptoglobin bound to hemoglobin and presumably free haptoglobin are removed from the blood by the reticulo-endothelial system, although the rate of removal of bound haptoglobin is much faster

than the rate of removal of free haptoglobin. The rate of clearance of haptoglobin under normal condition is not yet known, but after experimental intravenous administration of free hemoglobin, haptoglobin is cleared from the plasma at a rate of 15 mg. hemoglobin binding capacity per 100 ml. of serum.

Degradation of haptoglobin and haptoglobin-hemoglobin occurs within the reticulo-endothelial (RE) cells. An enzyme has been discovered in RE cells which specifically attacks the complex of hemoglobin and haptoglobin at the heme moiety: heme 2-methenyl oxygenase

The combination of haptoglobin-hemoglobin is too large to be filtered by the glomerulus, and under normal condition neither haptoglobin nor hemoglobin appears in the urine. However, after the hemoglobin binding capacity of the serum has been exceeded, free hemoglobin will be cleared both by the kidney and by the RE system, and will appear in the urine.[34]

When free hemoglobin is released into the intravascular compartment, the hemoglobin is bound within a matter of minutes and the complex is taken up by the reticuloendothelial system at greatly increased rates, but the synthesis of new haptoglobin is slow. Consequently, the serum haptoglobin falls to very low levels, often to 0, within a matter of hours and remains low for several days following hemolysis, a fact of diagnostic importance.[6]

Abnormal Quantities of Haptoglobins - the normal serum haptoglobin level ranges from low values of 20 to 50 mg.% hemoglobin binding capacity to high values of 150-190 mg.%. These vary with the type of haptoglobin.[15]

Haptoglobin levels are markedly lowered in hemolytic

conditions, liver disease and pernicious anemia. Haptoglobins are absent in intravascular hemolysis because free hemoglobin quickly combines with haptoglobin and the complex is quickly taken up by the reticulo-endothelial system.[35,36] The low levels in liver disease are the result of decreased synthesis due to impaired cellular metabolism.[37,38,39] The mechanism of decreased haptoglobin levels in pernicious anemia is unclear.[40]

Haptoglobin levels are elevated in other situations: acute and chronic infections,[41,42,43,44] burns, trauma,[45] collagen disease,[46,47,48] scurvy, amyloidosis, biliary obstruction, renal disease, Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma.[1,6,49] Haptoglobins are also elevated by the administration of androgens, glucocorticoids, parathormone and the purified enzymes papain, elastase and hyaluronidase.[1]

The effect of surgery on haptoglobin levels is controversial. Crockson reported a definite rise in serum haptoglobin in most patients following surgery, but the rise was extremely variable (25 - 100)mg.% and in some patients the hemoglobin level actually decreased.[50,51] More recently, Drak in Poland analyzed the haptoglobin levels of 40 male patients following surgery.[52] In patients who had no local inflammatory or generalized systemic reactions, a significant drop in haptoglobin levels from 125 $\pm$ 28 mg.% to 82 $\pm$ 26 mg.% occurred during the first few days and persisted 14 to 16 days later. In patients with distinct local or systemic reactions postoperatively, haptoglobin levels rose sharply the day after the inflammation was noted to a peak level of 195 $\pm$ 45 mg.%. then fell back to the normal starting

levels within 14 to 16 days postoperatively. Patients with febrile reactions had rises to significantly higher levels than the levels of non-febrile patients. Drak concluded that without complications, surgery caused a decrease in haptoglobins and hypothetically attributed this decrease to "trace hemolysis caused by the crushing of blood filled tissues" by surgical manipulation.

Compatible blood transfusions have no predictable effect on haptoglobin levels; they may cause either an increase or a decrease.[53] However, an incompatible blood transfusion with hemolysis will of course reduce the haptoglobin level to zero.

Haptoglobins are also related to age: they are absent or consistently low during the first two months of life; to sex: they are slightly higher in men; and to pregnancy: they are elevated in puerperal women.[54,55]

Clinical applications of the measurement of haptoglobins are presently: 1) to diagnose hemolytic transfusion reactions; 2) to diagnose hemolytic anemia,e.g.,in sickle crisis, in leukemia, and other acquired hemolytic anemias; 3) to determine hereditary haptoglobin deficiency;4) to aid in the differential diagnosis of jaundice; 5) to assess the degree of hepatocellular damage and to prognosticate in patients with cirrhosis and hepatitis.[1,35,36]

Haptoglobins and Cancer - Attempts to Associate Serum Haptoglobin Phenotype and Cancer Type - In early studies, Keviatoski found no significant difference between the distribution of types of haptoglobins in patients with carcinomas of the breast, skin, uterus and lips and the distribution among the control population.[56] However, A.C. Peacock at the National Cancer Institute

later found that a leukemic population of 79 individuals contained an excess of individuals with type 1-1 and a deficiency of individuals with type 2-2 when compared to the inidviduals of the control population.[57] The relative risk of having leukemia (acute lymphocytic, chronic myelogenous or acute myelogenous) among people with type 1-1 as compared with people of 2-2 type was approximately fourfold. The conclusion held true for subgroups of people with each type of leukemia, as well as for the group of leukemic patients as a whole ($.02 < p < .05$).

Subsequently, Larkin typed 526 cancerous patients with two interesting results.[53] First, not only in the leukemias, but also in the reticuloses group as a whole (consisting of 36 patients suffering from leukemia, myeloma, Hodgkin's disease, or lymphosarcoma), there was an increased tendency toward a high frequency of the gene Hp1 and phenotype 1-1. Secondly, 53 females with genital cancers showed a considerably lower frequency of the Hp1 gene than the controls (0.264 for patients vs. 0.388 for controls); thus women with genital cancers showed a preference for type 2-2 haptoglobins. This group of patients contained 9 with cancer of the ovary, not further specified.

Haptoglobin Type	1-1	2-1	2-2
Number of Patients	0	2	7

Larkin's findings concerning female genital cancer were not corroborated by a subsequent investigation in 1969.[16] Mulinicove,

Jandova, and Skoda studied 291 females in Prague with genital malignancies and found no relation between the distribution of haptoglobin types in either the entire group of 291 patients with genital cancers or the 50 patients with carcinoma of the ovary, not further specified, and the distribution of haptoglobin types in the control population.

Attempts to Associate Serum Haptoglobin Level and the Existence of Malignancy - Cancer in General - In 1956, Jayle, Serpicelli, and Robert studied 37 patients with cancer, not further specified, whose haptoglobin levels ranged from 60-540 mg.%. They concluded that cancer could result in either a normal or a high haptoglobin.[59] In a 1959 study of 127 Scandinavian patients, Nyman reached the following conclusions:[26]

1) In localized cancer, even when complicated by infection, the serum haptoglobin level is only slightly raised.

2) In cancer with widespread metastases, haptoglobin levels may be high, normal or low.

3) High haptoglobin levels are most common in patients with skeletal and lung lesions and in patients with fever; normal values are most often seen in patients with liver metastases and with cancer of the mammary glands.

His study contained two patients with cancer of the ovary, both with widespread metastases. One had a level greater than 190 mg.%; the other had a level below 190 mg.%. In summary, Nyman found that high haptoglobin levels may be of some very limited value in determining the location of the cancer, but are of no use in determining the amount of tumor.

Reticuloses - In 1964 Owen found haptoglobin levels of patients with lymphomas, leukemias, Hodgkin's, and myeloma to be

significantly higher than that of controls.[60]

	Mean Hemoglobin Binding Capacity	Standard Deviation	Actual Range
64 reticulosis patients	147 mg.%	91 mg.%	10-420 mg.%
152 controls	93 mg.%	40 mg.%	10-220 mg.%

Malpas, in a series of 78 patients with reticuloses, likewise noted high serum haptoglobins. He also demonstrated elevations in ceruloplasmin, another alpha$_2$ globulin, as well as elevations in the alpha$_2$ globulins as a whole.[61] However, the rise in alpha$_2$ globulin could not be correlated with an increased amount of haptoglobin or ceruloplasmin. Consequently, the rise in alpha$_2$ globulins must have been caused by several components, not merely haptoglobin or ceruloplasmin; and conversely, an elevated haptoglobin or ceruloplasmin level was not necessarily manifested by a rise in alpha$_2$ globulins.

Carcinomas - Owen also noted a rise in haptoglobin levels in patients with carcinomas.[60]

	Mean Hemoglobin Binding Capacity	Standard Deviation	Actual Range
54 cancer patients (not further specified)	216 mg.%	93 mg.%	62-510 mg.%
152 controls	93 mg.%	40 mg.%	10-220 mg.%

Of recent interest is a report of fever, refractory anemia, and hyperhaptoglobinemia in two patients which were later found to have renal cell carcinomas.[62] Nephrectomy corrected these abnormalities. The haptoglobin level of the first patient fell

from 435 mg.% preoperatively to 310mg.% on the 14th postoperative day, to 80 mg.% 6 months postoperatively. The haptoglobin level of the second patient fell from 286 mg.% preoperatively to 111 mg.% on the 4th postoperative day.

Changes in hemoglobin binding capacity may be an accurate indication of the affect of radiotherapy on carcinoma of the cervix, Stages II and III.[63] The average haptoglobin level of 8 patients with Stage I disease was found to be normal prior to radiotherapy and throughout a course of treatment. The average haptoglobin level of eight patients with Stage II disease and eight patients with Stage III disease was high (234mg%) before the onset of radiotherapy. The onset of radiotherapy was accompanied by a slight rise in the average haptoglobin level, followed by a decrease to a normal haptoglobin level at 6 months with successful treatment and no signs of tumor recurrence. In two patients who had recurrences of tumor at notime did the haptoglobin decline to a normal level; and at the time of recurrence, haptoglobins in these two patients rose to astronomical levels.

Haptoglobin Turnover Rate and Cancer - Turnover rate has been calculated using I^{131} - labeled human haptoglobin in control persons and in eight patients with malignant neoplasms whose haptoglobin concentrations ranged from 42 mg.% to 702 mg.%.[31,32] In spite of this wide range of concentrations, the turnover rate and the half-life of haptoglobin remained relatively constant; T 1/2 varied from 2.0 to 3.0 days. The constant half-life indicates that high haptoglobin levels are not caused by a decreased rate of removal of haptoglobin from serum .

The turnover rate of haptoglobin in cancer patients appears to remain constant in spite of elevated haptoglobin levels.

Errata:

p. 13, lines 11-12

2) tumor mass less than 6 cm.
3) tumor mass greater than 6 cm.

8 women

Maclyn

Department

e Yale-

ne patient

a tissue diagnosis of the tumor with microscopic examination was obtained. Evidence of tumor by physical or surgical examination and roentgenology at the time of the sample was noted. On the basis of existing evidence patients were classified into three groups depending on the amount of tumor they contained: 1) no tumor, 2) tumor mass larger than 6 cm. in diameter, 3) tumor mass smaller than 6 cm. in diameter. Patients with numerous widespread metastases were placed in the third category. Patients were also classified according to the three major forms of treatment administered to them before the serum haptoglobin sample was drawn: surgery, radiotherapy and chemotherapy, within 0-2 months, 2 months to 1 year, or 1 year to 5 years prior to the sample. Surgery was laporotomy with total resection, partial resection or, if the tumor could not be resected, tumor biopsy. Surgery was followed by radio-and/or chemo-therapy. Radiotheapy consisted of 3,000 rads to the pelvis with or without an additional 2,000 rads to the abdomen. Chemotherapy was one of three types:

1) 5 Fluorouracil 7.5 mg./kg., I.V./o.d. x 5d./mo. and Uracil mustard 1 mg. p.o./o.d.

2) Leukeran 4 mg. p.o. o.d. and Velban 0.01 mg./kg. I.V. q. 2 wks.

3) 1,3-Bischloronitrosourea 100 mg./m^2 I.V. on 2 successive days 4-6 wks. apart.

In the nonserial studies, to avoid biasing results in patients with serial haptoglobin determinations, only the initial serum sample was used. Thirty-five patients were followed with serial haptoglobin determination for periods of up to one year. In the patients who were followed serially, tumor mass size, surgery, radio- and chemo-therapy, intercurrent infections, blood transfusions and paracentesis were noted.

The average age of the ovarian cancer patients was 51.9 years with a standard deviation of 14 years and an actual range of 18 years to 84 years.

Controls - There were two control groups: a preliminary control group (D-Controls) of thirty hospital personnel - nurses, doctors and medical students who were in good health with no known illnesses ; and a second control group (C-Controls) more closely approximating the target population in sex and age: 50 women admitted to Yale-New Haven Hospital for elective surgery with no signs of active inflammation or malignancy. The average age of the C-Controls was 43.7 years with a standard deviation of 12 years and an actual range of 23 to 72 years. Comparisons between patients and controls were always comparisons between patients and C-Controls.

Serum Samples - Blood was collected in a 10cc. tube without anticoagulant, allowed to stand for one hour, then centrifuged. The serum was extracted by pipette, then fresh frozen at -15°C. until use.

Methods of Typing Haptoglobin - All methods for typing haptoglobins involve separation of serum components by gel

electrophoresis, followed by examination of the distribution of bands in the alpha$_2$ range to determine the type of haptoglobin. Type 1-1 contains only one band, but types 2-1 and 2-2 contain multiple bands due to various polymer linkages in between the haptoglobin beta and haptoglobin alpha$_2$ chains.[64]

The original studies utilized starch gels stained with a non-specific protein stain amidoblack.[13,65] A new more elegant method utilizes polyacrylamide gels stained to reveal only the haptoglobin.[15] There is no stain which is specific for haptoglobin, but there is a stain specific for the peropidase activity of hemoglobin. Free hemoglobin added in excess to serum combines with haptoglobin readily but changes its electrophoretic mobility only slightly. Gel electrophoresis of serum to which hemoglobin has been added in excess separates the free hemoglobin and the various polymers of haptoglobin bound to hemoglobin. Staining with ortho-dianisidine, specific for peroxide activity, reveals the band of free hemoglobin and the various bands of haptoglobin polymers containing hemoglobin.

Preparation of Specimens - A freshly prepared hemolysate of adult human hemoglobin A was diluted to a final concentration 10 mg. per 100 ml. 0.01 ml. of hemoglobin hemolysate was added to 0.25 ml. of serum and the mixture was incubated in a water bath at 30^{o} C. for 30 min.

Electrophoresis - Electrophoresis was performed with an E-C Apparatus electrophoresis cell using a 7% polyacrylamide gel and a Tris ethylenediaminotetraacetic acid (EDTA) - boric

acid buffer, pH 8.3 to 8.4. After the gel was formed, 10 lambda of complexed serum were inoculated into each slot with a micropipet. The samples were allowed to settle in the slots for 5 minutes, and electrophoresis was then carried out at 200 volts for 3 hours at 8° C.

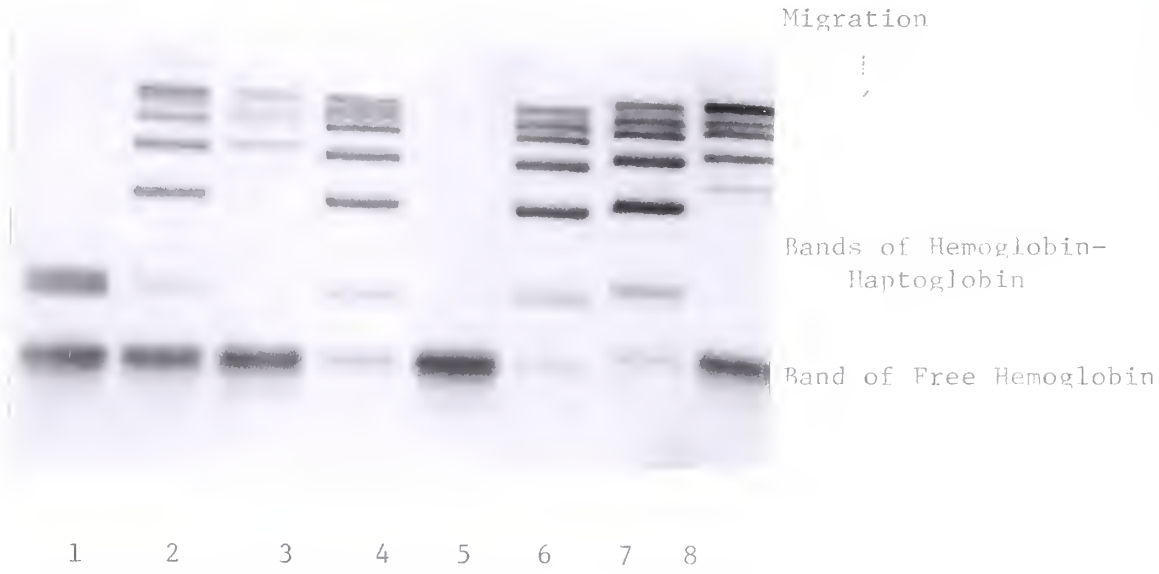
Staining - Following electrophoresis, the gel slab was submerged in o-diansidine solution buffered to pH 4.7 with acetate and agitated on a shaker table for thirty minutes. The gels developed a cloudy color which was cleared by soaking them for 30 minutes in an acetate buffer solution. The details of the above three steps are reported in the Ferris article.[15] A picture of a typical gel with haptoglobin types is seen in Figure I.

Method of Determination of Hemoglobin-Binding Capacity - Serum haptoglobin concentrations are not reported in mg. of haptoglobin per 100 ml. of serum but in mg. of free hemoglobin bound to haptoglobin per 100 ml. of serum. There are four basic methods of quantifying haptoglobins: 1) electrophoretic techniques [15,66,67,68,69], 2) spectrophotometric techniques,[70] 3) immuno diffusion,[71] and 4) gel filtration[72,73,74].

Quantitation of a Standard Serum by Electrophoretic Techniques - The original concentration of the hemoglobin in the hemoglobin serum mixture prepared above to type hemoglobin is 385 mg./100 ml. By running the gel slab containing the stained hemoglobin and separate hemoglobin-haptoglobin bands through a densimeter, one can calculate the proportion of free and bound hemoglobin. The absolute hemoglobin binding capacity of serum

FIGURE I

Haptoglobin Types



1. Type 1-1
2. Type 2-1
3. Type 2-2
4. Type 2-1
5. Absent Haptoglobin
6. Type 2-1
7. Type 2-1
8. Type 2-2

can easily be computed. In this manner the hemoglobin binding capacity of patient X-69 was found to be 277 mg. hemoglobin binding capacity/100 ml. serum.

However, in practice, the initial band types 1-1 and 2-1 of the gels was quite close to the band of the free hemoglobin band, sometimes merging with it,prohibiting proper use of the densimeter to demonstrate the densities of the separate bands. In addition, densimeter and integration procedures were time-consuming and cumbersome. Accordingly, an alternate method of quantifying haptoglobins was chosen: the spectophotometric method of Tarukoski.[70]

Quantitation of Serum Samples by Spectophotometric Techniques with a Standard Serum - The method used was a very simple colorimetric reaction which, unlike all other methods of quantification did not require either the separation of free hemoglobin from bound hemoglobin or the use of special apparatus. The final color could be read by any photometer with a light source of 395 mμ.

