AN INVESTIGATION OF THE LOCALIZATION
OF $^{131}$ LABELLED FIBRIN ANTIBODIES ON THROMBI

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I. Formulation of the problem

Thrombosis and blood coagulation are dynamic processes necessary for the maintenance of circulatory hemostasis. As well as preventing life threatening loss of blood during surgery or accidental wounding, the process is concerned with the more subtle preservation of the integrity of small blood vessels and quickly plugs vessels when a solution of continuity occurs.

Hemostasis depends on a combination of three factors. These are the integrity of the capillary and blood vessel wall, circulating blood platelets and a number of clotting factors of which eleven have been isolated. Inefficiency of one or a combination of these factors leads to a thrombocytopenic purpura, thrombocytopenic purpura, hemophilia and various other abnormal bleeding states.

Thrombosis becomes pathological when it occurs and obstructs normal or abnormal vessels not as a life saving hemostatic process, but as a life threatening impedance to necessary blood flow. Directly or indirectly, thrombosis and vascular disease are the major causes of human death at the present time.

The surgeon is intimately involved with this mechanism depending on it to prevent his patient's death from blood loss during surgery, while also fearing it as a cause of post-operative death from coronary thrombosis, thrombophlebitis and pulmonary embolism.
Thrombosis and the inflammatory process constitute the most important tissue and body response to injury, and both are necessary as a prelude for the reparative process. The natural course of both can be altered by the administration of drugs such as the anticoagulants, cortisone and antibiotics. Obstruction to flow can be relieved surgically and embolism can be prevented by vessel ligation.

Pulmonary embolism secondary to phlebothrombosis and thrombophlebitis usually involving the pelvic or deep veins of the leg accounts for two percent of all deaths and is found as a contributing factor in ten percent of all post-mortems. Since methods exist to prevent or alter the course of this phenomenon, it would be of considerable value to the physician to be able to diagnose and localize a thrombophlebitic process before embolism occurs. Unfortunately a deep venous thrombosis is more often than not an occult process, first announcing its presence with a lethal pulmonary embolism. Non-specific changes such as a low grade fever or slight tachycardia often occur before the embolic phenomenon, and it is at this time that a diagnostic test might be of value.

The purpose of this investigative work was to assess the possible use of a radioactive antibody to fibrin for the detection of intravascular thrombi by surface counting techniques. In order to anticipate the localization of an antibody on a substrate for this purpose, one must assume that the complementary antigen does not normally exist in the experimental animal.
The antigen which appears to meet this requirement is fibrin which is not a normal body constituent, but which is involved at a basic level in the formation of thrombi or intravascular clots. Fibrin is the copolymer of fibrinogen which is a normal plasma protein. In order for antigenic specificity to occur it must be assumed that the molecular configuration of fibrinogen is changed during the polymerization process. The observation that radioactive fibrin antibodies localize in rat tumors, containing a high proportion of fibrin in their stroma, suggests that this may indeed happen.

If a radioactive fibrin antibody could be shown to localize on in vivo fibrin a very important clinical and experimental method would become available for the diagnosis and investigation of thrombosis, intracranial hemorrhage and intrathoracic or intra-abdominal inflammatory processes.

II The hemostatic mechanism

A general review of the coagulation mechanism is necessary in order to elucidate the structure and metabolism of fibrinogen. In 1954 the International Committee for the Nomenclature of blood clotting factors was set up with Irving S. Wright as Chairman. Eleven clotting factors are now accepted.

1. Fibrinogen
2. Prothrombin
3. Thromboplastin
4. Calcium
5. Labile factor, Proaccelerin, Accelerator Globulin.
6. Non-existent
7. Stable factor, Proconvertin, Serum Prothrombin Conversion Accelerator.
8. Antihemophilic Factor
9. Plasma thromboplastin component
10. Stuart Prower Factor
11. Plasma thromboplastin antecedent
12. Hagemen Factor

A composite scheme for the interaction of these various factors in the coagulation process is represented in Figure I.
FOREIGN SURFACE