An excess of hemoglobin was again added to the serum and then o-dianisidine and ethyl hydrogen peroxide were added to give a colorimetric reaction. The order and the timing of adding the reagents, adjustments in pH, and selection of wave length used in the photometer were such that the color produced by the reaction between free hemoglobin and the reagents was negligible, while the reaction between hemoglobin bound to haptoglobin and the reagents was maximal. Thus, this quantitation procedure had an additional advantage over other quantitation procedures: prior

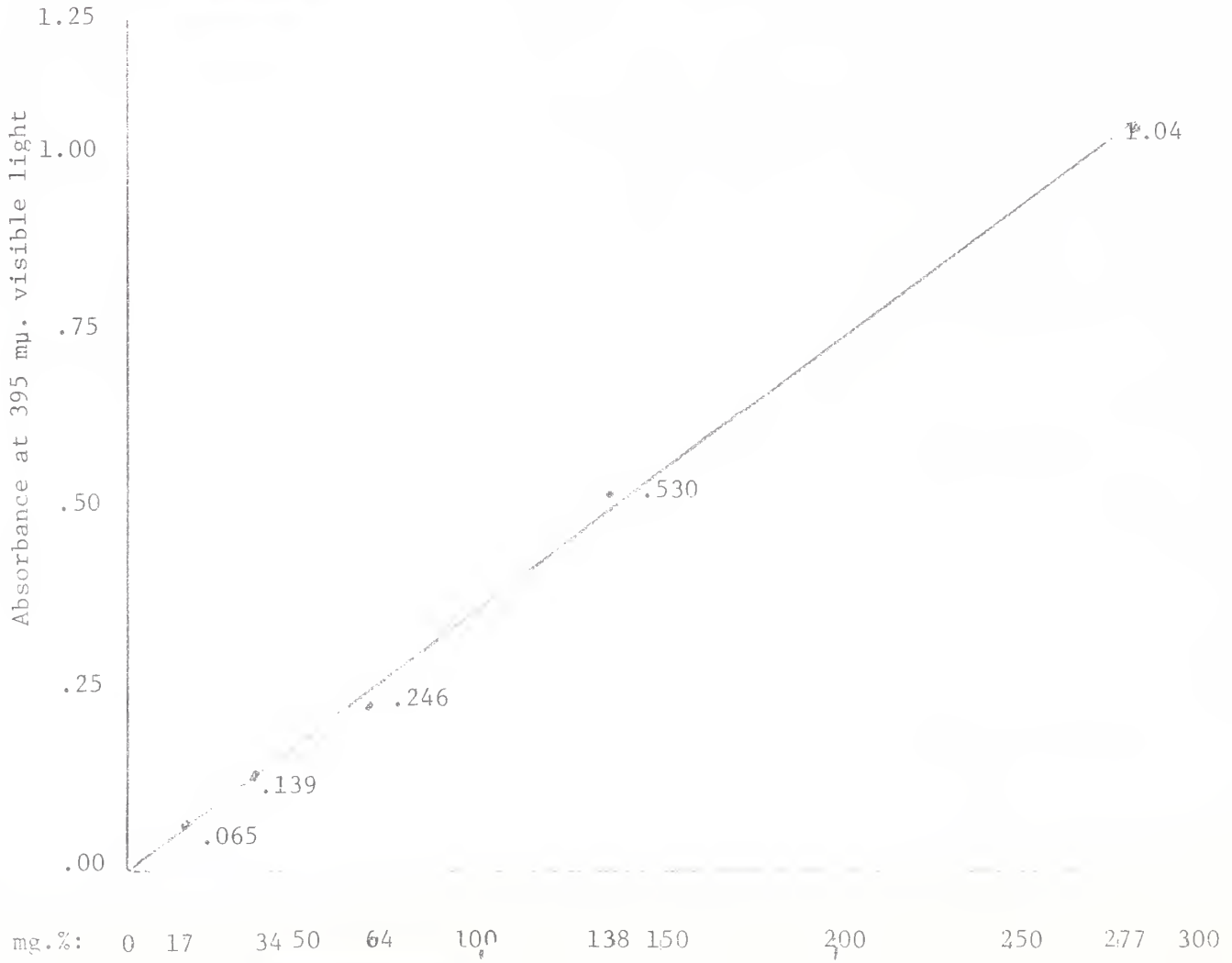
trace hemolysis of the serum samples with binding of haptoglobin and hemoglobin did not affect the results. A standard curve was constructed using the spectrophotometric readings of dilutions of a .5 ml. aliquot of the standard sera whose full strength hemoglobin combinding capacity was known to be 277mg.%. The spectrophotometric readings of the unknown sera could easily be translated in hemoglobin binding capacity using the standard curve. (See Figure II).

Reagents - 1) o-Diansidine reagent - The reagent is prepared by dissolving 1g. o-Dianisidine, 0.5g. EDTA and 15.6g. (0.1 mole) of $NaH_2PO_4 \cdot 2H_2O$ in one liter of distilled water overnight, the unidssolved residue filtered off and the solution carefully adjusted to pH 4.10± .05 with two to four drops of concentrated H_3PO_4. The reagent is stable for two weeks when stored at room temperature, and may be used for several more weeks if traces of undissolved precipitate resulting from auto-oxidation are filtered off before use. 2) Ethylhydroperoxide, 0.03N - 10 per cent aquaeous stock solution (3.0N)* is diluted 100 times with distilled water. The reagent is stable for months when stored in a refrigerator. 3) Hemoglobin A Stock Solution - Centrifuge whole blood. Remove plasma. Wash the red blood cells three times with plasma. Remove supernate and record volume of packed cells. For each 1 ml. of packed cells add 1.4 ml. distilled water and 4 ml. of toluene. Shake vigorously for 5 minutes. Centrifuge for 15 minutes at 3,000 RPM. Remove top toluene layer by pipette;

*Supplied by Dr. Halbren, Polysciences Corporation Research Laboratory, Betharyes, Penn. 19006

FIGURE II

Standard Curve for Haptoglobin Quantitation



then remove hemoglobin solution by inserting a pipette below the plug of capillary material. Filter the solution through Whatman 42 Filter Paper. Filtrate should be clear. Determine hemoglobin content of filtrate. If greater than 10 mg.%, dilute to 10 mg.%. Freeze in 5 ml. aliquots.

Standard Estimation Procedure - Thaw serum samples to be analyzed, one 0.5 ml. aliquot of standard serum, and one ampule of stock hemoglobin solution. Dilute stock solution of hemoglobin and dilute to 60 mg./100 ml. Prepare dilutions of standard serum 1/2, 1/4, 1/8 and 1/16 of undiluted concentration, leaving at least 0.1 ml. of serum undiluted. Add 0.1 ml. of standard serum or diluted standard serum into a small test tube containing 0.5 ml. of dilute hemoglobin. Prepare serum samples by pipetting 0.1 ml. of serum and 0.1 ml. of water into a small test tube containing 1.0 ml. of dilute hemoglobin solution. Mix. Pipette 20 lambda of solution of standard sera or unknown sera prepared above into a 15 ml. test tube containing 5.0 ml. diansidine solution. Mix. Let sit for 15 minutes. Add 1.0 ml. of 0.1% aqueous ethylhydroperoxide to each large test tube. Mix. Let sit for 1 hour. Add 3 drops concentrated phosphoric acid to each tube. Mix. Add 6 ml. of distilled water to each tube. Mix. Prepare blank solution by adding 20 lambda of hemoglobin to 11.0 ml. distilled water. Read smaples in spectrophotometer at 395 millimicrons, using visible light source. Concentrations of unknown sera prepared in this manner and read from standard curve are 1/2 the concentrations of the original undiluted sera.

Therefore, the concentration from the curve must be doubled to obtain the true hemoglobin binding capacity. Should the concentration of the unknown sera diluted to 1/2 the original concentration be greater than 277 mg.% hemoglobin binding capacity, the serum must be further diluted so that the reading will fall on the stardard curve. (This final procedure was not necessary in the present study.)

RESULTS

Distribution of Haptoglobin Types - The following tables state the distribution of haptoglobin types in control populations, in patients with malignant ovarian tumors and in patients with particular types of malignant ovarian tumors.

Distribution of Haptoglobin Types in Control Populations:

Control Pops.	Total #	# Type 2-1	% Type 2-1	# Type 2-2	% Type 2-2	# Type 1-1	% Type 1-1
D-controls	30	13	43.3	7	23.3	10	33.3
C-controls	50	26	52.0	19	38.0	5	10.0
Controls of Ferris Study[15]	182	83	45.6	69	37.9	30	16.5

Distribution of Haptoglobin Types in Patients with Malignant Ovarian Tumors vs. Distribution of Haptoglobin Types in Controls:

Patients	58	31	53.4	22	39.9	5	8.6
C-Controls	50	26	52.0	19	38.0	5	10.0

$X^2_{(2)} = .07$ $p > .70$

Distribution of Haptoglobin Types in Cancer Patients Subgrouped by Tumor Type vs. Types of Controls:

Granulosa-thecal cell	2	2	(100)				
Malignant teratoma	1	1	(100)				
Embryonal cell	2	1	(50.0)	1	(50.0)		
Adenocarcinoma	52	27	51.9	20	38.5	5	9.6
C-Controls	50	26	52.0	19	38.0	5	10.0

$X^2_{(2)} = .00$ for adenocarcinomas $p > .95$

Distribution of Haptoglobin Types in Patients with Differentiated Carcinomas vs Undifferentiated Carcinomas:

Anaplastic	16	8	50.0	6	37.5	2	12.5
Differentiated	36	19	52.7	14	38.9	3	8.3

$X^2_{(2)} = .12$ $p > .70$

Distribution of Haptoglobin Types in Patients with Mucin-Producing Adenocarcinomas vs. Nonmucin Producing Adenocarcinomas -

	Total #	# Type 2-1	% Type 2-1	# Type 2-2	% Type 2-2	# Type 1-1	% Type 1-1
Mucin	6	3	50.0	2	33.3	1	16.7
Non-mucin	46	24	52.2	18	39.1	4	8.7

$X^2_{(2)} = .39$ $p > .50$

Results of Single Determinations of Haptoglobin Levels -

Haptoglobin levels in patients with ovarian tumors are related to the amount of tumor, type of tumor, degree of differentiation in tumor and to the death of the patient:

	TYPE 2-1			TYPE 2-2			TYPE 1-1		
Control Pops.	Mean	S.D.	Mean ± 2S.D.	Mean	S.D.	Mean ± 2S.D.	Mean	S.D.	Mean ± 2S.D.
D-controls	92	41	10-174	82	44	0-170	66	33	0-132
C-controls	76	31	14-138	83	53	0-189	96	18	50-132
Controls of Ferris Study[15]	108	36	36-180	83	32	19-147	124	34	58-194

The C-controls are the actual controls of the experiment. Considing the range of normal to be the mean ± 2 standard deviation units, the upper limit of normal for all types of haptoglobin is 189mg.%. Haptoglobin levels of 190mg.% and above were considered grossly elevated, while haptoglobin levels below 190mg.% were considered grossly normal.

Haptoglobin levels above and below 190mg.% of controls and patients subdivided according to amount of tumor, and haptoglobin levels of patients who died of tumor within two months of determination are noted. See Figure III.

In patients with widespread tumor, haptoglobin levels are related to histologic type, to degree of differentiation of tumor, and to mucin production by tumor.

Haptoglobin Levels vs Type of Ovarian Carcinoma in Patients With Widespread Tumor:

Type	# of Patients	Hp Level mg.%	Average Hp Level
Granulosa-thecal cell	1	440	440
Malignant teratoma	1	164	164
Embryonal cell carcinoma	2	360;420	390
Adenocarcinoma	29		260

FIGURE III

Haptoglobin Levels Above and Below 190 mg.% of Controls And Patients Subdivided According to Amount of Tumor And Haptoglobin Levels of Patients Who Died of Tumor Within Two Months of Haptoglobin Determination

	No. with Hp. Level > 190mg.%	No. with Hp. < 190mg.%	Total No. Pts.	Average Hp. Level mg.%	Fisher's Exact Test (x); X^2 Test (*)	X^2 vs. C-controls	p
C-controls	1	49	50	81	-	-	-
D-controls	0	30	30	81	-	-	-
Ovarian pts. with no tumor	0	15	15	122	x	-	p = .77
Ovarian pts. with tumor < 6 cm. diameter	0	9	9	144	x	-	p = .77
Ovarian pts. with tumor > 6 cm. diameter	25	9	34	268	*	48.47	p<.001
Ovarian pts.who died of tumor within 2 mo. of last hap. determination	16	4	20	263	*	47.23	p<.001

Haptoglobin Levels vs Degree of Differentiation in Patients With Widespread Adenocarcinoma:

	No. of Patients	Sum of Ranks	Average Hp. Level mg.%
Undifferentiated adenocarcinoma	10	132	271
Differentiated adenocarcinoma	19	-	264

$\tau' = 168$
$p > .05$ by Wilcoxson Rank Sum Test[75]

Haptoglobin Levels vs Mucin or Lack of Mucin Producing Tumor in Patients with Widespread Adenocarcinomas:

	No. of Patients	Haptoglobin Level mg.%	Average Hp. Level
Mucin producing	2	272;380	326
Non-mucin producing	27	-	255

In patients with widespread ovarian malignancy, haptoglobin levels are related to surgery, radiotherapy or chemotherapy within two months prior to obtaining the sample.

	No. of Patients	Sum of Ranks	Average Hp. Level mg.%
Lap. in last 2 mo.	13	170.5	291
No Lap. in last 2 mo.	21	-	268

$\tau' = 284.5$
$.01 < p < .05$ by Wilcoxson Rank Sum Test

Radio.in last 2 mo.	5	80.5	275
No Radio in last 2 mo.	29	-	267

$\tau' = 94.5$
$p > .05$ by Wilsoxson Rank Sum Test

Chemo. in last 2 mo.	17	336	247
No Chemo. in last 2 mo.	17	-	290

$\tau' = 259$
$p > .05$ by Wilcoxson Rank Sum Test

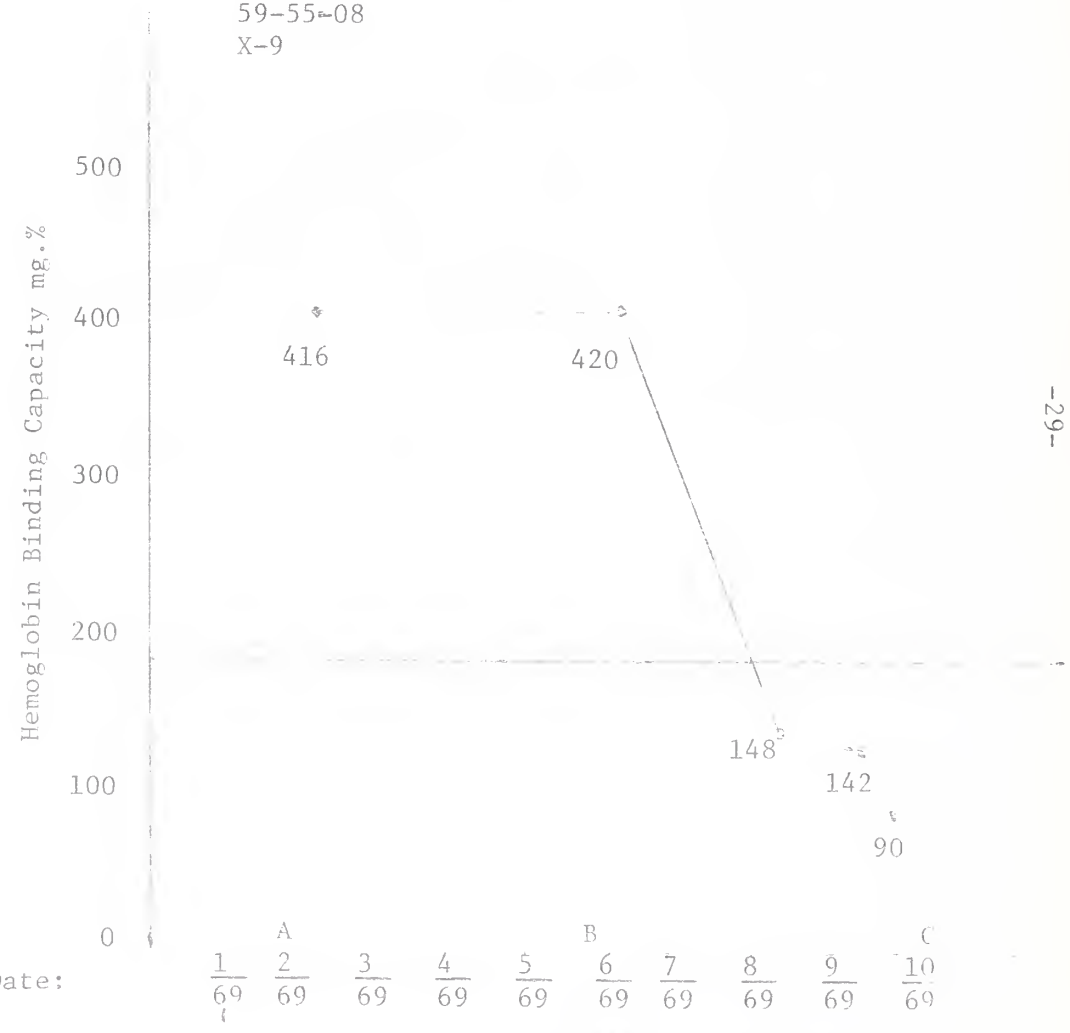
Results of Serial Determinations of Haptoglobin Levels -

Group A	No. of Patients
Patients who had a marked decrease of tumor from > 6 cm. to < 6 cm. because of tumor therapy and whose haptoglobin level decreased from abnormally high values to normal ones: X-9, X-25, X-44, X-34, X-43, X-46, X-68.	7

A. Large pelvic mass.

B. Laporotomy - removal of cell tumor.

C. No tumor.

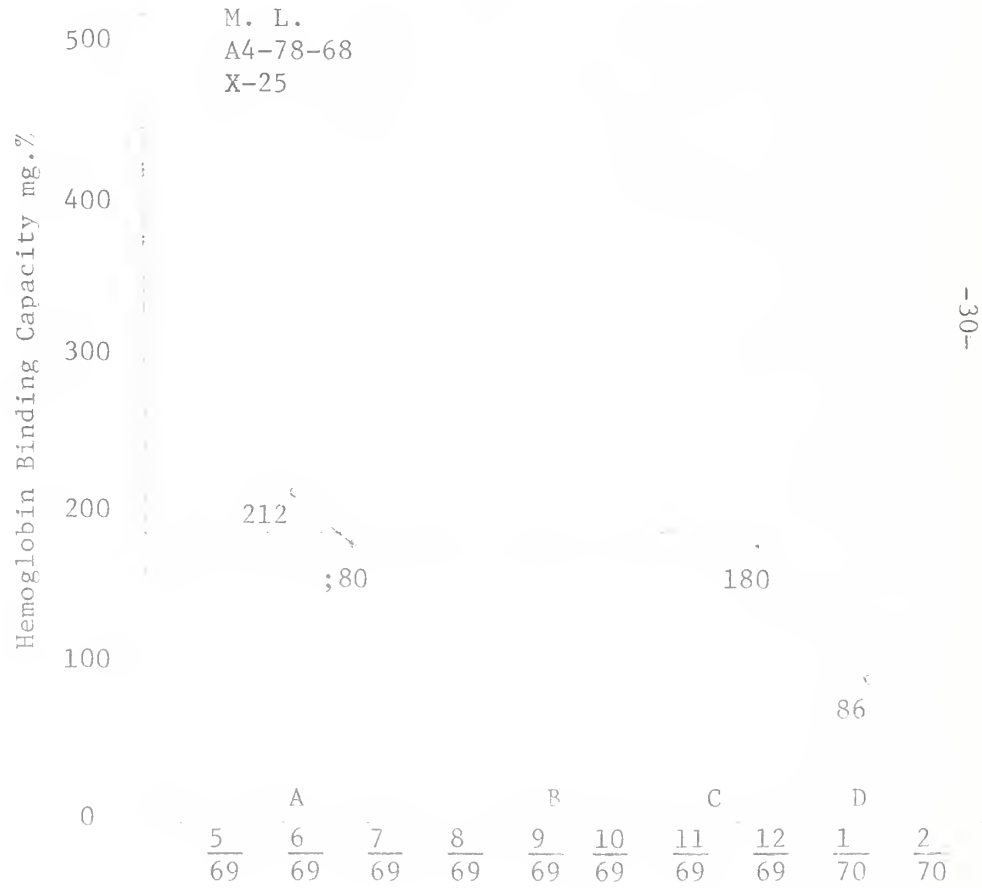


A. Laporotomy - large cystic pelvic mass and ascitic fluid removed.

B. No tumor.

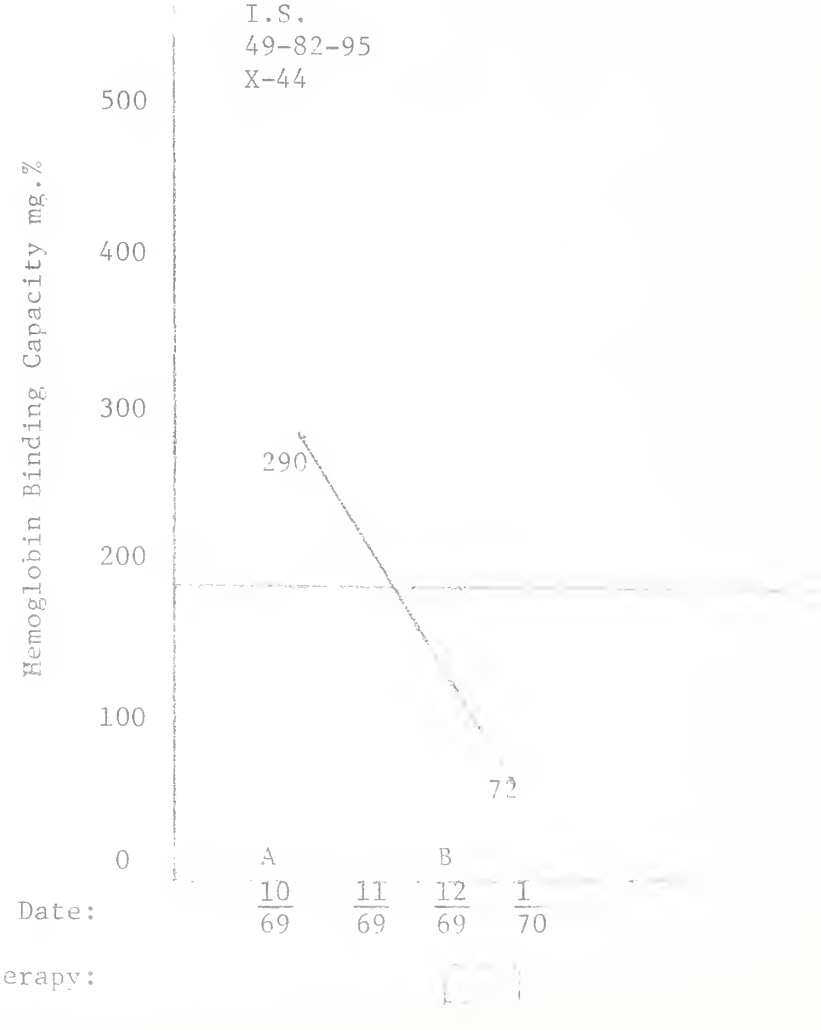
C. No tumor.

D. No tumor.



A. Laporotomy - massive tumor left.

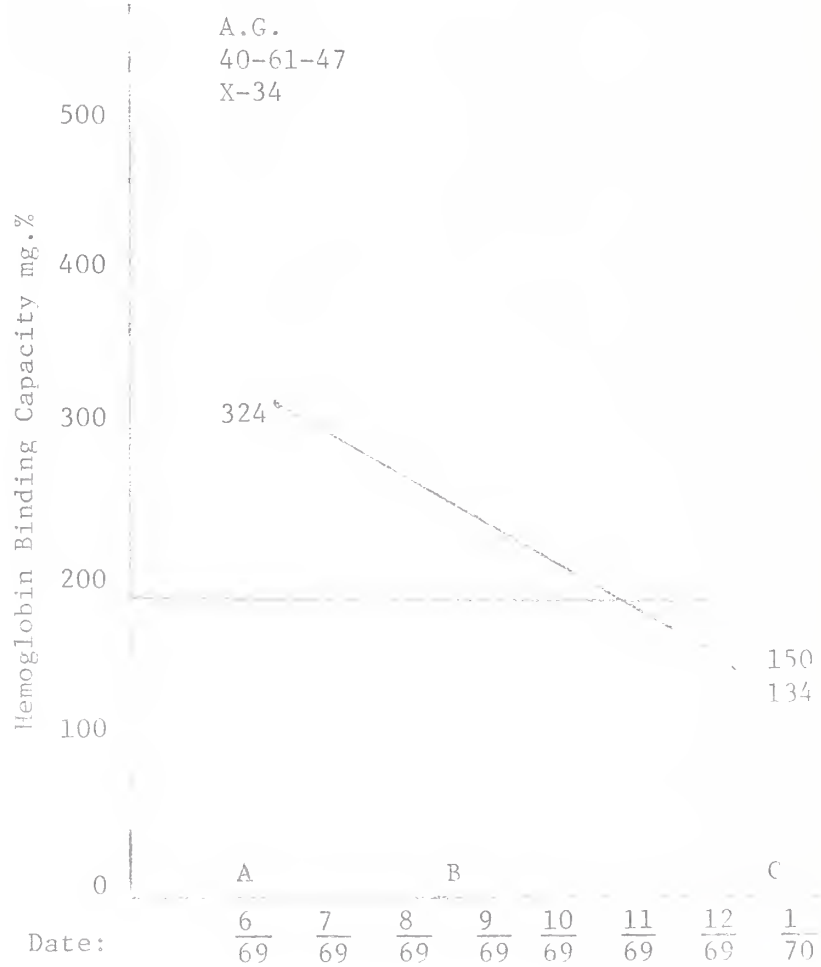
B. No tumor.



A. Laporotomy - tumor metastases to sigmoid removed.
No other tumor seen.

B. No tumor.

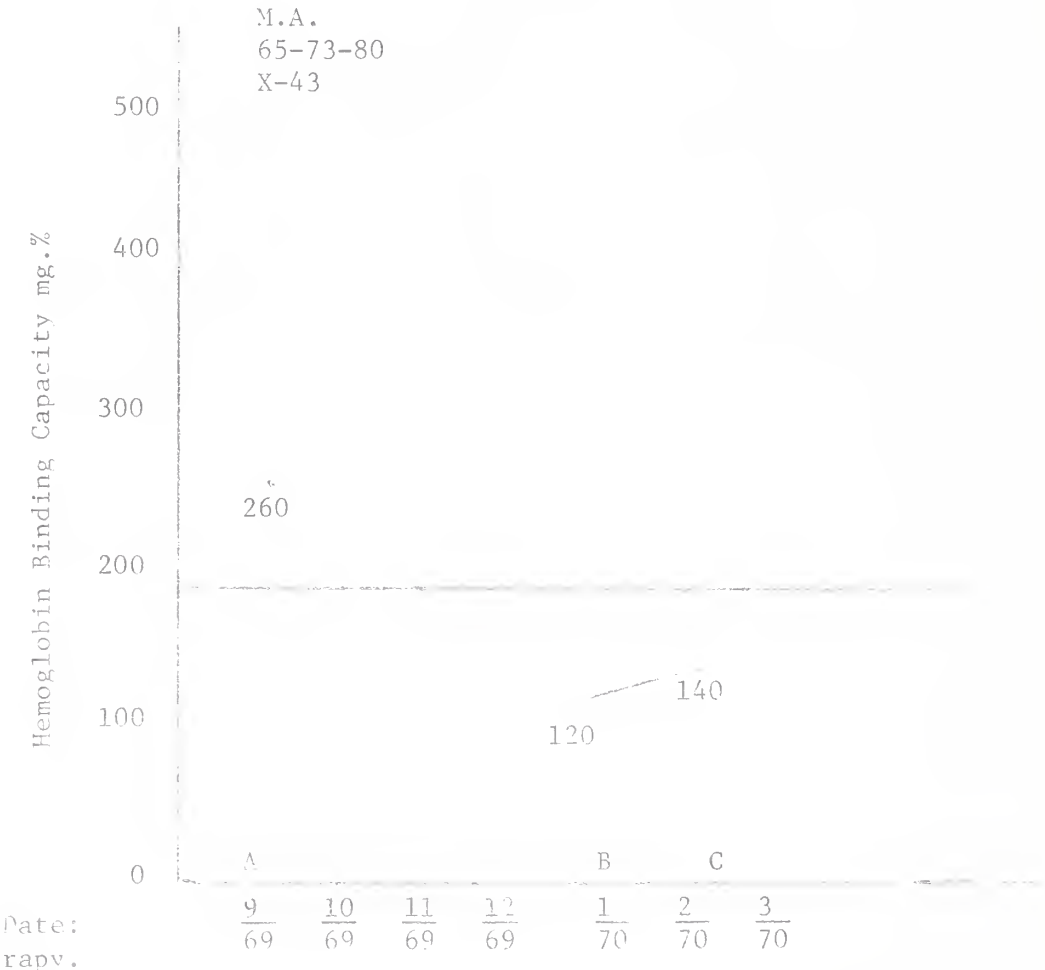
C. No tumor.



A. Surgery 1/67; 1/69 .
9/69 No tumor; had upper respiratory infection.

B. No tumor.

C. No tumor.

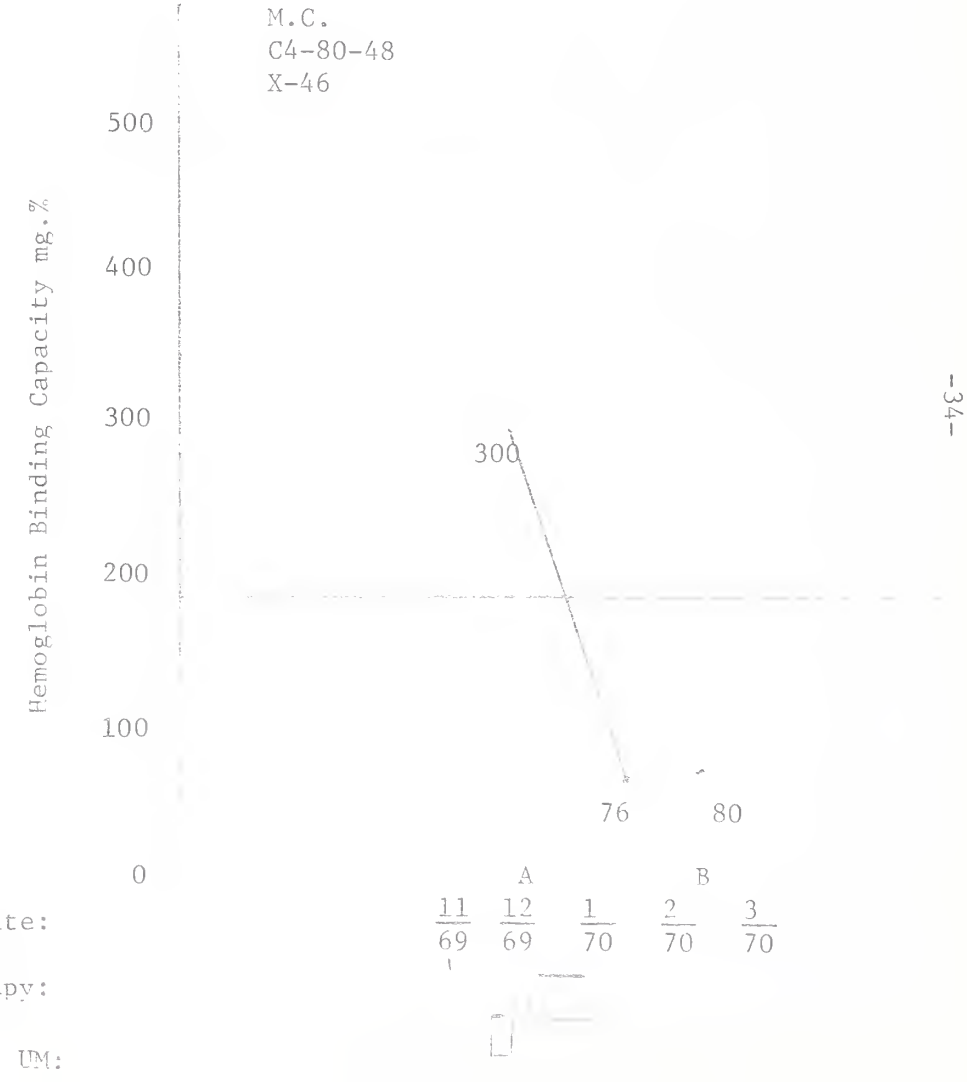


Date:
2/67 Radiotherapy.

Chemotherapy 5FU, UM:

A. Surgery 10-69 - Large amount of tumor left.
11/69 - widespread tumor.

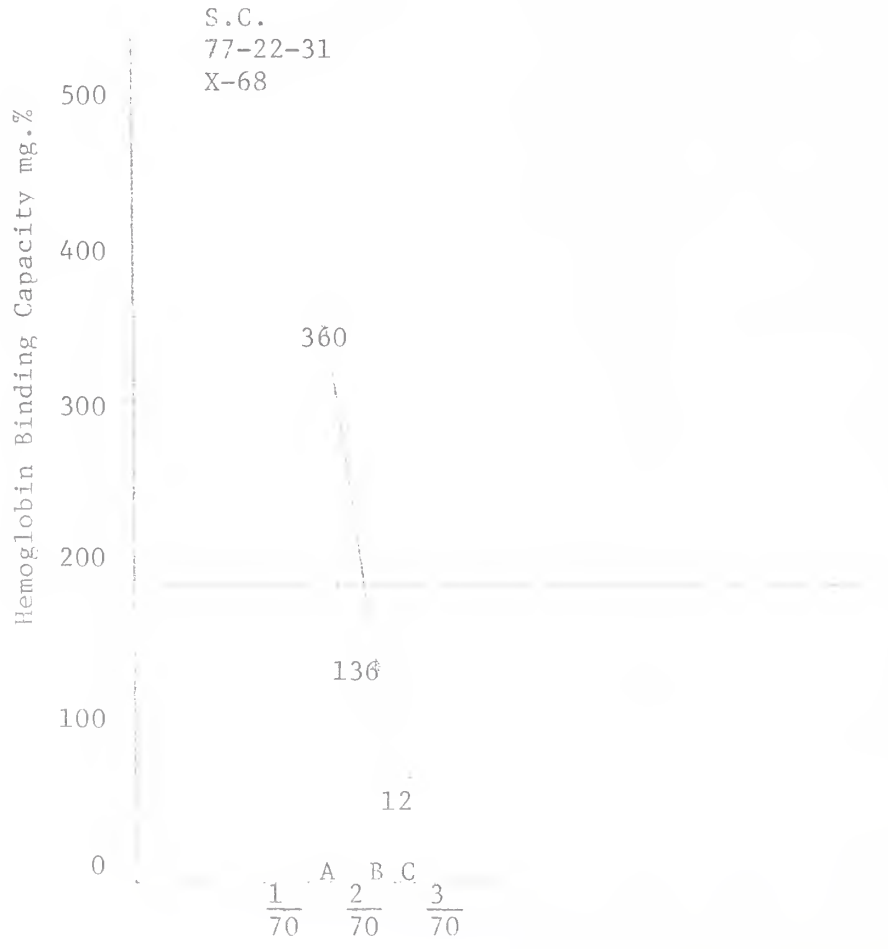
B. Laporotomy. No tumor found.



A. Laporotomy - massive tumor all removed.

B. ? of tumor.

C. Laporotomy - 6 cm. tumorous mass removed.
No visible or palpable tumor left.



Group B	No. of Patients
Patients who continued to have or who developed widespread tumor and whose haptoglobin levels remained high or rose from normal to high values: X-15, X-20, X-32, X-33, X-55, X-31, X-45, X-39, X-62, X-42, X-47, X-64, X-57, X-70.	14

A. Breast surgery - 1.5 cm. adenocarcinoma removed. No pelvic mass.

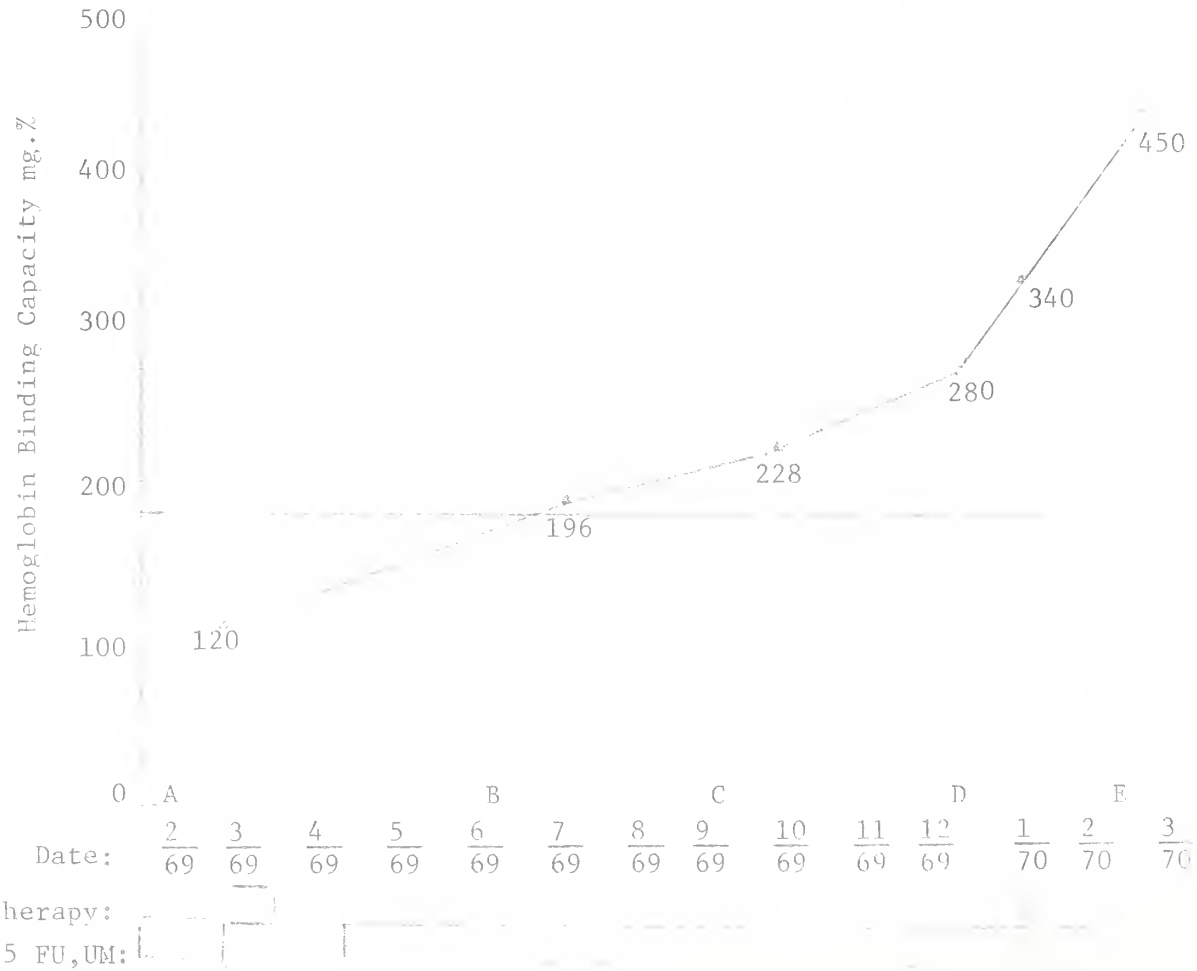
B. No tumor.

C. L.L.Q. mass - 4 cm.

D. L.L.Q. mass - 9 cm.

E. Paracentesis 4,600 ml. removed.

J.C.
72-24-51
X-15



A. Surgery 4/68. 2/69 No tumor.

B. Cul-de-sac mass 3cm.

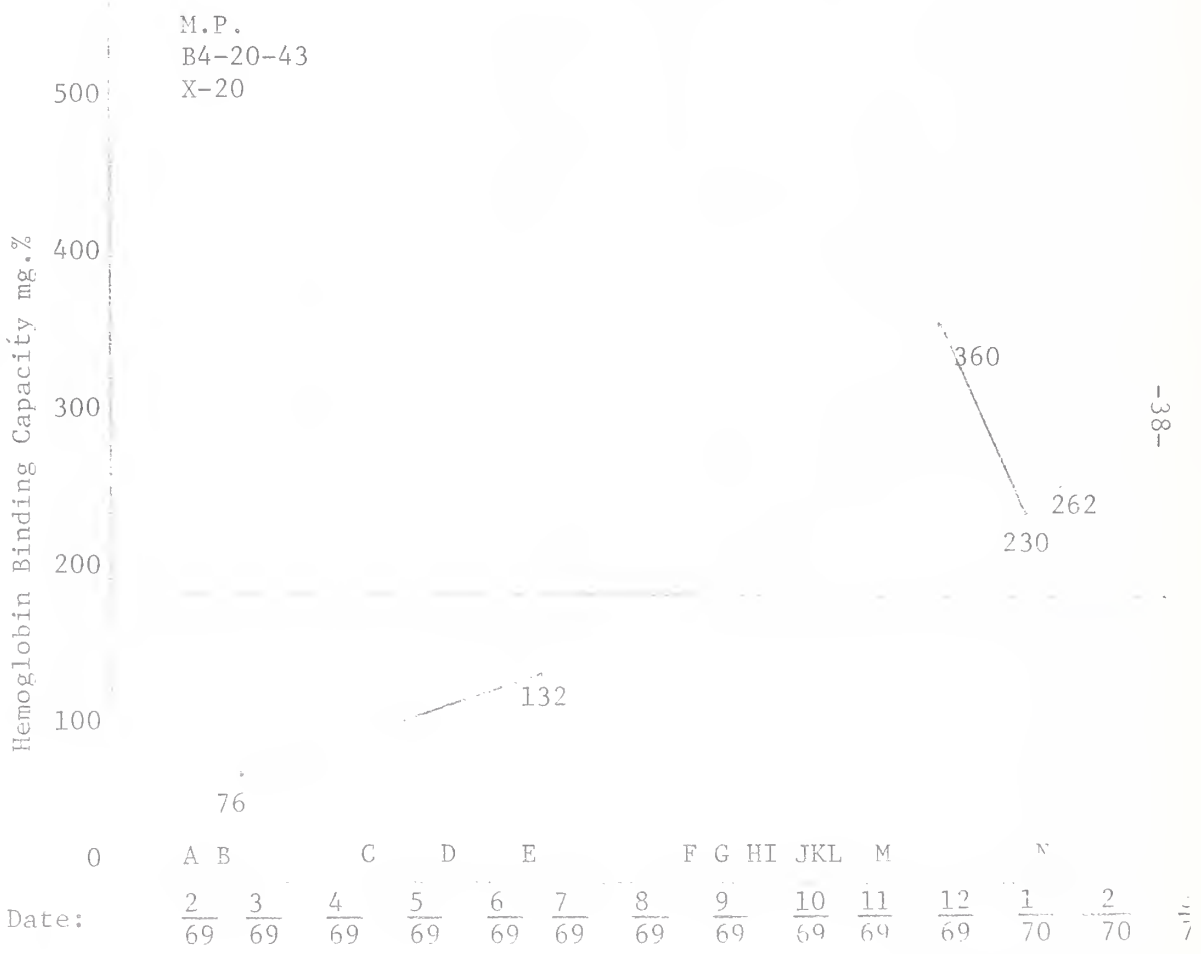
C. No change.