HAGEMAN FACTOR ➔ PTA

TISSUE JUICE ➔ PLATELET FACTOR ➔ PLATELETS

VIII

Ca++

IX

Tissue VII Factor Ca++ ➔ Intermediate Product

V

PLASMA PROTEOLYTIC FACTORS ➔ Thromboplastin ➔ PROTHROMBIN

PAIN ➔ PERMEABILITY ➔ FIBRINOLYSIN ➔ FIBRIN MONOMER ➔ STABILIZING FACTOR Ca++

LYSED FIBRIN ➔ THROMBIN ➔ FIBRIN POLYMER

FIBRINOGEN ➔ FIBRIN POLYMER

FIGURE 1
Fibrinogen is a spindle shaped protein molecule with a molecular weight of 300,000 (Figure II). It has a half life of four days\(^8\). Coons' fluorescent antibody studies indicate that it is limited to vascular channels, interstitial spaces and connective tissues\(^9\). Normal fibrinogen levels vary from 200 to 300 mgm per 100 ml of plasma in the human, and are slightly higher in the dog. Fibrinogen therefore constitutes four to five percent of the total plasma protein.

Thrombin acts as a proteolytic enzyme splitting arginine-glycine bonds which bind two fibrinopeptides to the fibrinogen molecule. Fibrinopeptide A has a molecular weight of 20,000 and is situated on the end of the molecule. Fibrinopeptide B has a molecular weight of 24,000 and is situated on the middle of the molecule. These polypeptides are vasoactive in vitro. The fibrin monomer thus formed polymerizes to fibrin polymer through the formation of hydrogen bonds. Disulphide linkages are developed in the presence of fibrin stabilizing factor and calcium, forming the very insoluble fibrin copolymer\(^10\).

Thrombosis occurs only in vivo. Platelets adhere to and build up on damaged endothelium as a series of masses. The factors responsible for the adherence of platelet masses are not known. It has been postulated that a change in the wetability of the endothelium might be responsible. Other possibilities include the local release of thromboplastin, the uncovering of subendothelial mucopolysaccharides following desquamation and the lack of local anticoagulants such as heparin produced by local mast cells. Normal endothelium carries a negative charge
as do the circulating platelets. Loss of this electrical repulsing force is a third possible cause for the adherence phenomenon.

**FIGURE II**

**CHEMISTRY OF FIBRINOGEN**

![Chemical reaction diagram showing the conversion of fibrinogen to fibrin polymer.]

**The fibrinogen → fibrin transition**
Interstices containing whole blood are formed between the platelet masses. Thromboplastin is generated but is at first carried away by the still flowing stream of blood. Later as the mass builds to occlude the vessel lumen, the local concentration of thromboplastin increases and leads to coagulation of the interstitial blood and the deposition of fibrin on the surface of the thrombus. Thus is formed the white agglutination or conglutination thrombus which displays the characteristic lines of Zahn. Coagulation of the static column of blood behind the thrombus occurs giving rise to its "stasis" tail. This latter process results in the formation of the red or coagulation thrombus.

Blood clotting occurs both in vivo and in vitro and refers to the coagulation of whole blood by the conversion of fibrinogen to fibrin. Microscopic examination of platelet plasma clots indicates the normal histological pattern to be one of rather compact structure with fibrin threads radiating from central small platelet masses forming a mosaic like structure. A change in the quality or quantity of either the platelets or the blood clotting factors affects the integrity of platelet plasma clot.\[11\]
III. The fibrinolytic system.

The phenomenon of fibrinolysis was first recorded by Morgagni in 1761. Tillett and Garner in 1933\textsuperscript{12} demonstrated that the lysis of human plasma clots could be induced by the filtrates of certain streptococci. Tagnon showed that the enzyme fibrinolysin mediates the proteolysis of the fibrinolytic system\textsuperscript{13}. Profibrinolysin can be isolated by an acid extraction technique from plasma fraction III\textsuperscript{14}.

A striking similarity exists between the activation and inhibition of the fibrinolytic and blood coagulation systems. A blood proactivator activated by streptokinase is postulated. The activator thus formed converts plasminogen or profibrinolysin to plasmin or fibrinolysin which in turn is responsible for fibrin lysis. Various inhibitors are present which act at different stages of the process. These are antistreptokinase, blood activator inhibitor and the anti-plasmins.