D. Umbilical hernia secondary to the increasing abdominal mass.

E. Surgical repair of umbilical hernia.

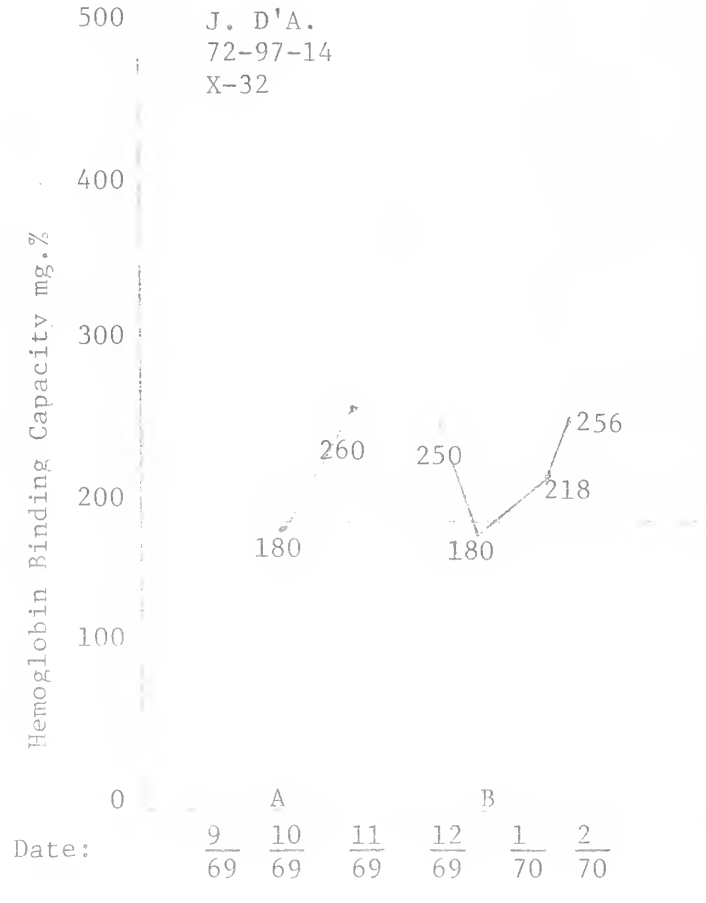
G.H.I.J.K.L.M. Paracenteses.

N. Expired.



A. Surgery 8/68-Metastases left.
9/69 - Large cul-de-sac masses increasing in size

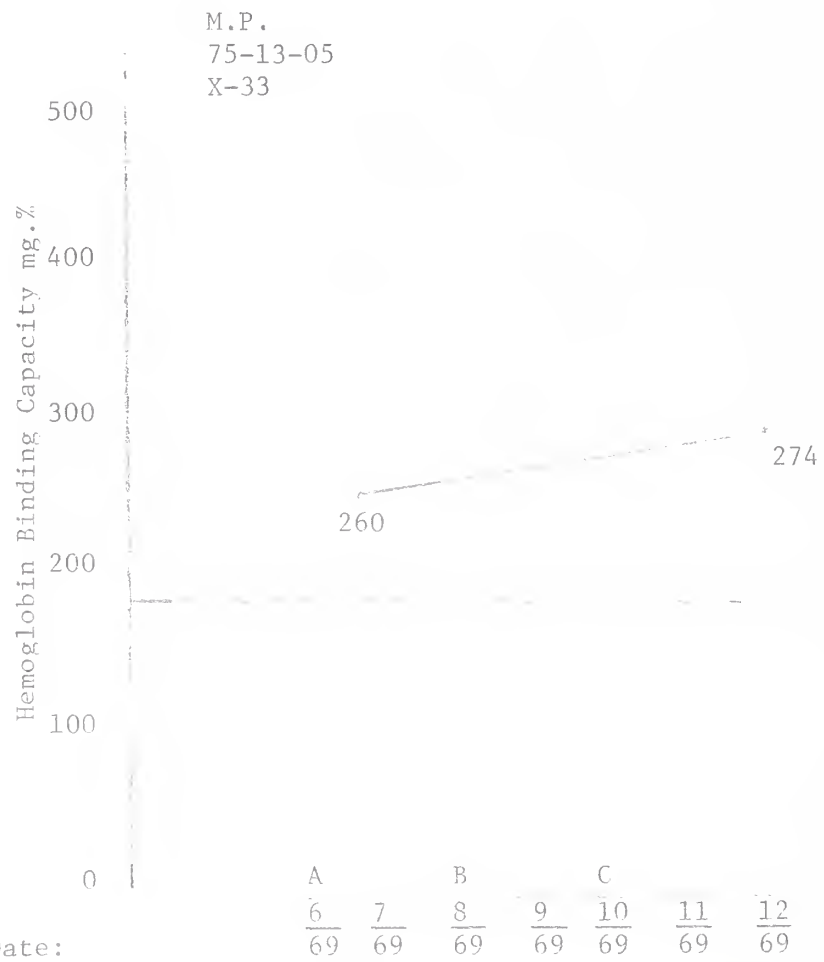
B. Tumor increasing, causing partial colonic obstruction.



A. Laporotomy - large widespread metastases left.

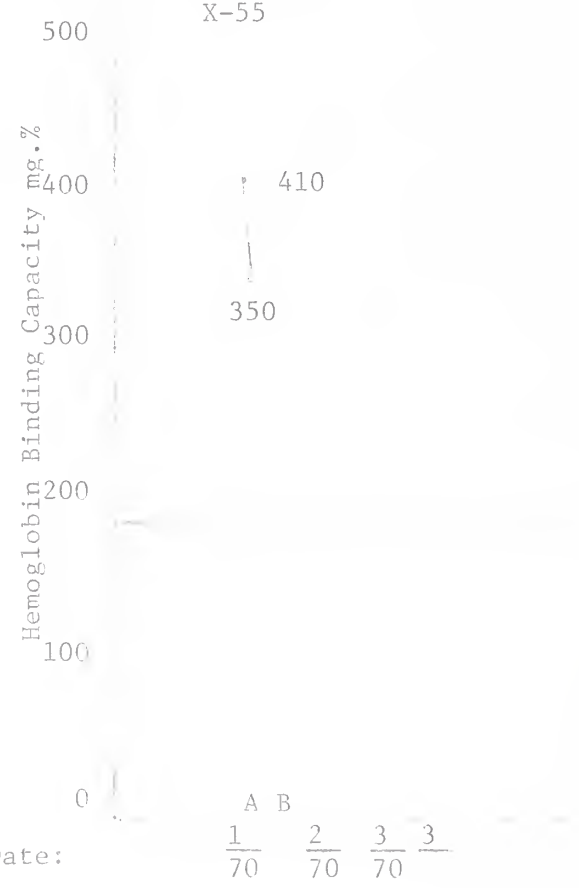
B. Laporotomy - most tumor removed.

C. Widespread tumor.



A. 11/69 Laporotomy. 1/70-Large tumor mass.

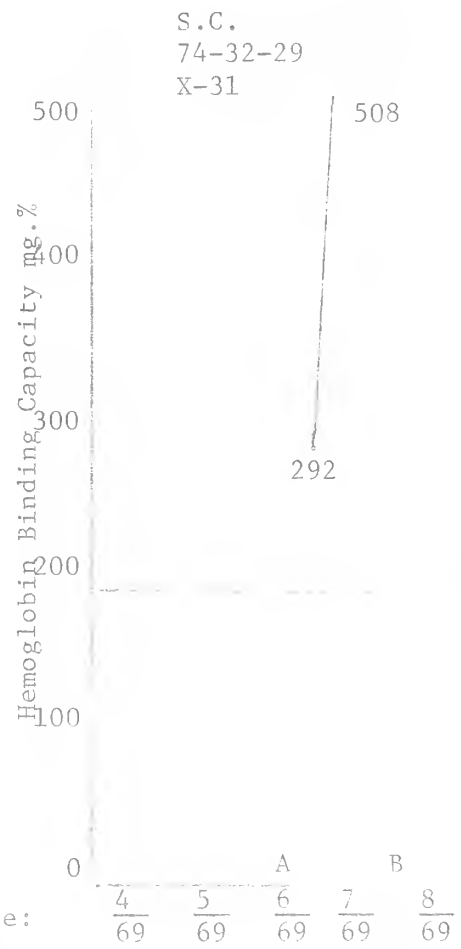
B. Vast amount of tumor.



12/69 Radiotherapy.
No Chemotherapy.

A. Laporotomy 4/69 - widespread tumor left.
5/69-widespread tumor.

B. Expired.

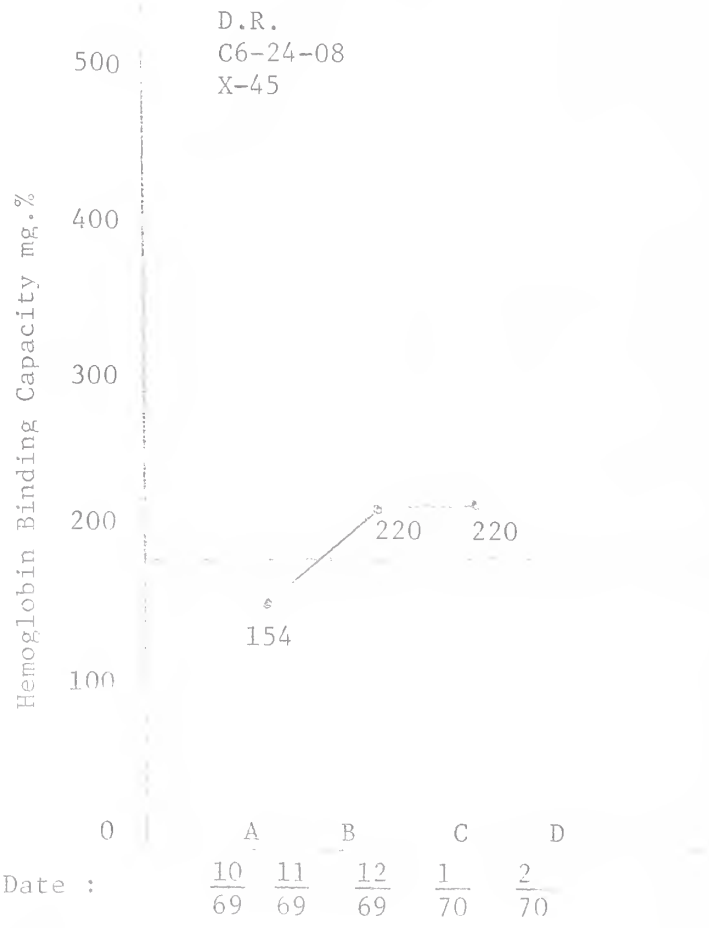


A. Laporotomy - Large amount of tumor left.

B. 2 large tumor masses.

C. Very large amount of tumor.

D. Expired.

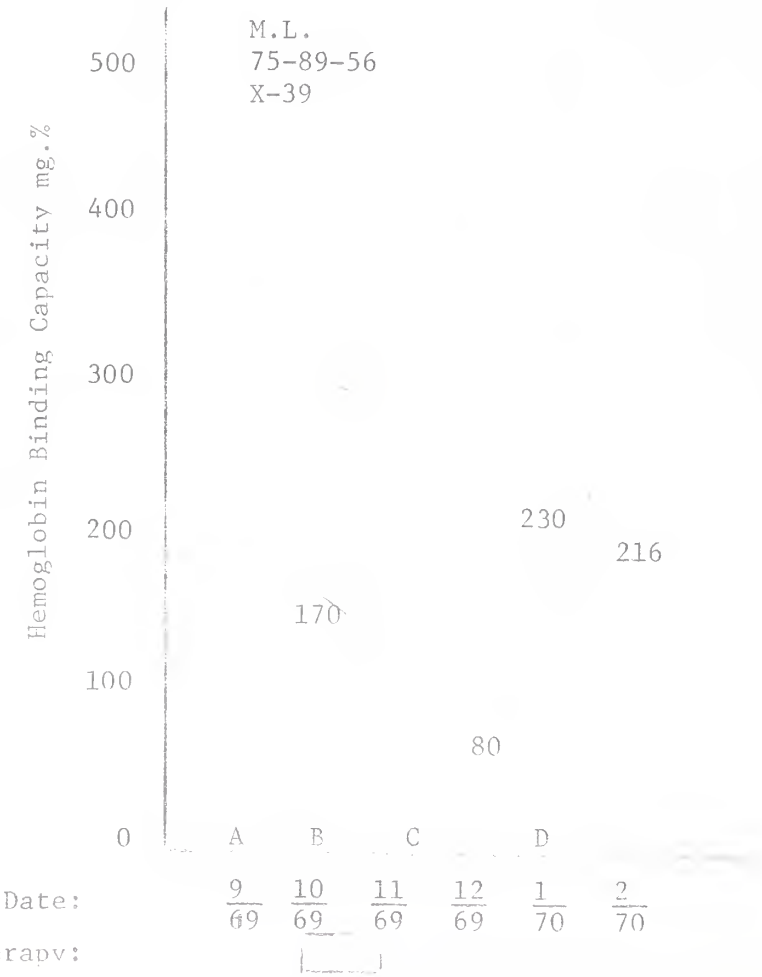


A. Large pelvic mass.

B. Tumor still large but decreased to 30% of its original size.

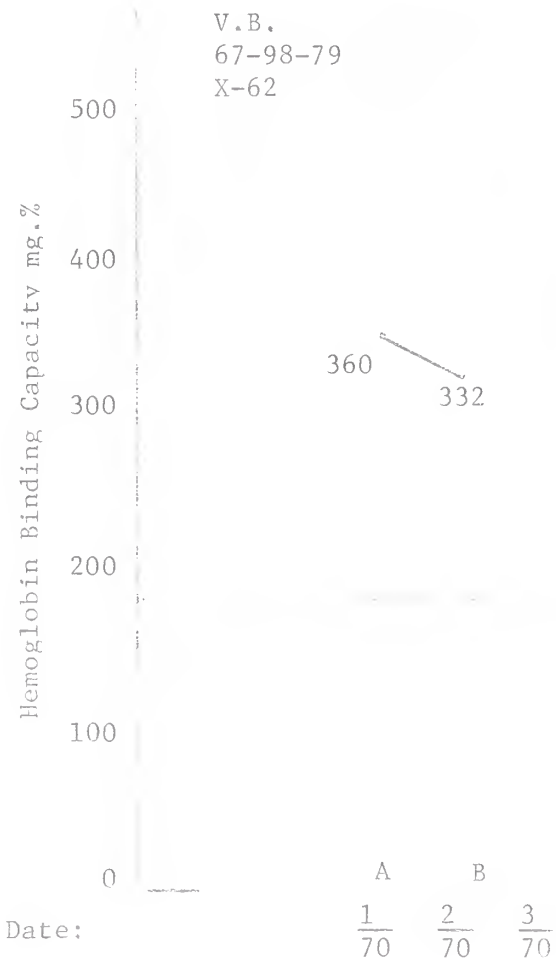
C. Laporotomy - Vast amount of tumor left.

D. Tumor increasing in size.



A. Surgery 2/66
1/70 - Pelvic mass extending above umbilicus

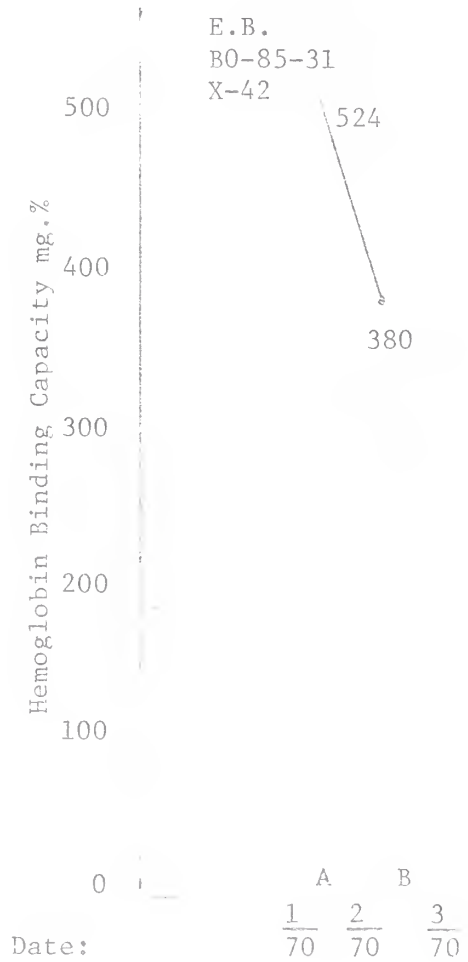
B. No change.



2/67 Radiotherapy.
Chemotherapy 5FU, UM:

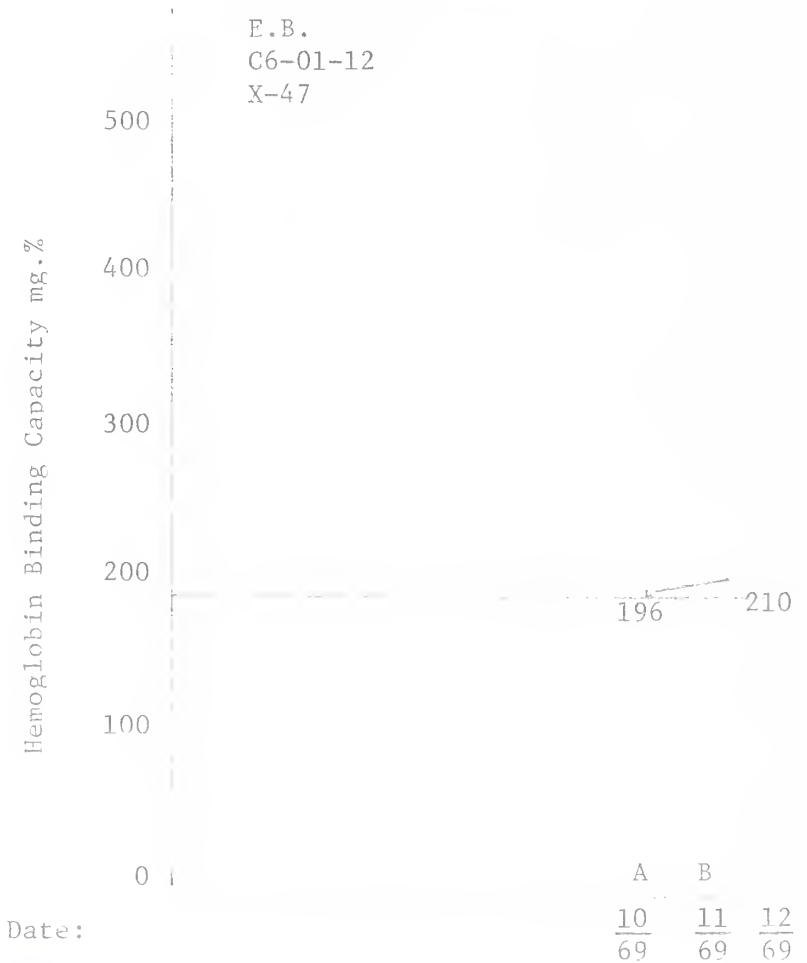
A. Laporotomy 10/69. Gross tumor left.
1/70 - Large tumor with abcess.

B. Large tumor with fistula.



A. Surgery 9/68 - Tumor left.
Surgery 8/69 - Metastases to spinal cord removed.
10/69 - Large amount of tumor.

B. Chest metastases.



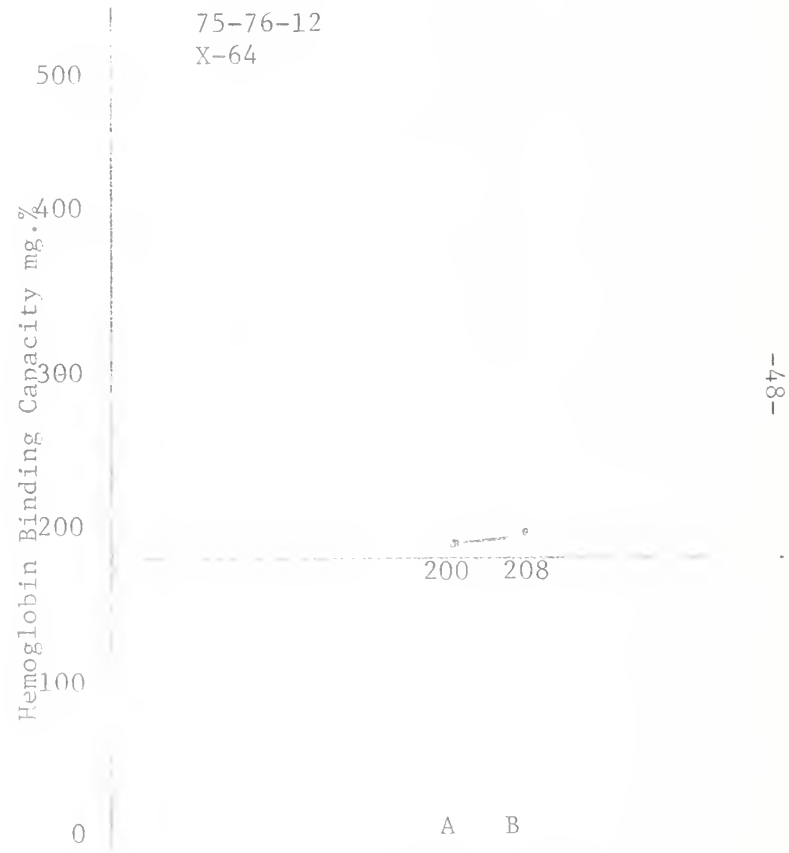
8/69 Radiotherapy.

Chemotherapy 5FU, UM

A. Large abdominal mass.

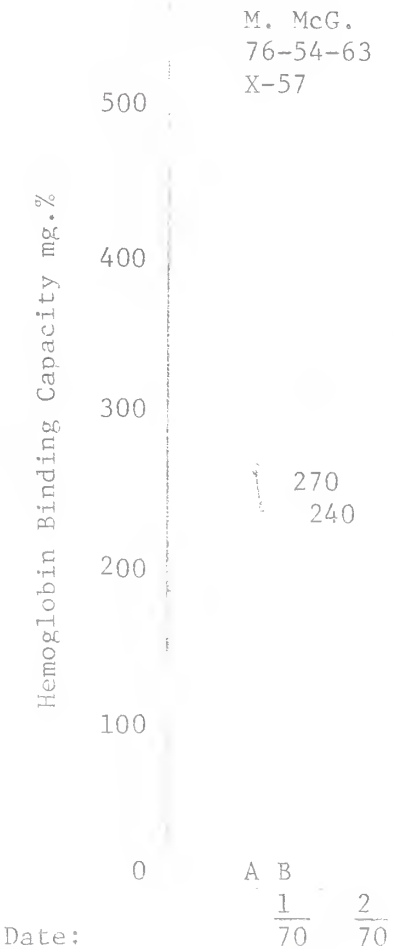
B. Mass extends from pelvis to umbilicus.

L.C.
75-76-12
X-64



A. Surgery 11/69 - Vast amount of tumor left.
12/69 Large amount of tumor.

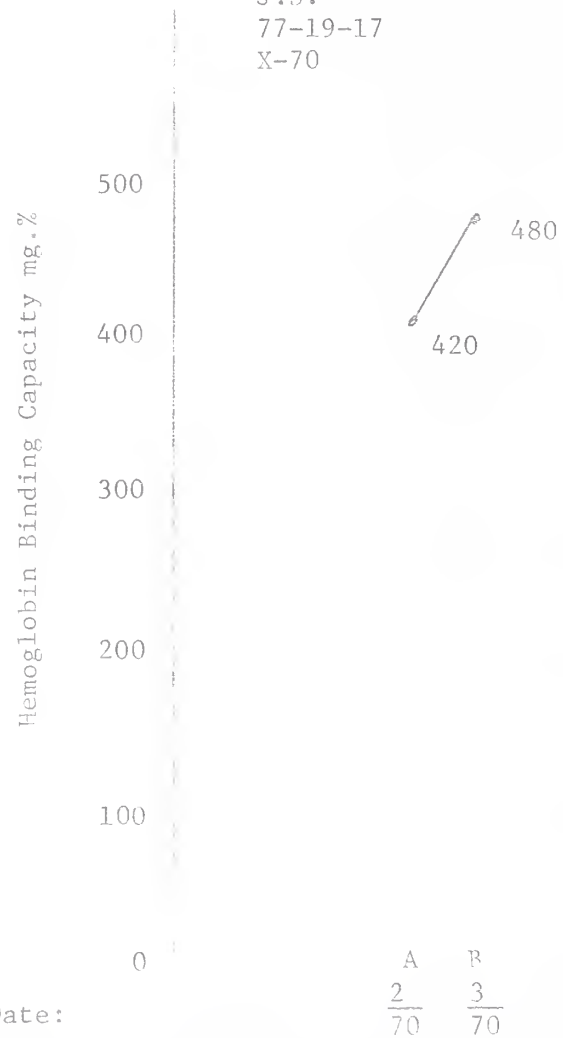
B. Expired.



A. Laporotomy 10/69.
2/70 - Tumor with partial small bowel obstruction and ascites.

B. Tumor and ascites.

J.S.
77-19-17
X-70



Date:
No Radiotherapy.
No Chemotherapy.

Group C	No. of Patients
Patients with former ovarian malignancies who had evidence of either no tumor or tumor < 6 cm. during serial studies and who continued to have normal haptoglobin levels: X-23, X-27, X-16, X-37, X-50, X-58, X-61, X-65.	8

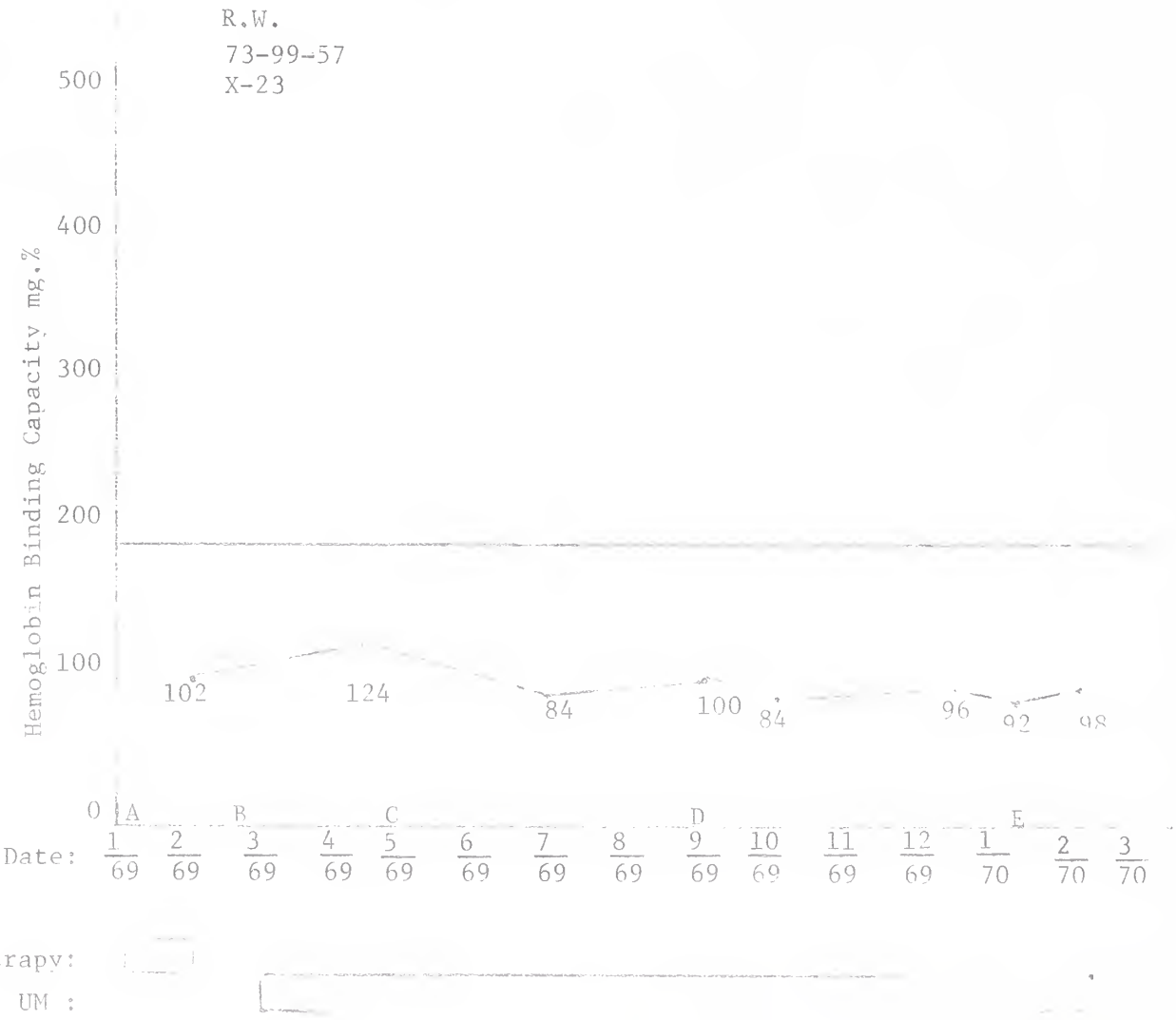
A. Surgery 1/68- Cul-de-sac mass left. P.E. 1/69 - Large cul-de-sac mass.

B. Laporotomy - Removal of 4 cm. of tumor.

C. Very small cul-de-sac mass.

D. No change.

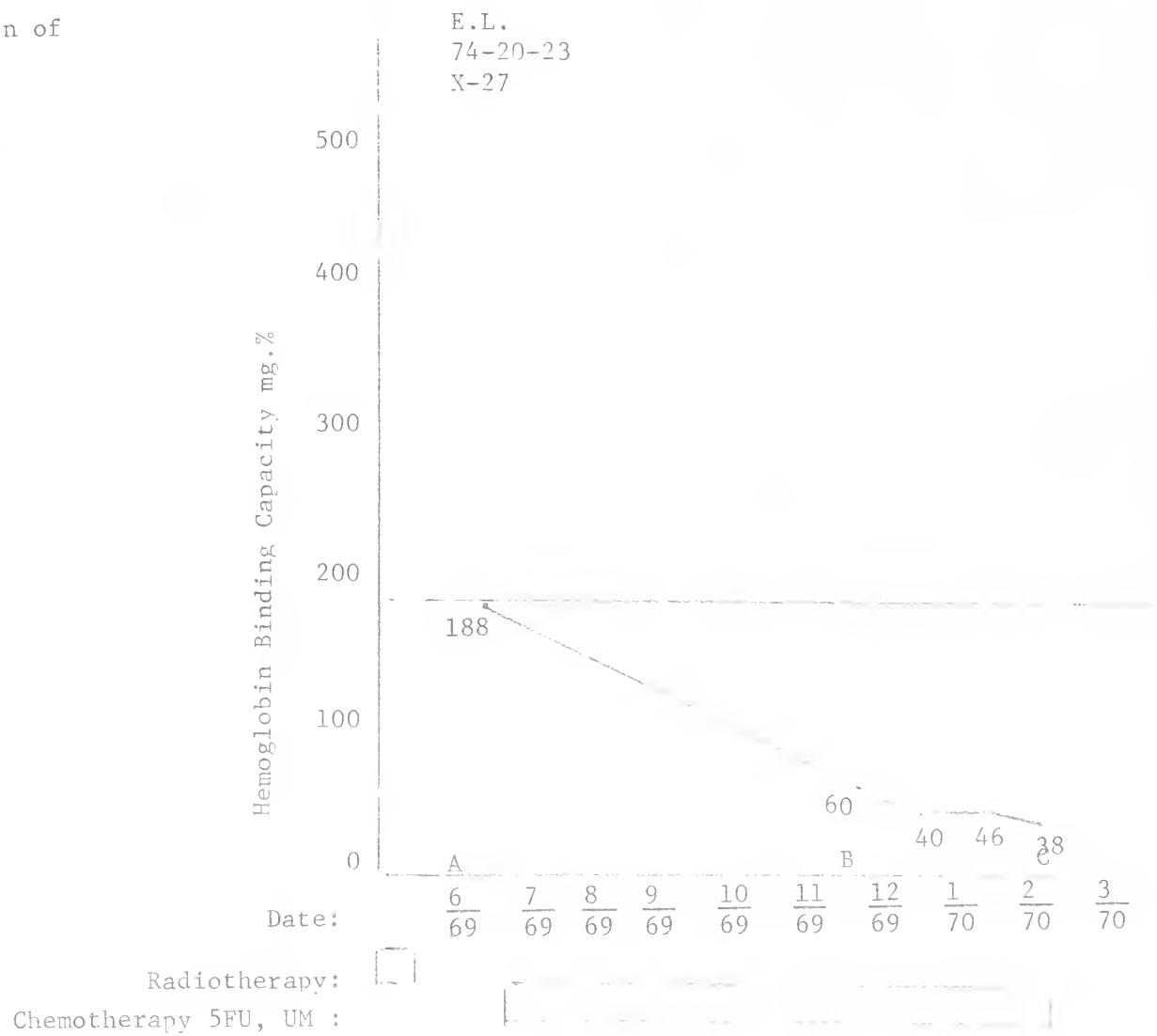
E. Laporotomy - Removal of 2cm. mass. Very small amount of tumor left.



A. Surgery in 4/69. Large amoutn of metastases left.

B. No tumor.

C. No tumor.

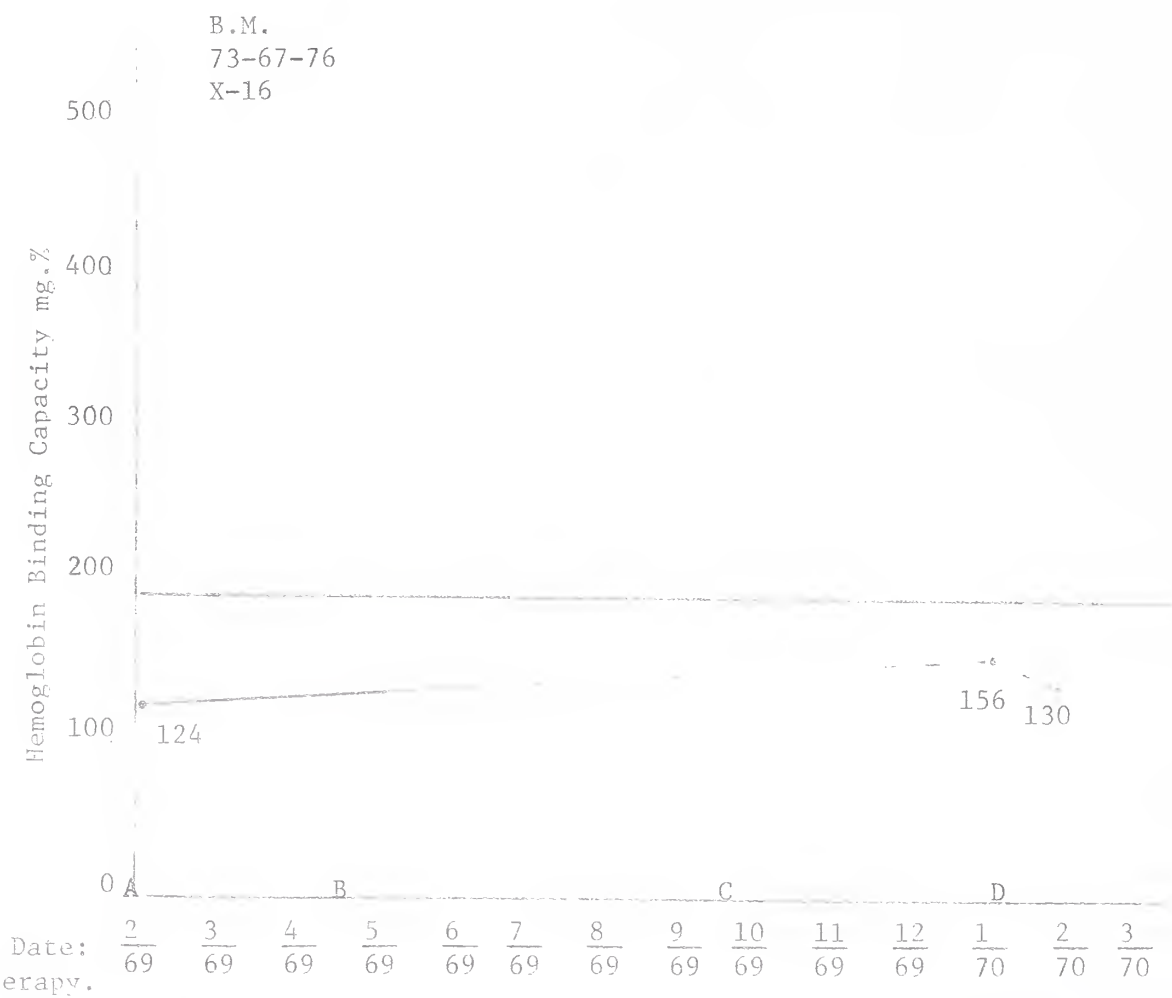


A. Surgery 11/68 - < 6 cm. tumor left.

B. No tumor.

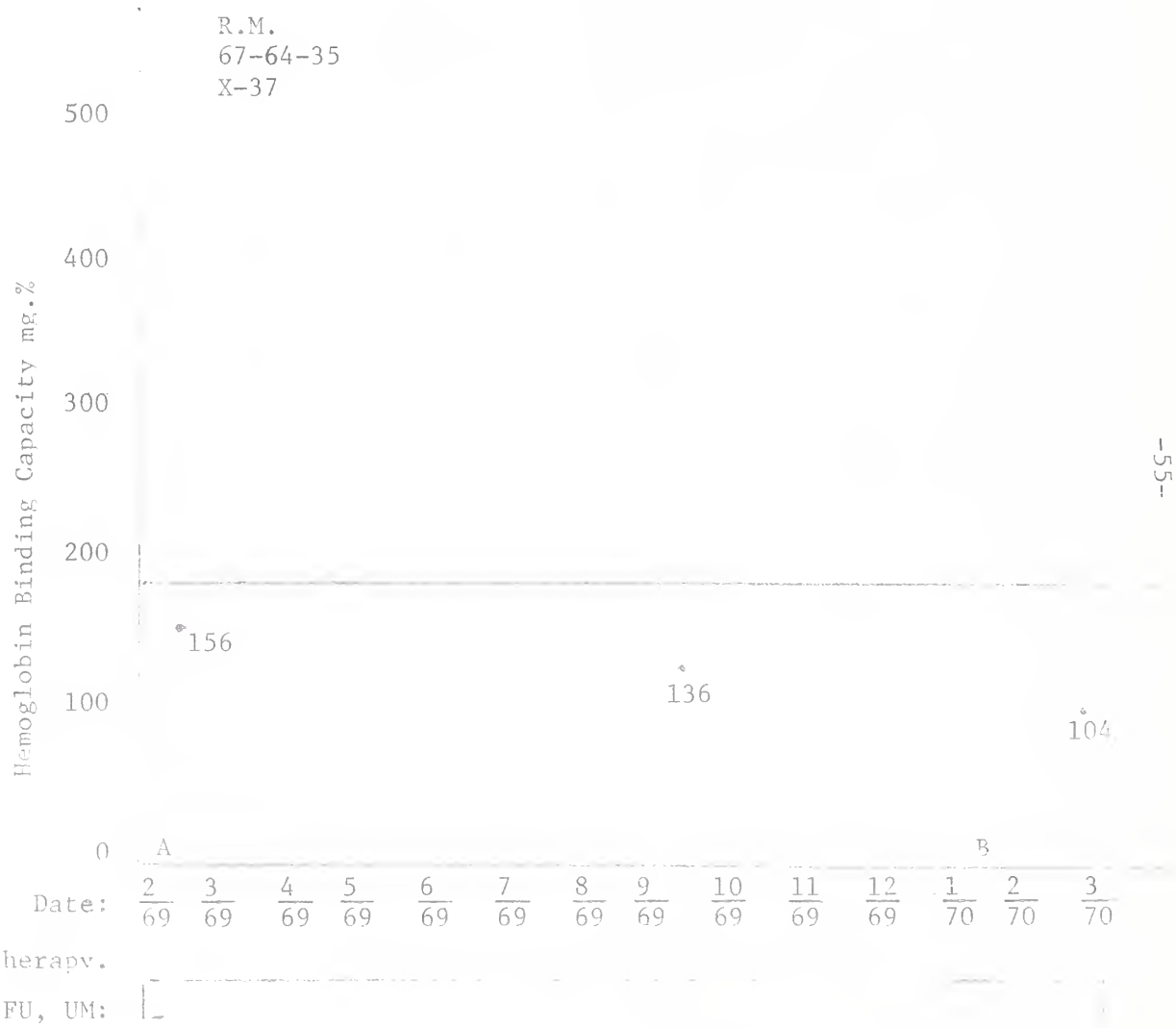
C. No tumor.