Figure III represents these reactions in diagramatic form.
THE FIBRINOLYTIC SYSTEM

TISSUE

[Diagram with arrows indicating flow and interactions between TISSUE and BLOOD components]

TISSUE ACTIVATOR

PLASMINOGEN (PROFIBRINOLYSIN)

PLASMIN (FIBRINOLYSIN)

FIBRIN LYSIS

BLOOD

PROACTIVATOR ANTISTREPTOKINASE

STREPTOKINASE

BLOOD ACTIVATOR

PLASMINOGEN

ALPHA - 1 ANTIPLASMIN

ALPHA - 2 ANTIPLASMIN

BLOOD ACTIVATOR INHIBITOR

FIGURE III
Activators

Natural occurring activators are urokinase, trypsin, streptokinase and staphylokinase. Others include protamine, nicotinic acid, pyrogens, adrenalin and acetylcholine. The natural occurring activators are increased by stress, hypoxia, surgery (especially lung, cardiovascular and hepatic), electroshock, carcinoma of the prostate, cirrhosis and leukemia\textsuperscript{15}.

Inhibitors

Natural occurring inhibitors are antistreptokinase, antistaphylokinase, antiblood activator and the antiplasmins. Antiplasmin exists as two alpha globulins. Alpha-1 globulin antiplasmin reacts slowly with plasmin and the reaction is temperature sensitive. The complex formed is not reversible in the presence of a fibrin substrate. Alpha-2 globulin antiplasmin reacts rapidly with plasmin and the reaction is not temperature dependent. The complex is reversible in the presence of a fibrin substrate\textsuperscript{16}. The amount of antiplasmin available as an inhibitor in the human exceeds by thirty times the potential proteolytic activity of intrinsic plasminogen\textsuperscript{17}.

The intravenous injection of radio-iodinated plasmin is followed by a rapid fall in the plasma plasmin activity but a slow fall in radioactivity. Some localization with subsequent clot lysis occurs. This is taken as an indication of at least a partial reversibility of the antiplasmin-plasmin inhibition system\textsuperscript{17}.

Synthetic inhibitors of the system are soybean trypsin inhibitors, amines (methylamine, laurylamine, quaternary amines), basic amino
acids (epsilon amino caproic acid, lysine and arginine ester), and urea\textsuperscript{15}. The administration of EACA following transurethral prostatectomy results in a four-fold reduction in blood loss\textsuperscript{18}. EACA treated rats retain 77.5\% of an injected dose of radioactive plasma clot at the site of subcutaneous injection while control animals retain only 13.5\% of the injected dose\textsuperscript{19}.

In contrast to the antiplasmin-plasmin reversible complex theory of clot lysis, Sherry et al\textsuperscript{20,22} believe that plasminogen is incorporated into a thrombus or clot at the time of its formation, and that lysis is brought about by activation of this "trapped" plasminogen, either by local or circulating activator. Inhibitors are not thought to be present in a thrombus and the plasmin thus formed is uninhibited in its action.

Future elucidation of the fibrinolytic problem is necessary before well controlled, standardized and effective clinical use of these agents becomes a matter of course.
HISTORICAL REVIEW

The first well documented use of antibodies to fibrin in clinical work occurred in 1953 when the report of Gitlin and Boyers dealing with the study of the metabolism of fibrinogen in two patients with congenital afibrinogenemia. The same group in 1953 studied the localization of homologous plasma proteins in the tissues of young human beings using the Coons' fluorescent antibody technique. These two studies indicated that the half life of circulating fibrinogen is approximately four days and it is found in the extravascular as well as the intravascular space. The antibody to fibrin used in these studies was specific for the species fibrin used as the original antigen. The fluorescent antibody was found limited to the vascular channels, connective tissues and interstitial spaces. None was demonstrated in the cerebral cortex.

In 1956 a group headed by Eugene Day from Buffalo, N.Y., attempted to produce antibodies to the Murphy Strum lymphosarcoma which they were growing in rats. A tumor cell suspension was injected into rabbits and the antisera collected. This is a rapidly growing tumor with a considerable inflammatory reaction in and around its stroma, and therefore contains a considerable amount of fibrin. They observed that the precipitation of their tumor cell suspension by the rabbit antisera in vitro was interfered with by a factor present in cleared ascitic fluid and plasma, but not present in rat serum. Suspecting that the interfering substance was fibrinogen
and that the active antigen in their tissue cell suspension was rat fibrin and not the tumor cells, they immunized rabbits with rat fibrin. Subsequent studies with antifibrin $^{131}$I revealed that tumor $^{131}$I uptake exceeded normal tissue uptake by a factor of fourteen. When normal rabbit gamma globulin $^{131}$I was injected tumor uptake exceeded normal tissue uptake by a factor of three. Rat antifibrin was subsequently purified and radioiodinated to a high degree of specific activity$^{23,24,25}$. Complete and rapid regression of tumors was noted when two to four millicuries of rat antifibrin $^{131}$I were given to tumor bearing rats$^2$. Administration of dog antifibrin $^{131}$I to dogs bearing various tumors resulted in significant uptake in only one$^{26}$. This was a synovial sarcoma which is a very vascular and rapidly growing neoplasm. It was suggested that specific localization of antifibrin $^{131}$I occurred in the tumor, either because of an antigen antibody reaction, or because of the deposition of a performed antifibrin $^{131}$I fibrinogen complex as an inflammatory exudate.
1. Inflammatory reaction of host tissues.