C. No tumor.



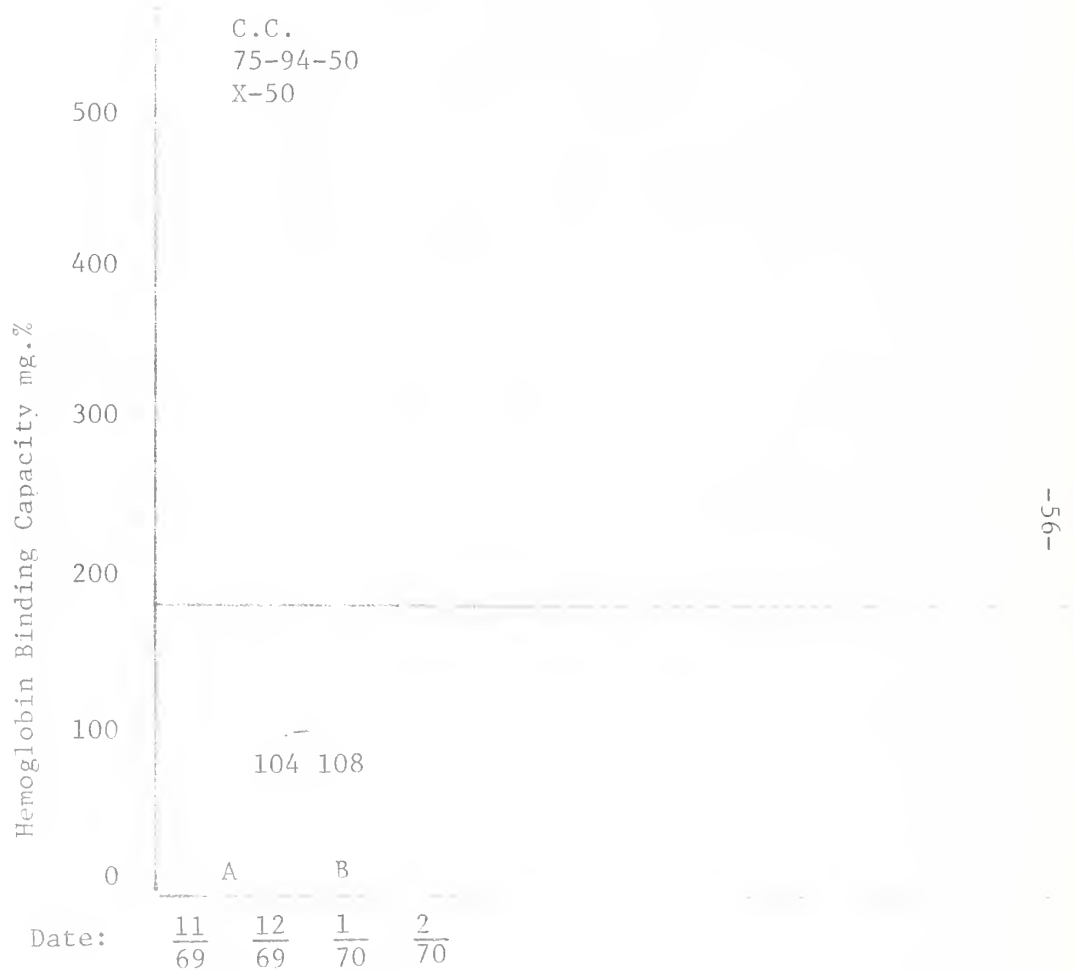
A. Surgery 11/66.
No tumor 2/69.

B. No tumor.



A. Surgery 9/69 - 9 x 4 cm. mass left. P.E. 11/69-No tumor.

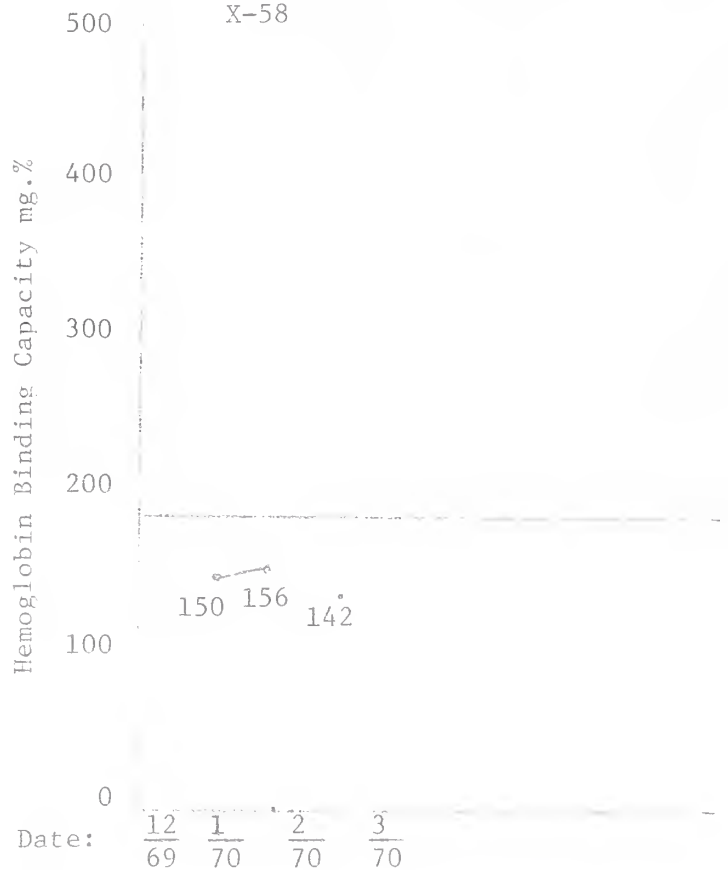
B. No tumor.



A. Surgery 6/67
12/69 - 6 cm. mass

B. Tumor 4 cm. mass.

D.S.
07-33-09
X-58

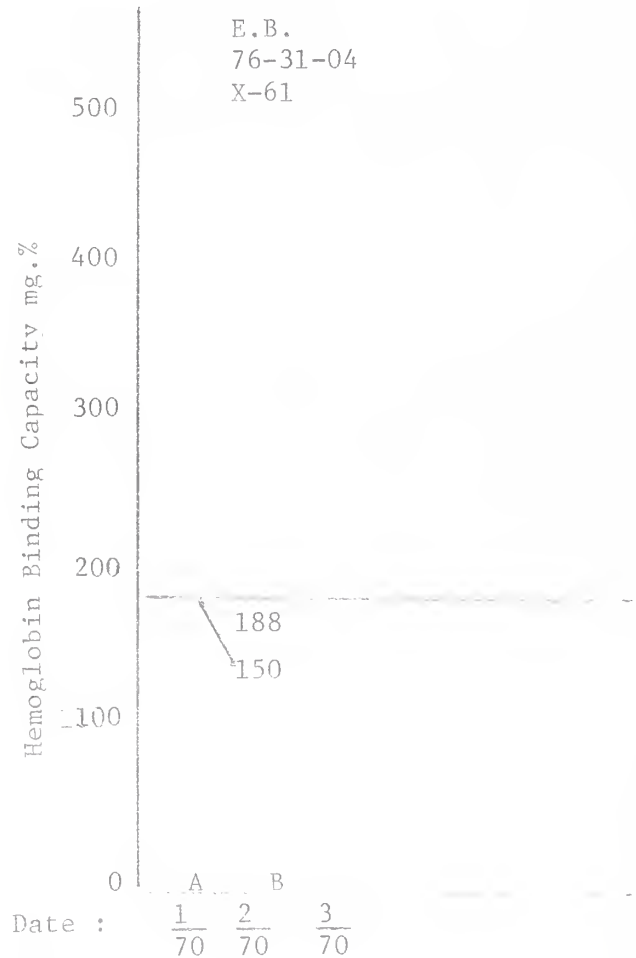


1/67 Radiotherapy.

Chemotherapy 5 FU, UM:

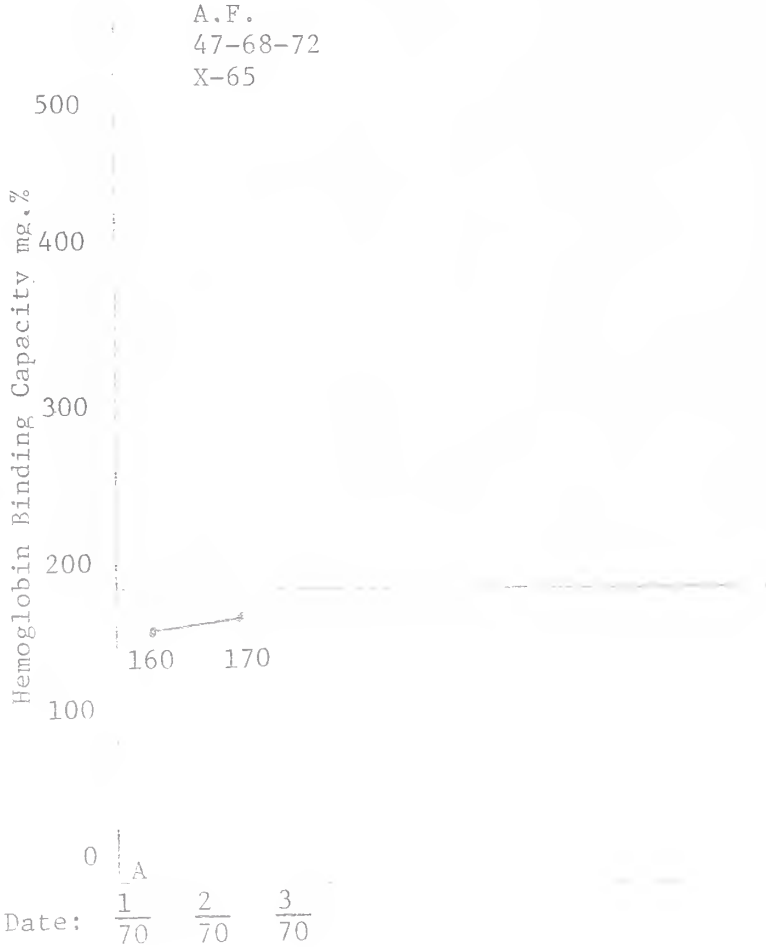
A. Surgery 1/69-all endometrial carcinoma removed.
Surgery 10/69-all ovarian carcinoma removed.
1/70 No tumor.

B. 2/70 No tumor.



11/69 Radiotherapy.
No chemotherapy.

A. Surgery - very small amount of tumor left in multiple peritoneal implants.



No Radiotherapy.

No Chemotherapy .

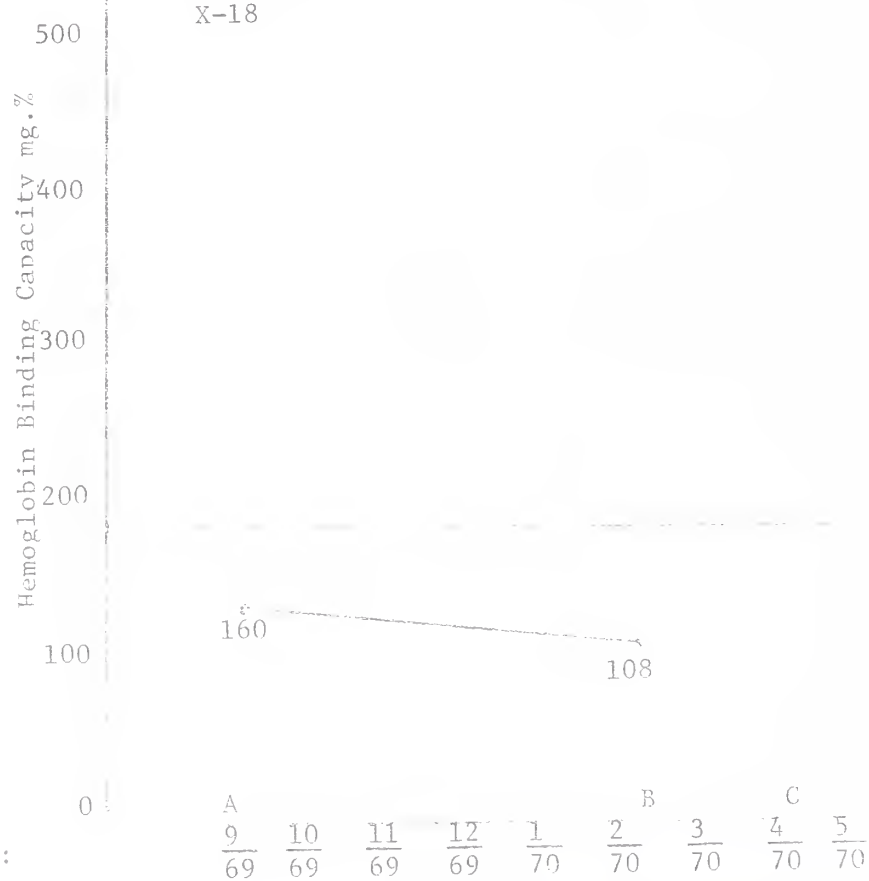
Group D	No. of Patients
Patients in whom haptoglobin levels did not consistently correlate with the amount of tumor:	
1) Widespread tumor with consistently low haptoglobins: X-18, X-19	2
2) Patients with widespread tumor who had elevated haptoglobins at the time of widespread disease, but had a normal haptoglobin level on one or two occasions: X-22, X-35	2
3) One patient with no present evidence of tumor whose haptoglobin level rose: X-53	1
4) One patient whose haptoglobin level was elevated long before there was any physical evidence of tumor: X-13	1

A. Surgery 5/68.
9/69 Widespread tumor.

B. Widespread tumor with ascites.

C. Expired.

F.L.
71-33-64
X-18



2/68 Radiotherapy .

Chemotherapy 5FU, UM :

A. Surgery 2/67.
2/69- Tumor 5 cm.

B. No Change.

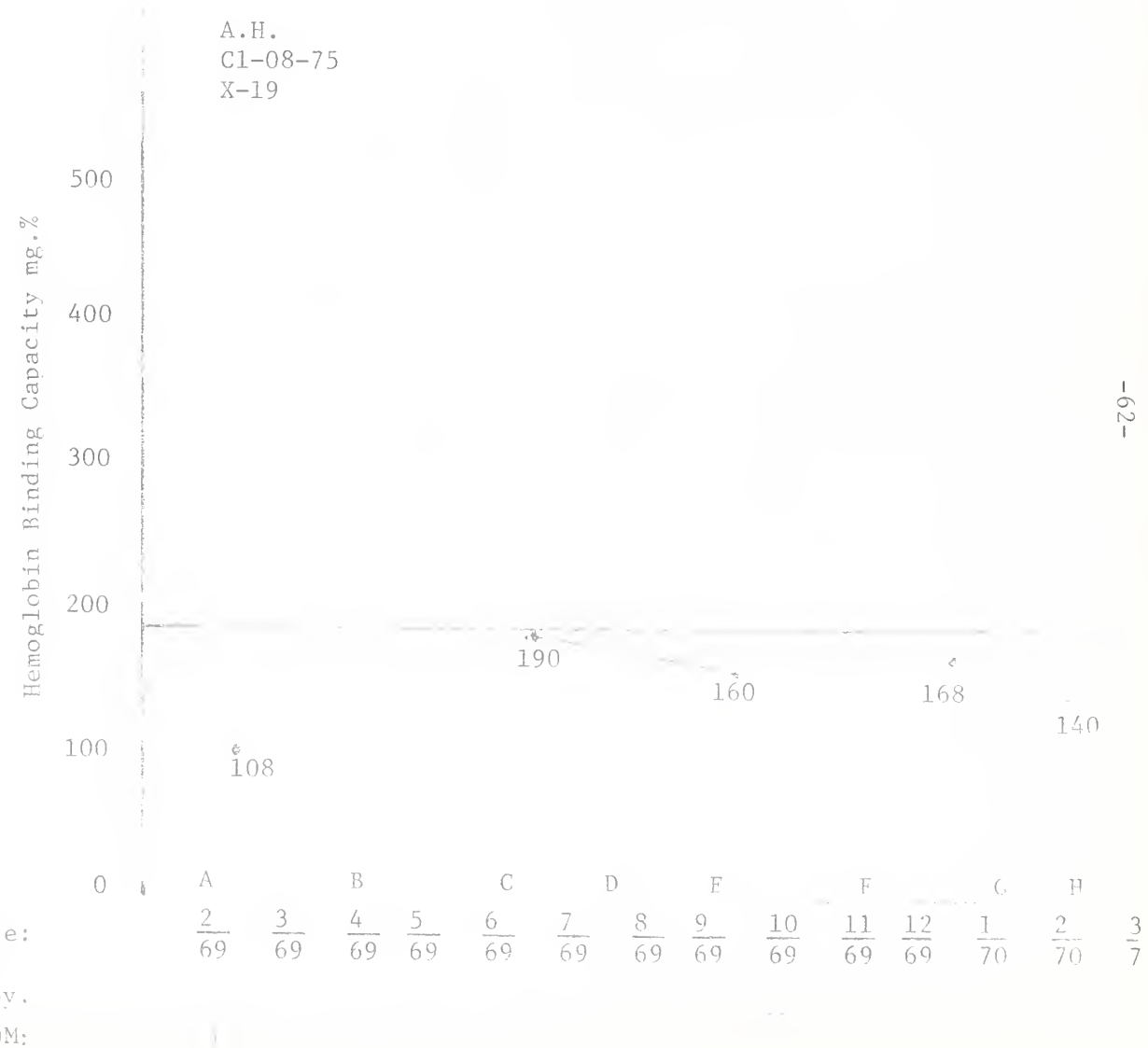
C. Tumor 8 cm.

D. Laporotomy - Widespread tumor left.

E. Widespread tumor

F., G. No change

H. Expired.



3/67 Radiotherapy.

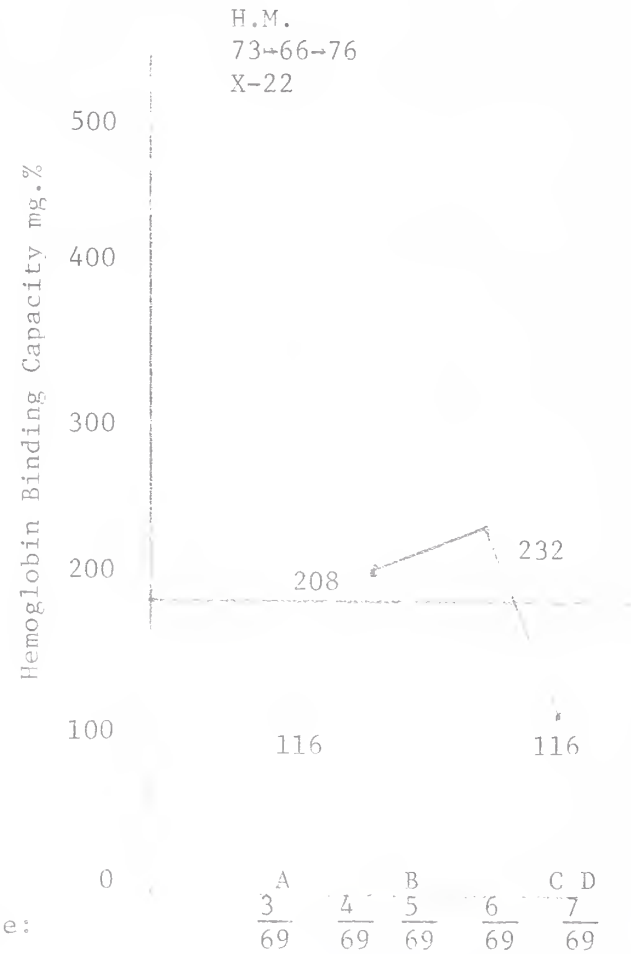
Chemotherapy 5FU, UM:

A. Laporotomy 11/68; 1/69.
3/69 Widespread tumor.

B. Widespread tumor.

C. No change.

D. Expired.



12/68 Radiotherapy.