TUMOR GROWTH

2. Blood vessel injury of supporting stroma or invaded tissue.

Deposition of fibrin derived from blood fibrinogen

Deposition in tumor of antibody to fibrin.

(a) secondary to fibrin deposition

(b) As a part of a fibrin antibody complex

Our investigation of the possible use of antifibrin $I^{131}$ for the detection of intravascular thrombi stemmed from the possibility that a specific antigen-antibody system was responsible for the localization reaction.
MATERIALS AND METHODS

I. MATERIALS

(a) Fibrinogen

Fibrinogen was prepared from citrated dog plasma as Cohn's fraction 127,28,29,30,31. One hundred ml. of citrated dog plasma was adsorbed with ten grams of barium sulphate for thirty minutes. Centrifugation at 3000g for thirty minutes was carried out. The Ph of the supernatant was adjusted to 7.2 with either 0.05 molar acetic acid or 0.05 molar disodium phosphate and 50 percent ethanol added to a final concentration of eight percent. All procedures were done at four degrees centigrade. Centrifugation at 3000g for three minutes gave a fibrinogen precipitate. This was washed with eight percent cold ethanol and then dissolved by gentle agitation in citrate saline (one part 0.11 molar sodium citrate to 19 parts 0.154 molar sodium chloride) equal to one third of the original plasma volume. The protein in the final solution was estimated by the micro Kjeldahl method. The clottable protein in the solution was determined as follows32.

One ml. of the fibrinogen solution was added to 30 mls. of 0.85 percent sodium chloride solution containing 1 ml. of M/5 phosphate buffer at pH 6.5 and 10.5 ml. of a one percent calcium chloride solution. To this diluted fibrinogen was added 0.5 ml. of a 50 percent glycerol solution containing 100 units of bovine thrombin (Parke-Davis). The fibrin clot which formed was rolled out on filter paper and the clot protein estimated by the micro
kjeldahl technique. The proportion of clottable protein varied from 75 to 86 percent of the total protein.

(b) Fibrin

One hundred mls. of the fibrinogen solution prepared above was placed in a Waring blender. Five hundred mls. of 0.9 percent sodium chloride and 500 mls. of two percent calcium chloride were added. Ten thousand units of bovine thrombin were added rapidly and just as clotting commenced the blender was turned on. A very fine suspension of fibrin particles formed. The fibrin was washed with saline and stored at four degrees centigrade.

(c) Normal gamma globulin

Rabbits were bled using an aortic catheter into glass centrifuge tubes of 15 ml. capacity. The blood was allowed to clot and incubated overnight at 37°C. The tubes were centrifuged at 3000g for 30 minutes and the serum collected. Subsequent procedures were carried out at -4 degrees centigrade. One hundred mls of the serum was diluted 1 to 3 with distilled water and the pH adjusted to 7.7 with 0.05 molar disodium hydrogen phosphate. A solution of 50 percent ethanol was added to a final concentration of 20% and the precipitate which formed, collected by centrifugation. The precipitate was dissolved in 0.0425 percent saline to a final protein concentration of approximately 1 percent. The pH was now adjusted to between 5.0 to 5.2 with 0.05 molar acetic acid. A solution of 50%
ethanol was added to a final concentration of 10% and the precipitate which formed was removed by centrifugation. The volume of the supernatant was measured and the pH adjusted to 7.3 with 0.05 molar disodium hydrogen phosphate. A final ethanol concentration of 25% was obtained by adding 0.6 ml of 50% ethanol for each ml of the supernatant measured above and one ml of 50% ethanol for each ml of 0.05 molar disodium hydrogen phosphate added during the pH adjustment. The suspension was centrifuged at -10°C and the precipitate collected. The precipitate was dissolved in 50 mls of normal saline.