Chemotherapy 5FU, UM:

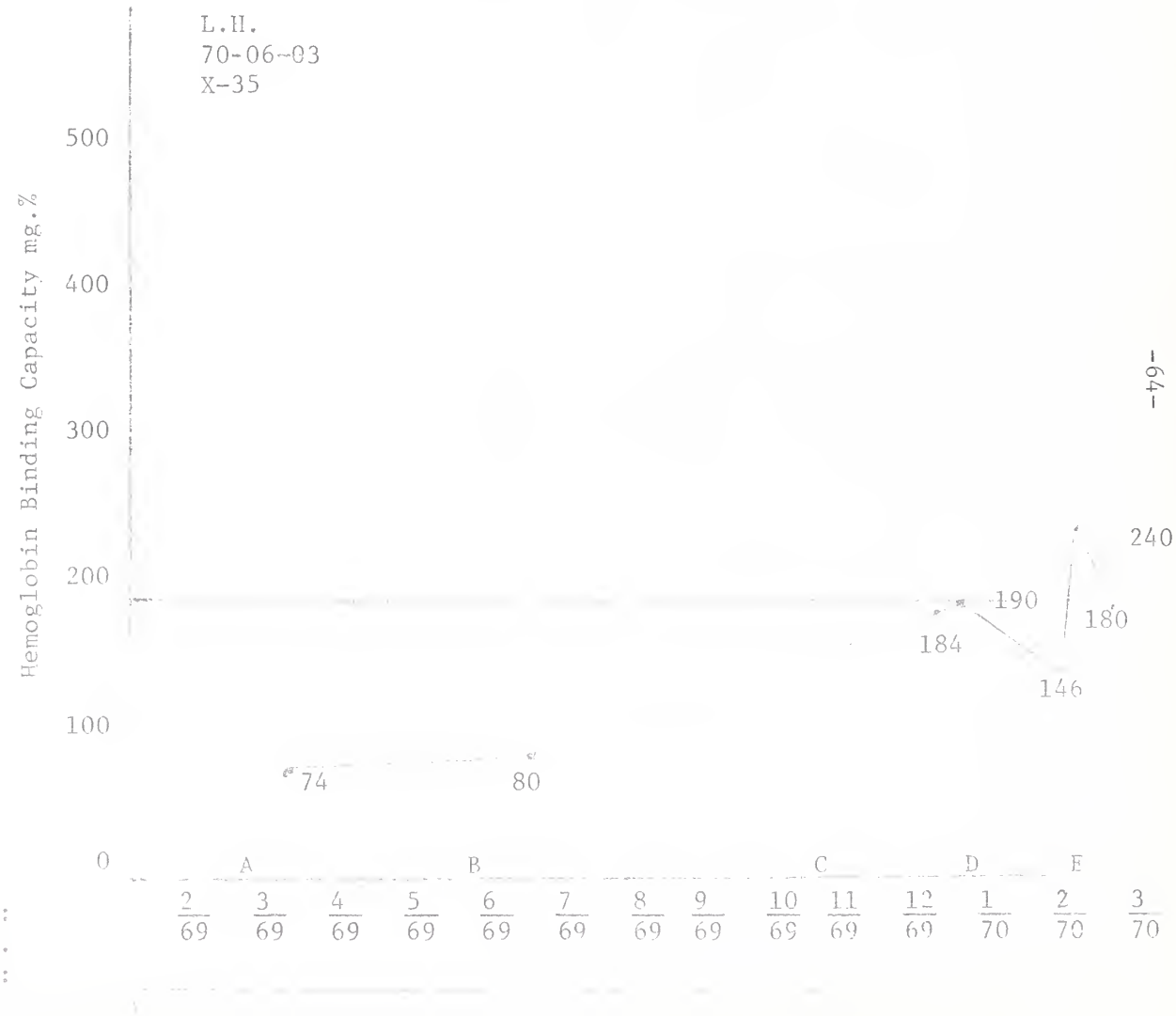
A. Surgery 8/67 - Removal of all tumor.
3/69 - No tumor.

B. Tumor 2 cm.

C. No tumor.

D. Ascites with positive cell block.

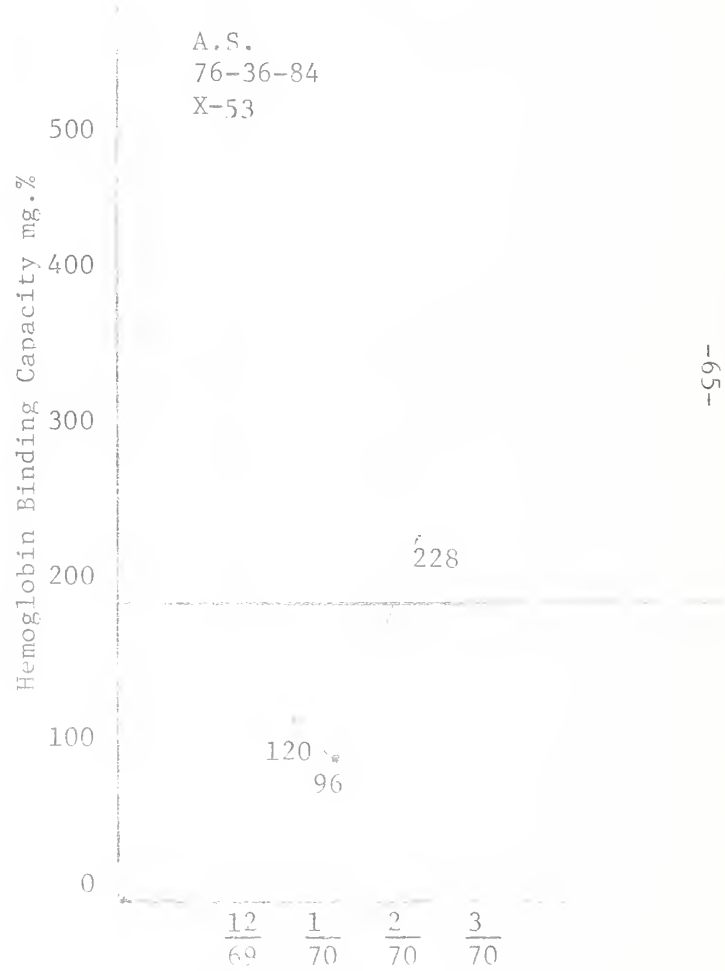
E. Widespread tumor.



Date:
9/67 Radiotherapy.
Chemotherapy 5FU, UM:

A. Laporotomy 11/69. No tumor left.

B. No tumor.



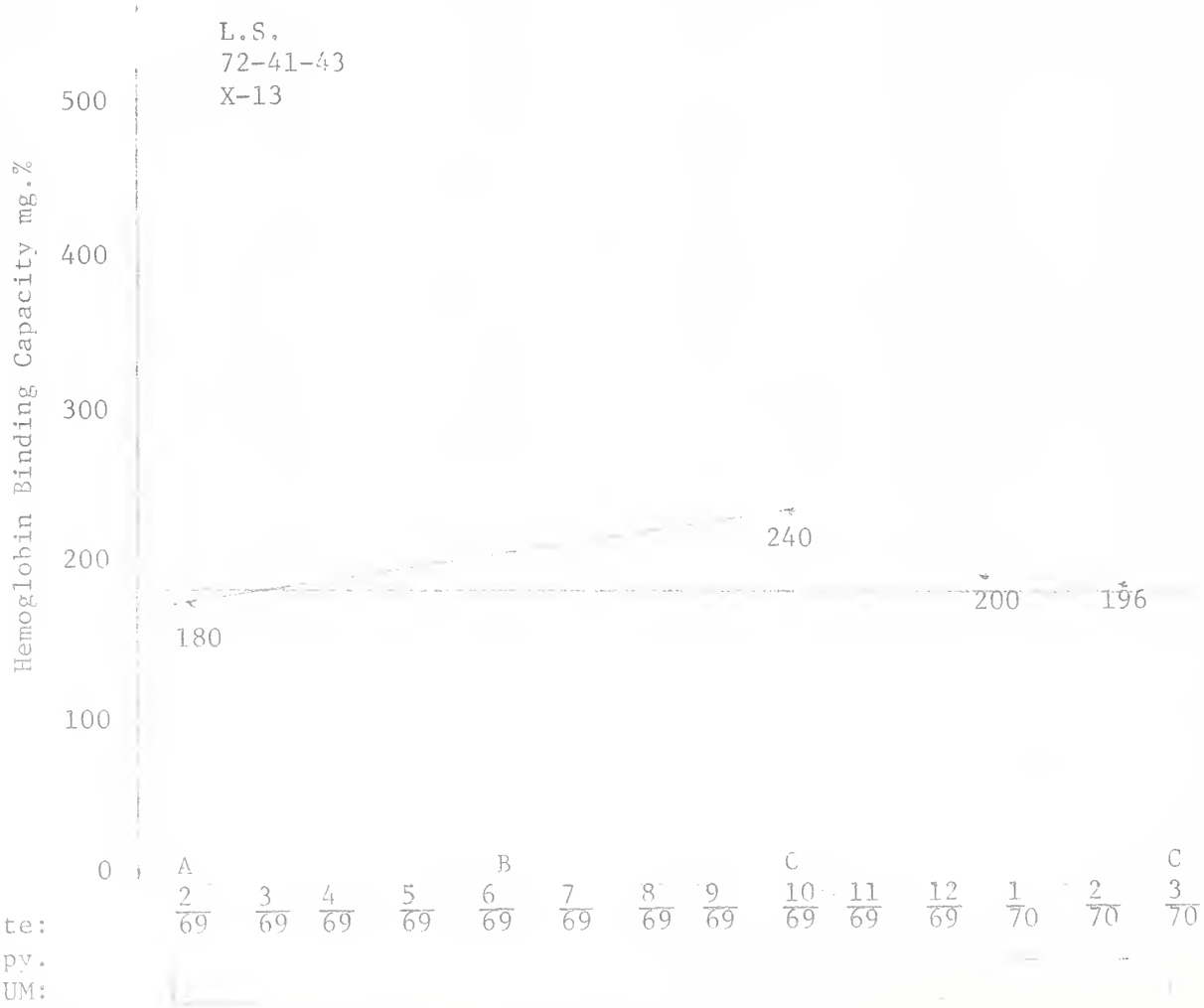
Radiotherapy:
No Chemotherapy.

A. Surgery 5/68
2/69 No tumor.

B. No tumor.

C. No tumor.

D. Increasing abdominal girth with ascites.



Summary of Results of Serial Haptoglobin Determination -

Group 1	No. of Patients
Patients whose haptoglobin levels rose from normal to high levels at the same time as or shortly after evidence for tumor mass increasing to > 6cm. was discovered: X-20, X-32, X-45, X-39, X-19, X-22.	6
Group 2	
Patients whose haptoglobin levels rose from normal to high before evidence of recurrence of tumor mass: X-15, X-35, X-13.	3
Group 3	
Patients who persistently had tumor mass > 6 cm. and who had elevated haptoglobin continuously: X-33, X-55, X-31, X-62, X-42, X-47, X-64, X-57, X-70.	9
Group 4	
Patients of Groups 1 and 2 who had tumor mass > 6 cm. of and had elevated haptoglobins for most of the time studied but who had a transient decrease or a decrease to normal haptoglobin level on one or two occasions: X-32, X-22, X-35	3
Group 5	
Patients of Groups 1 and 2 who had tumor mass > 6 cm. and who had elevated haptoglobin on at least one occasion but who for most of the time had grossly normal haptoglobin levels: X-19.	1
Group 6	
Patients who had tumor > 6 cm. but at no time developed an elevated haptoglobin level: X-18.	1
Group 7	
Patients whose haptoglobin level decreased from high to normal at the same time as or shortly following evidence that tumor mass had decreased from widespread disease to estimated tumor mass < 6 cm. in diameter: X-9, X-34, X-44, X-68.	4

Group 8	No. of Patients
Patients whose haptoglobin levels decreased from high to normal prior to evidence that tumor mass had decreased from widespread disease to estimated tumor mass < 6 cm. in diameter: X-25, X-46.	2

Group 9	
Patients who had < 6 cm. tumor at all times and persistently low haptoglobin levels: X-23, X-27, X-36, X-16, X-50, X-61, X-58, X-65.	8

Group 10	
Patients who had < 6 cm. tumor at all times and persistently high haptoglobins:	0

Group 11	
Patients who had < 6 cm. tumor and had a high haptoglobin at any time that could not be explained by active infection: ?X-53	?1

Patient X-43 consistently had no tumor and had low haptoglobin level except for one high level at the time of an obvious upper respiratory infection.

Patient X-53 developed an elevated haptoglobin at the time of writing but did not yet have any sign of tumor recurrence.

In summary, the total number of patients followed serially was 35. The number of patients with tumor mass >6cm. who failed to develop an elevated haptoglobin level at any time was 1 (X-18). The number of patients who had tumor mass > 6 cm. and who did develop an elevated haptoglobin at least once but who for the most part of this study had normal haptoglobins was 1 (X-19). The number of patients with tumor mass < 6 cm. who had normal haptoglobin levels for most of the study but whose haptoglobin rose to abnormal level which could not be explained by infection or recurrence of widespread tumor was ?1 (?X-53). There was one patient with tumor mass < 6 cm. who had elevated haptoglobin levels over 4 months prior to physical evidence of recurrence.

The total number of negative correlations between amount of tumor and level of haptoglobin was 2:

Tumor > 6 cm. with normal haptoglobin level for most of the time of this study: 2 (X-18, X-19)

Tumor < 6 cm. with elevated haptoglobin level for most of the time of this study: 0

The total number of positive correlations was 33.

Tumor > 6 cm. with elevated haptoglobin level for most of the time of this study or tumor < 6 cm. with normal haptoglobin level for most of this study.

DISCUSSION AND CONCLUSIONS

Types of Haptoglobin

1. There is no difference between the distribution of haptoglobin types in patients with malignant ovarian tumors and the distribution of haptoglobin types in the controls ($p > .70$).
2. There is no difference between the distribution of types in patients with ovarian carcinomas and the distribution in controls ($p > .95$).
3. There is probably no difference between the distribution of haptoglobin types in patients with granulosa-thecal cell tumors, malignant teratomas, and embryonal cell tumors and the distribution of controls, but the number of patients was to small to state this with certainty.
4. There is no difference between the distribution of haptoglobin types in patients with mucin producing adenocarcinomas and the distribution in patients with non-mucin producing adenocarcinomas ($p > .50$).
5. There is no difference between the distribution of haptoglobin types in patients with differentiated ovarian carcinomas and the distribution in patients with poorly differentiated adenocarcinomas ($p > .70$).

Genetic haptoglobin types do not appear to be related to malignant ovarian tumors in general, to any specific type of ovarian tumor, to mucin production by tumor, or to the degree of anaplasia of tumor.

Amount of Haptoglobin - Single Determinations of Haptoglobin Quantity.

1. Grossly elevated haptoglobin levels (i.e. levels of 190 mg.% and above) seen in patients with ovarian tumor mass > 6 cm. in diameter are highly significant when compared to controls ($p < .001$).
2. The gross elevations in serum haptoglobins in patients who died from ovarian malignancy within two months of the date of obtaining the serum are highly significant compared to controls ($p < .001$).
3. In patients with a previous history of malignant ovarian tumor but no present physical, radiological, or surgical evidence of tumor, haptoglobin levels are not elevated when compared to controls ($p = .77$).
4. In patients with tumor mass < 6 cm. haptoglobin levels are not grossly elevated when compared to controls ($p = .77$).
5. Patients with widespread (tumor mass > 6 cm.) granulosa-thecal cell tumors or widespread embryonal cell tumors possibly may have considerably larger concentrations of haptoglobins than patients with widespread carcinomas, but the number of patients with these rare tumors was too few to allow the results to be significant.
6. In patients with widespread tumor, haptoglobin levels probably are not influenced by the presence or absence

of mucin production. Again, the number of patients was too small to state this conclusion unequivically.

7. In patients with widespread tumor, the degree of differentiation of the tumor does not influence the haptoglobin level ($p > .05$).
8. In patients with widespread tumor the use of radiotherapy or chemotherapy within two months prior to the obtaining of the serum sample has no effect upon haptoglobin level ($p > .05$).
9. In patients with widespread tumor, laporotomy within 2 months of the obtaining of the serum sample significantly raises the average haptoglobin approximately 23 mg.% (from 268 to 291) ($.01 < p < .05$).

Amount of Haptoglobin - Serial Determinations of Haptoglobin Quantity -

1. In 94.3% of the patients with malignant ovarian tumors (33 out of 35 patients) serum haptoglobin levels correlate with the amount of tumor mass:

   < 6 cm. mass yields normal haptoglobin levels
   > 6 cm. mass yields elevated haptoglobin levels

2. A decrease in tumor mass to < 6 cm. is accompanied by a decrease from abnormally high haptoglobin levels to normal levels. With successful chemo- or radio-therapy, this decrease may precede clear physical or radiologic

evidence of decreasing tumor size. (Of the 7 patients whose tumor was completely removed, none had elevated haptoglobin levels that remained elevated.)

3. An increase in tumor mass to > 6 cm. is accompanied by an increase in haptoglobin from normal to high levels. This increase in haptoglobin levels may precede clear physical or radiologic evidence of increasing tumor mass. (25 out of 26 patients with widespread tumor developed high haptoglobin levels.)
4. A single isolated normal haptoglobin level in the presence of widespread tumor is not significant. (3 out of 19 patients with continued widespread tumor showed transient normal levels. 1 out of 19 patients with continued widespread tumor showed a drop to a normal level which persisted. 1 out of 19 patients with continued widespread tumor showed no elevation in haptoglobin.)
5. A single high haptoglobin unexplained by intercurrent infection or other diseases which cause elevated haptoglobin is a probable indication of recurrent tumor. (Only 1 of 9 patients with continued evidence of tumor mass ≤ 6 cm. developed an elevated haptoglobin.) Addendum: two weeks after the study was concluded the patient developed ascites due to recurrent tumor.

Possible Pathophysiological Mechanisms of Increased Haptoglobin Production with Widespread Ovarian Tumor

How do ovarian tumor with tumor mass ≥ 6 cm. cause

elevation in serum haptoglobin levels? Theoretically there are three possible mechanisms for increased levels:

1) Increased synthesis by liver and/or synthesis by another organ

2) Increased release of stored haptoglobin

3) Decreased uptake or removal of haptoglobins

Studies of patients with Hodgkin's tumor show that the rate of clearance of haptoglobin from the blood stream is directly proportional to the total amount of haptoglobin in the blood stream (i.e. the fractional removal rate is constant).[31,32] If this conclusion holds true for patients with ovarian carcinomas as well, decreased uptake of haptoglobins does not occur, there is no paralysis of the reticulo-endothelial system,and possibility 3) above is eliminated.

Release of stored haptoglobin might explain the elevations in serum haptoglobin following surgery. However, the elevation of serum haptoglobins seen in some patients with continued widespread tumor went on for extended time periods: patient X-15, for instance, had a marked elevation for seven months. There would have to be enormous depot stores of haptoglobin if increased release of previously synthesized stores is to account for continued as well as transient elevations; for the turnover rate of haptoglobin is relatively short - approximately four days. Thus, possibility 2) seems an unlikely explanation for long-standing elevations.

Only possibility 1) is left: increased synthesis of haptoglobin by the liver parenchyma or by other tissue. It is possible that in the process dedifferentiation previously repressed operators

could be derepressed to dictate the synthesis of haptoglobin. However, haptoglobin is not a simple polypeptide but a complex molecule with alpha and beta chains joined in a specific manner and linked between beta and $alpha_2$ chains to form polymers. It is unlikely that a dedifferentiated cell of a cell line which was originally incapable of producing haptoglobin would be able to produce such a complicated molecule. These ideas should, however, be validated experimentally with anti-haptoglobin antibody studies of normal and malignant ovarian tissue.

The most likely explanation is that tumor causes an alteration in the serum which stimulates the liver to increased synthesis of haptoglobins. This change could be a haptoglobin stimulator substance produced by tumor growth or tumor breakdown as suggested by Graf and Probst.[63]

There appears to be a gradient in haptoglobin levels in relation to amount of tumor:

	No. of Patients	Average Haptoglobin Level
C controls	50	81
Patients with no evidence of tumor	15	122
Patients with tumor < 6 cm.	34	144
Patients with tumor > 6 cm.	9	268

This gradient could best be explained by a haptoglobin stimulating factor produced by tumor or tumor necrosis.

Alternately, a haptoglobin stimulator substance could be produced by another organ, other than tumor tissue. An unanswered question is whether the increased synthesis of haptoglobin by the liver in patients with large amount of malignant tumor is mediated by the

adrendals. It is well known that androgen therapy raises haptoglobin levels and that androgen produced by the adrenals is converted to estrogen by the ovaries. Virilizing effects suggestive of increased androgen levels were not seen in these patients with ovarian tumors, but androgens might still be implicated in increased haptoglobin synthesis. Another possible mechanism involving the adrenal is that tumor causes a stress response with increased release of glucocorticoids and epinephrine which in turn stimulate the liver to produce haptoglobin.

In conclusion, the most likely mechanism of increased haptoglobin levels in patients with malignant tumors appears to be increased synthesis by the liver, stimulated by a haptoglobin stimulator substance from adrenal or from tumor. The pathophysiology of increased serum haptoglobins is an important field for new exploration.

Clinical Value of Haptoglobin Studies in Malignant Ovarian Tumors

Haptoglobin types appear to be unrelated to ovarian malignancies and therefore are of no clinical value.