(d) Immunization schedule and collection of antiserum

Fibrin prepared as described above was suspended in saline at a concentration of 10 mgms per ml. and emulsified volume for volume with Freunds adjuvant. Adult white rabbits with an average weight of three kilograms were used. Four mls. of the emulsion prepared above were given to each rabbit in two different sites in the thigh muscles once a week for four weeks. Ten days after the final injection the rabbits were exsanguinated. This was done by placing a catheter in the abdominal aorta using a midline abdominal incision. Blood was collected in 15 ml centrifuge tubes and allowed to clot. Following incubation and clot syneresis the serum was collected. Approximately sixty mls of serum was collected from each animal.

(e) Purification of fibrin antibodies

Two hundred mls of the rabbit antiserum was incubated with approximately 2 grams of washed dog fibrin for four hours
at 37°C to allow for the formation of an antifibrin-fibrin complex. The suspension was then centrifuged at 3000g for 30 minutes. The precipitate was washed four times with 0.9 percent sodium chloride. The final washing was carried out with 0.9 percent saline, the pH of which had previously been adjusted to 2.0 using one normal hydrochloric acid. The buffering effect of the protein usually raised the pH to about 2.5. The supernatant was collected after centrifugation at 3000g for 15 minutes. The pH of the supernatant was adjusted to 7.2 using a dilute solution of sodium hydroxide. A fine precipitate formed as the pH approached six and was most marked at pH 7.2. This was removed by centrifugation. Approximately 30 mls. of supernatant was obtained and placed in a well washed dialysis sac. Concentration of the solution was achieved by pervaporation in front of a fan in the cold room to a volume approximately one third of the original. The final protein yield was about three mgms. per ml. in a total volume of ten mls.

(f) Iodination of antifibrin

It has been shown that iodination of an immune globulin can be carried out to a level of two atoms of $\text{I}^{131}$ per protein molecule without altering antibody activity$^{37}$. In these experiments a modification of Mc Farlane's jet iodination technique$^{35,36}$ as proposed by Franks et al$^{34}$ was used. The iodination apparatus is shown in Figure IV.
Reagents.

(a) **PH 9.3 glycine buffer**

2.93 grams of sodium chloride and 3.75 grams of glycine were dissolved in 50 mls. of distilled water. Four parts of this solution were mixed with one part of one normal sodium hydroxide.

(b) **Mc Farlane's iodide-iodate solution**

200 mgms. of sodium iodide and 50 mgms. of sodium iodate were dissolved in 50 mls. of distilled water.

(c) **0.25 normal hydrochloric acid**

(d) **1.0 normal hydrochloric acid**

(e) **2 millicuries of carrier free sodium iodide^{131}**
THE IODINATION APPARATUS
Method

Two millicuries of carrier free sodium iodide$^{131}$ were added to tube A and the side arm attached to water trap d. Two or three drops of one normal hydrochloric acid were added to the tube and washed in with a few drops of distilled water. The tube was heated in a boiling water bath for two minutes following which it was immediately immersed in a beaker of ice water. Just before iodination 0.15 to 0.20 ml. of McFarlane's iodide-iodate solution were added to tube A and a brown colour, indicating free iodine obtained.

While the $^{131}$ solution was being prepared three mls. of the protein solution containing a total of nine mgms. of protein were added to the iodination chamber B, and 1.5 ml. of McFarlane's pH 9.3 glycine buffer was added to the protein solution and briefly mixed with the electric stirrer. The top cap was placed on the chamber and a negative pressure of ten pounds developed in reservoir "C" with Tap I open. Tap I was closed, the electric stirrer turned on and Tap 2 opened allowing the $^{131}$ to enter the chamber under negative pressure. Tap 2 was closed as soon as one or two bubbles of air entered the chamber, and Tap I was opened to bring the mixing chamber to atmospheric pressure. One minute later one ml. of 0.25 normal hydrochloric acid was added to the solution in the mixing chamber to bring the pH to neutral.
The contents of the chamber were run into a well washed dialysing sac and the sac placed in a rocking type dialyser. Dialysis against 40 litres of 0.9 percent saline over a twenty-four hour period was carried out. A small precipitate formed during dialysis which was removed by centrifugation. A sample of the solution was diluted one in ten and counted in a well-type scintillation counter which had a counting efficiency of 50 percent. The amount of unbound $^{131}I$ still present after dialysis was estimated by adding one ml. of normal serum and one ml. of a 20 percent trichloracetic acid solution to one ml. of the dilute antifibrin $^{131}I$ solution to precipitate all of the radioactive protein. Following incubation for one hour the suspension was centrifuged and the amount of radioactivity in the precipitate and in the supernatant determined. The latter reflects the amount of unbound $^{131}I$ present in the antifibrin $^{131}I$ solution.