A hemoglobin binding capacity of 190 mg./100ml. serum is a dividing line between grossly normal and grossly elevated haptoglobin levels. Variations in haptoglobin level which do not cross the 190 mg.% boundary line have as yet no discernible clinical significance.

Haptoglobin levels do not appear to be influenced by the type of ovarian malignancy. The results suggest that widespread

granulosa-theca cell tumors and embryonal cell tumors are associated with especially high haptoglobin levels , > 300mg.%, but a larger number of patients should be studied to reach a definite conclusion. Furthermore, haptoglobin levels do not appear to be influenced by the degree of anaplasia of the tumor or by mucin production.

Haptoglobin levels are definitely affected by the amount of tumor present. Because they are minimally affected by the type of ovarian tumor or by chemotherapy, radiotherapy, or surgery *per se* they are of value in indicating the amount of tumor irrespective of therapy or tumor type.

Although an isolated normal haptoglobin determination (<190mg.%) is not of value in determining the amount of tumor, continued normal haptoglobin levels in the vast majority of cases indicate that tumor mass is < 6 cm. and that radio-, chemo-, and surgical therapy have been effective in containing the tumor. However, continued normal haptoglobin levels of themselves are not conclusive evidence that tumor remains < 6 cm.

An isolated elevated haptoglobin level which cannot be explained by infection or other disease known to cause elevation is very strongly suggestive of tumor > 6 cm: tumor has recurred, or previously existing tumor is failing to respond to radio-, chemo-, or surgical therapy. Continued elevated haptoglobins imply continued existence of tumor >6cm.

A decrease of haptoglobin level from ⩾ 190 mg.% to ⩽ 190 mg.% followed by continued normal haptoglobin levels implies successful therapy. An increase of haptoglobin level from < 190 mg.% to

190 mg.% in the absence of infection, etc., is strongly suggestive of tumor mass increasing to < 6 cm. and of recurrence of tumor. Decreases and increases in haptoglobin levels may precede the clinical evidence of decreasing or increasing tumor: an unexplained increase in haptoglobin from < 190 mg.% to > 190 mg.% is an early indication of tumor recurrence.

SUMMARY

Sera of 58 patients with ovarian cancers were typed and quantified for haptoglobins. The relationships between haptoglobin type and tumor type, mucin production and the degree of differentiation were investigated. The relationships between haptoglobin level and tumor type, amount of tumor, death from tumor, mucin production, degree of differentiation, recent radio-, chemo- and surgical therapy were also investigated. 35 patients were followed with serial haptoglobin levels. In addition to haptoglobin level, the amount of tumor, use of radiotherapy, chemotherapy, and surgery, and the occurence of death were noted. The study posed some interesting questions concerning the pathophysiology of increased haptoglobin levels seen with ovarian cancers and with other conditions. The results and conclusions suggest that haptoglobin levels either in single instances or serial studies may be of clinical value in following the course of malignant ovarian tumors and in suggesting tumor recurrence before it is physically or radiologically evident.

APPENDIX

D CONTROLS

Study #	Initials	Haptoglobin Type	Haptoglobin Quantity: mg.% Hemoglobin Binding Capacity
D-1	P.S.	2-1	60
D-2	K.P.	2-2	38
D-3	E.DeS.	2-1	40
D-4	A.M.	2-1	110
D-5	G.W.	1-1	80
D-6	P.H.	2-1	120
D-7	J.C.	2-1	42
D-8	B.P.	1-1	72
D-9	W.M.	1-1	58
D-10	E.W.	2-2	20
D-11	K.B.	2-1	90
D-12	C.D.	2-2	150
D-13	D.W.	2-2	136
D-14	M.H.	2-1	60
D-15	T.G.	1-1	72
D-16	B.P.	1-1	66
D-17	S.A.	2-1	170
D-18	J.V.	1-1	38
D-19	C.A.	2-1	162
D-20	C.Y.	1-1	38
D-21	S.Q.	1-1	76
D-22	J.R.	2-1	96

Study #	Initials	Haptoglobin Type	Haptoglobin Quantity: mg.% Hemoglobin Binding Capacity
D-23	L.N.	2-2	72
D-24	R.S.	2-2	88
D-25	E.A.	1-1	146
D-26	A.C.	1-1	118
D-27	M.D.	2-1	60
D-28	D.R.	2-2	68
D-29	S.G.	2-1	70
D-30	M.H.	2-1	118

C CONTROLS

Study #	Init.	Hospital Unit #	Age	Date	Diagnosis	Hap. Type	Hbg. Binding Capacity
C-1	G.K.	A2-59-59	39	06/30/69	Menorrhagia	2-2	56
C-2	U.M.	43-11-44	41	06/30/69	Uterine leiomyoma	2-1	67
C-3	L.C.	75-38-35	36	06/30/69	Anovulatory bleed	2-1	58
C-4	L.L.	61-19-66	30	06/30/69	Venous varicosities	2-1	44
C-5	H.A.	41-99-96	44	07/02/69	Menorrhagia	2-2	59
C-6	H.B.	74-94-31	24	07/02/69	Bartholin cyst	1-1	128
C-7	D.F.	A0-61-84	72	07/02/69	Vaginal band	2-2	38
C-8	M.H.	73-80-85	39	07/02/69	Deviated septum	2-2	26
C-9	B.J.	33-02-98	42	02/17/70	Hemorrhoids	2-2	92
C-10	P.W.	39-83-75	45	07/02/69	Uterine leiomyoma	2-2	65
C-11	V.I.	31-65-67	49	07/02/69	Uterine leiomyoma	2-2	69
C-12	M.B.	54-90-27	50	07/03/69	Menopause	2-1	128
C-13	A.M.	60-54-90	53	07/03/69	Inguinal hernias, diverticulosis, leiomyoma	2-1	102
C-14	M.F.	36-84-48	48	07/01/69	Chronic cystic mastitis	2-1	92
C-15	C.W.	42-21-22	43	07/03/69	Cervical polyp-Class I, uterine leiomyoma	2-1	88
C-16	T.S.	C3-61-74	62	07/07/69	Hemorrhoids	2-1	103
C-17	J.M.	60-79-27	38	07/07/69	Elective tubal ligation	2-1	112
C-18	E.S.	75-45-57	55	07/07/69	Endometriosis	2-1	39
C-19	R.M.	68-21-08	30	07/08/69	Chronic cystic mastitis	2-2	36
C-20	A.Z.	75-45-88	23	07/08/69	Bunion	2-1	38
C-21	S.L.	75-45-89	51	07/08/69	Disuse atrophy of meniscus	2-1	52
C-22	B.M.	36-79-98	68	07/08/69	Enterocele	2-1	60
C-23	A.E.	B6-07-15	24	07/08/69	Chronic cystic mastitis	2-1	62
C-24	L.B.	75-40-91	54	07/08/69	Menorrhagia	1-1	100
C-25	E.J.	75-46-16	69	07/09/69	Ganglion	2-1	116

Study #	Init.	Hospital Unit #	Age	Date	Diagnosis	Hap. Type	Hbg. Binding Capacity
C-26	T.K.	64-85-66	37	07/09/69	Uterine prolapse	2-1	44
C-27	P.M.	71-88-73	23	07/09/69	Benign laryngeal cyst	2-1	42
C-28	M.M.	06-92-99	42	07/09/69	Venous varicosities	2-2	128
C-29	R.M.	63-45-95	27	07/09/69	Hemorrhoids	2-1	76
C-30	J.W.	57-21-39	43	07/09/69	Anovulatory bleed	2-2	40
C-31	V.V.	75-46-17	51	07/10/69	Bunions	2-1	126
C-32	M.K.	37-14-13	53	07/10/69	Hemorrhoids,anal fissure	2-2	230
C-33	M.H.	C3-74-99	56	07/10/69	Chronic pelvic inflammatory disease	2-1	156
C-34	F.E.	75-46-41	51	07/10/69	Essential hypertension	2-2	84
C-35	M.M.	B2-79-76	59	07/10/69	Ganglion	2-1	112
C-36	N.M.	52-13-86	49	07/10/69	Fibroadenoma of breast	2-1	134
C-37	M.S.	42-97-97	43	07/11/69	Herniated disk	2-2	38
C-38	M.V.	C3-32-96	43	07/11/69	Uterine leiomyomata	2-2	168
C-39	H.F.	A1-32-15	43	07/11/69	Uterine prolapse	2-2	34
C-40	C.F.	45-38-17	45	07/11/69	Hemorrhoids,anal fissure	2-2	68
C-41	D.S.	69-28-61	27	o7/11/69	Venous varicosities	1-1	84
C-42	N.M.	72-76-67	39	07/14/69	Benign endometrial polyp	1-1	76
C-43	M.B.	02-87-82	46	07/14/69	Hemorrhoids	2-2	148
C-44	D.H.	47-45-89	31	07/14/69	Carcinoma in situ of cervix	1-1	94
C-45	J.B.	48-73-96	48	07/17/69	Venous varicosities	2-1	52
C-46	F.W.	08-88-81	48	07/17/69	Scar and neuroma	2-2	132
C-47	M.H.	B1-08-43	52	07/17/69	Uterine leimyoma	2-2	68
C-48	E.J.	49-12-08	33	07/17/69	Benign breast cyst	2-1	68
C-49	C.H.	75-54-99	44	07/17/69	Chronic cystic mastitis	2-1	30
C-50	J.S.	77-32-34	31	02/17/70	Tubal adhesions	2-1	40

PATIENTS WITH MALIGNANT OVARIAN TUMORS

Study #	Init.	Hospital Unit #	Age	Date	Radio-therapy			Chemotherapy			Surgery		
					1*	2*	3*	1*	2*	3*	1*	2*	3*
X-1	M.P.	52-02-92	62	08/20/68	0	+	0	0	0	0	0	0	+
X-2	A.D'E.	46-55-58	41	08/12/68	0	+	0	0	A	B	0	+	0
X-3	E.S.	68-10-16	48	09/05/68	0	0	0	0	A	B	+	0	0
X-4	B.F.	38-93-67	46	09/05/68	0	+	0	0	A	A	0	+	0
X-7	L.A.	71-08-07	42	09/17/68	0	+	0	0	A	B	0	+	0
X-8	B.H.	71-24-03	47	09/13/68	0	+	0	0	0	A	+	0	0
X-9	L.H.	59-55-08	51	02/04/69	0	0	0	0	0	0	0	0	0
X-11	J.S.	72-48-63	57	09/09/68	0	0	+	0	0	A	0	+	0
X-12	A.P.	B0-52-13	47	02/04/69	+	0	0	A	A	A	+	0	0
X-13	L.S.	72-41-43	50	02/06/69	0	+	0	0	A	A	0	+	0
X-14	R.H.	A3-06-98	61	02/05/69	0	+	0	0	A	A	0	+	0
X-15	J.C.	72-24-51	42	02/04/69	0	+	0	0	A	A	0	+	0
X-16	B.M.	73-67-76	42	02/04/69	0	0	+	0	0	0	0	+	0
X-17	R.C.	73-93-73	43	02/12/69	0	0	0	0	0	A	0	0	+
X-18	F.L.	71-33-64	56	09/01/68	0	+	0	0	0	A	0	+	0
X-19	A.H.	C1-08-75	56	07/23/69	0	0	0	A	A	A	+	0	0
X-20	M.P.	B4-20-43	59	02/11/69	0	0	0	0	A	A	0	+	0
X-21	P.P.	73-58-61	55	02/04/69	0	0	0	0	0	A	0	+	0

*1. Within 5 years to 1 year prior to date of serum sample

2. Within 1 year to 2 months prior to date of serum sample

3. Within the two months predeing the date of the serum sample

Chemotherapy symbols:

0: no chemotherapy

A: uracil mustard and 5-fluorouracil

B: Leukeran and Velban

C: 1,3-bischloronitrosourea

Study #	Tissue Diag.*	Mucin	Hap. Type	Amount Tumor**	Evidence	Hbg. Binding Capacity	Hbg. Binding Capacity at Death***
X-1	Au	0	2-2	2	P.E.	256	256
X-2	Ad	0	2-1	2	P.E.	176	176
X-3	G	0	2-1	2	P.E.,autopsy	440	440
X-4	Ad	0	2-1	2	P.E.	228	228
X-7	Au	0	2-1	2	X-ray	282	282
X-8	Ad	0	2-2	2	Laporotomy	196	-
X-9	Ad	0	2-1	2	P.E.	416	-
X-11	Ad	0	2-1	2	P.E.	208	208
X-12	Ad	0	2-1	0	Autopsy	156	-
X-13	Au	+	2-1	0	P.E.,X-ray	180	-
X-14	Ad	0	2-2	0	P.E.,X-ray	68	-
X-15	Ad	0	2-1	1	P.E.: breast ca.-1.5 cm.	120	-
X-16	Ad	0	1-1	0	P.E.,X-ray	124	-
X-17	Au	0	1-1	2	Laporotomy	154	-
X-18	Au	0	2-2	0	P.E.	160	-
X-19	Ad	0	2-2	0	P.E.	138	110
X-20	Ad	0	2-1	1	P.E.	76	262
X-21	Ad	0	2-1	2	P.E.	120	-

*Tissue diagnosis: Ad: Differentiated adenocarcinomas, including papillary, serous, cystic, and mucinous adenocarcinomas and combinations thereof
Au: Undifferentiated adenocarcinomas
G: Granulosa-theca cell tumors
T: Malignant teratomas
E: Embryonal cell tumors

** Amount tumor: 0: No evidence of tumor
1: Evidence of tumor <6cm. in diameter
2: Evidence of tumor >6cm. in diameter

*** Last hbg. binding capacity determination before expiration: date of serum sample was within two months of date of death from tumor

Study #	Init.	Hospital Unit #	Age	Date	Radio-therapy			Chemotherapy			Surgery		
					1	2	3	1	2	3	1	2	3
X-22	H.M.	73-66-76	38	03/10/69	0	+	0	0	0	A	0	+	0
X-23	R.W.	73-99-57	47	02/04/69	0	0	+	0	0	0	0	+	0
X-25	M.L.	A4-78-68	62	05/28/69	0	0	0	0	0	0	0	0	0
X-26	A.S.	B4-75-36	52	03/17/69	0	0	0	0	0	A	0	+	0
X-27	E.L.	74-70-23	66	06/16/69	0	0	+	0	0	0	0	+	0
X-31	S.C.	74-32-29	62	06/12/69	0	+	0	0	0	A	0	0	+
X-32	J.D'A.	72-97-14	49	09/23/69	0	+	0	A	B	B	+	0	0
X-33	M.P.	75-13-05	64	06/16/69	0	0	+	0	0	0	0	0	+
X-34	A.G.	40-61-47	65	06/12/69	0	0	0	0	0	0	0	0	+
X-35	L.H.	70-06-03	65	03/04/69	+	0	0	A	A	A	+	0	0
X-36	R.M.	67-74-35	74	02/17/69	+	0	0	A	A	A	+	0	0
X-38	A.J.	69-03-37	84	08/27/69	+	0	0	A	0	0	+	0	0
X-39	M.L.	75-89-56	51	08/29/69	0	0	0	0	0	0	0	0	+
X-40	B.C.	05-18-29	51	09/09/69	+	0	0	A	B	B	+	0	0
X-41	S.B.	65-14-06	45	06/20/69	+	0	0	A	0	0	+	0	0
X-42	E.B.	B0-85-31	69	01/07/70	0	0	0	0	0	A	0	+	0
X-43	A.M.	65-73-80	44	01/06/70	0	0	0	A	B	B	+	0	0
X-44	I.S.	49-82-95	70	10/06/69	0	0	0	0	0	0	0	0	+
X-45	D.R.	C6-24-08	49	10/16/69	0	0	0	0	0	0	0	0	0
X-46	M.C.	C4-80-48	46	11/24/69	0	0	+	0	0	0	0	0	+
X-47	E.B.	C6-07-12	48	10/07/69	0	0	+	0	0	A	0	0	+
X-48	R.S.	76-23-12	54	01/07/70	0	0	0	0	A	A	0	+	0
X-49	M.D.	C1-86-78	53	11/20/69	0	0	0	0	0	A	0	0	+
X-50	C.C.	75-94-50	57	11/2o/69	0	0	0	0	A	A	0	+	0
X-51	S.P.	76-78-19	25	12/15/69	0	0	0	0	0	0	+	0	+
X-53	A.S.	76-36-84	47	12/23/69	0	0	0	0	0	A	0	0	+

Study #	Tissue Diag.	Mucin	Hap. Type	Amount Tumor	Evidence	Hbg. Binding Capacity	Hbg. Binding Capacity at Death
X-22	Ad	0	2-2	2	P.E.	116	116
X-23	Ad	0	2-1	1	P.E.	102	-
X-25	Ad	0	2-2	2	Laporotomy	212	-
X-26	Ad	+	2-2	2	Autopsy	272	272
X-27	Ad	0	2-1	1	Laporotomy	180	-
X-31	Ad	0	2-1	2	P.E.	292	508
X-32	Au	0	2-2	2	P.E.	180	256
X-33	Au	0	2-1	2	P.E.	260	274
X-34	Ad	0	2-1	2	Laporotomy	324	-
X-35	Ad	0	2-2	0	P.E.,X-ray	74	-
X-36	Ad	0	2-1	0	P.E.,X-ray	156	-
X-38	Ad	0	2-1	0	P.E.,X-ray	108	-
X-39	Au	0	2-2	1	P.E.,laporotomy	170	-
X-40	Ad	+	2-2	0	P.E.,X-ray	56	-
X-41	Ad	0	2-1	0	P.E.,X-ray	110	-
X-42	Au	0	2-1	2	P.E.	524	380
X-43	Au	0	2-2	0	P.E.,X-ray	120	-
X-44	Au	0	2-1	2	Laporotomy	290	-
X-45	Ad	0	2-2	2	Laporotomy	154	220
X-46	Au	0	2-2	2	Laporotomy	300	-
X-47	Au	0	1-1	2	P.E.,X-ray	196	210
X-48	Ad	0	1-1	2	P.E.	170	-
X-49	Ad	0	2-1	1	P.E.	100	-
X-50	Au	0	2-1	0	P.E.	104	-
X-51	Ad	+	2-1	2	Laporotomy	380	380
X-53	Ad	0	2-2	0	P.E.	120	-

Study #	Init.	Hospital Unit #	Age	Date	Radio-therapy			Chemotherapy			Surgery		
					1	2	3	1	2	3	1	2	3
X-55	J.J.	44-18-38	43	01/06/70	0	0	+	0	0	0	0	0	+
X-56	S.G.	52-42-21	32	10/06/69	0	+	0	0	0	0	0	0	+
X-57	M.McG.	76-59-63	63	01/04/70	0	0	0	0	0	0	0	+	0
X-58	D.S.	07-33-09	70	12/15/69	+	0	0	0	0	0	+	0	0
X-59	E.S.	76-84-73	44	12/16/69	0	0	0	0	0	0	0	0	+
X-60	L.Z.	78-85-16	26	12/17/69	0	0	0	0	0	0	0	0	+
X-61	E.B.	76-31-04	45	01/07/70	0	+	0	0	0	0	0	+	0
X-62	V.B.	67-98-79	43	01/07/70	+	0	0	A	A	A	+	0	0
X-64	L.C.	75-76-12	73	01/14/70	0	0	0	0	0	0	0	0	0
X-65	A.F.	47-68-72	67	01/14/70	0	0	0	0	0	A	0	0	+
X-66	M.M.	66-51-38	65	01/14/70	+	+	0	0	0	0	+	0	0
X-68	S.C.	77-22-31	18	01/15/70	0	0	0	0	0	0	0	0	+
X-69	H.M.	75-48-25	58	08/08/69	0	+	0	0	0	A	0	+	0
X-70	J.S.	77-19-17	23	02/05/70	0	0	0	0	0	0	0	+	0

Study #	Tissue Diag.	Mucin	Hap. Type	Amount Tumor	Evidence	Hbg. Binding Capacity	Hbg. Binding Capacity at Death
X-55	Ad	0	2-1	2	Laporotomy	410	-
X-56	Ad	0	2-2	2	Laporotomy	396	-
X-57	Au	0	2-1	2	P.E.,laporotomy	270	240
X-58	Au	0	2-2	1	P.E.	150	-
X-59	Au	+	2-1	1	P.E.	140	-
X-60	T	0	2-1	2	Laporotomy	164	-
X-61	Ad	+	1-1	0	P.E.	188	-
X-62	Ad	0	2-2	2	P.E.	360	-
X-64	None	-	2-2	2	P.E.,X-ray	200	-
X-65	G	0	2-1	1	Laporotomy	160	-
X-66	Ad	0	2-2	2	P.E.	110	-
X-68	E	0	2-2	2	Laporotomy,P.E.	360	-
X-69	Ad	0	2-1	2	P.E.	277	277
X-70	E	0	2-1	2	P.E.	420	-

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