**Results**

0.1 ml of original antifibrin $^{131}I$ solution counted 54,320 c.p.m. The protein precipitate from the antifibrin $^{131}I$ counted 53,885 c.p.m. The supernatant from the antifibrin $^{131}I$ solution counted 421 cp.m.

Percentage of free $^{131}I$ \[ \frac{421}{54,320} \times 100 \approx 0.77\% \]
(g) Studies on the antifibrin solution

Various procedures were carried out to assess the purity and properties of the antifibrin solution.

(1) Electrophoresis

These studies were carried out in the Department of Metabolism of the Montreal General Hospital. A composite graphic representation of the results obtained are seen in Figure V. The protein solutions used were:

(a) Normal rabbit serum
(b) Immune rabbit serum
(c) Immune rabbit serum from which the antibody to fibrin had been removed.
(d) Antifibrin solution
**FIGURE V**

**NORMAL SERUM**
Gamma Globulin = 20%

**IMMUNE SERUM**
Gamma Globulin = 27%

**IMMUNE SERUM**
Minus Antibody
Gamma Globulin = 19.5%

**ANTIBODY SOLUTION**

**COMPOSITE GRAPH OF ELECTROPHORETIC PATTERNS**
Results:

Protein fractions expressed as a percentage of the total

<table>
<thead>
<tr>
<th>Albumin</th>
<th>Alpha-1</th>
<th>Alpha-2</th>
<th>Beta</th>
<th>Gamma Globulin</th>
<th>Grams %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Serum</td>
<td>43.60</td>
<td>4.60</td>
<td>13.62</td>
<td>18.18</td>
<td>20.00</td>
</tr>
<tr>
<td>Immune Serum</td>
<td>39.30</td>
<td>3.08</td>
<td>13.81</td>
<td>16.91</td>
<td>26.90</td>
</tr>
<tr>
<td>Immune Serum less antifibrin</td>
<td>42.40</td>
<td>4.76</td>
<td>13.90</td>
<td>19.47</td>
<td>19.47</td>
</tr>
<tr>
<td>Antifibrin</td>
<td>-------</td>
<td>-------</td>
<td>------------</td>
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</tr>
</tbody>
</table>

The quantitation of an immunological reaction using electrophoresis is fraught with possibilities for error, however, a general qualitative analysis of these results suggests that there is a definite increase in the gamma globulin fraction of immune serum and the extraction procedure removed gamma globulin in approximately the same proportion as it was increased by the immunization procedure. The product of the extraction appears to be about 80% gamma globulin.

2. Incubation of antifibrin solution with fibrin

To assess the amount of antifibrin globulin in the purified antibody solution a known amount of it was incubated with homogenized dog fibrin and the protein removed from the
solution by fibrin estimated. A control normal gamma globulin fraction was also used to rule out non-specific effects.

Results:

Total protein in 3 ml. of antifibrin solution = 6 mgms.
Total protein in 3 ml. of supernatant = 2.16 mgms.
Protein removed by fibrin = 3.84 mgms.
Percentage of protein removed from solution by fibrin = \( \frac{3.84}{6.00} \times 100 = 64\% \)

Total protein in 3 mls. of normal gamma globulin solution = 7.96 mgms.
Total protein in 3 mls. of supernatant = 7.38 mgms.
Total protein removed from solution by fibrin = 0.58 mgm.
Percentage of protein removed by fibrin \( \frac{0.58}{7.96} \times 100 = 7.26\% \)
Percentage of protein in antibody solution which is antifibrin \( 64.00 - 7.26 = 57.74\% \)

(3) Incubation of antifibrin \(^{131}\text{I}\) solution with washed dog fibrin

Two mls. of antifibrin \(^{131}\text{I}\) were incubated with homogenized dog fibrin and the amount of radioactivity remaining in solution after centrifugation determined.
Results:

Two mls. of antifibrin $^1$I$^3$I solution counted 8305 c.p.m.
Fibrin precipitate counted 4690 c.p.m.
Supernatant counted 3600 c.p.m.
Percentage of radioactivity removed from the antifibrin $^1$I$^3$I by fibrin $\frac{4690}{8305} \times 100 = 56.50\%$

(4) Globulin precipitations

Two mls. of a saturated solution of ammonium sulphate were slowly added to 4 mls. of antifibrin $^1$I$^3$I solution to produce a final concentration of 33% ammonium sulphate. After standing in the cold for four hours the solution was centrifuged and the supernatant and precipitate counted.

Results:

Four mls. of antifibrin $^1$I$^3$I solution counted 84,304 c.p.m.
Precipitated globulins counted 74,415 c.p.m.
Supernatant counted 9,889 c.p.m.
Percentage of radioactivity associated with globulins in the antifibrin $^1$I$^3$I solution

$\frac{74,415}{84,304} \times 100 = 88.10\%$

(5) Fibrinogen precipitation

As a further control the conditions of the antifibrin $^1$I$^3$I solution were adjusted with cold ethanol as previously described for the precipitation of fibrinogen. This was done
to rule out the possibility that the non-specific protein in the solution might be fibrin monomer or soluble fibrin polymer eluted from the fibrin-antifibrin complex during the elution procedure.

0.76 ml. of a 50 percent ethanol solution was added to 4 mls. of antifibrin $^{131}$ solution at a temperature of four degrees centigrade. The solution was centrifuged and the precipitate and supernatant counted.

**Results:**

<table>
<thead>
<tr>
<th>Precipitate counted</th>
<th>5,310 c.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant counted</td>
<td>92,215 c.p.m.</td>
</tr>
</tbody>
</table>

Percentage of radioactivity which precipitates as fibrinogen

$$\frac{5,310}{97,525} \times 100 = 4.55\%$$

(6) *Agar Gel precipitation*

These procedures were carried out by the Department of Immunology in the Montreal General Hospital. A saline agar gel was prepared on a number of glass slides and six wells punched in each preparation. Serial dilutions of dog fibrinogen solution were prepared and 200 units of heparin added to each ml. The fibrinogen solution was placed in the centre well and tested against a solution of antifibrin containing 4.19 mgms. of protein per ml. and a solution of normal rabbit gamma globulin containing 1.87 mgms. protein per ml. as control.
Results:

<table>
<thead>
<tr>
<th></th>
<th>Antifibrin</th>
<th>Normal Gamma Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mgm</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>8 mgm</td>
<td>Faint Band</td>
<td>Neg.</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4 mgm</td>
<td>Dense Band</td>
</tr>
<tr>
<td></td>
<td>2 mgm</td>
<td>Faint Band</td>
</tr>
<tr>
<td></td>
<td>1 mgm</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

A dense well defined precipitation band as shown in Figure VI occurred in the agar gel between the well containing one drop of fibrinogen solution in a concentration of four mgm. per ml. and the well containing antifibrin. No reaction occurred between any of the wells containing the normal gamma globulin solution and the well containing antifibrin.

(7) Test tube precipitation studies

One ml of antifibrin solution containing 1.2 mgm of protein was added to one ml. quantities of an increasing dilution of fibrinogen solution. One hundred units of heparin (Parke-Davis) were added to each tube. The test tubes were incubated in a water bath at 37 degrees for eight hours.
FIGURE VI

Agar oil precipitation showing well defined band between the well containing antifibrin and the well containing fibrinogen.
Results:

**Fibrinogen Solution**

<table>
<thead>
<tr>
<th></th>
<th>5 mgm.</th>
<th>2.5 mgms.</th>
<th>1.25 mgm.</th>
<th>0.625 mgm.</th>
<th>0.156 mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antifibrin 1.2 mgm.</strong></td>
<td>++</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Conclusions from the above studies of the antifibrin solution.

Antibodies to dog fibrin were produced in rabbits and purified by an elution technique from an antifibrin-fibrin complex. The eluate gave positive agar gel and test tube precipitation tests when fibrinogen was used as the antigen. The protein of the eluate was approximately 80% gamma globulin and 70% of this was fibrin antibody. The non-gamma globulin fraction of the antifibrin solution did not precipitate when exposed to conditions for fibrinogen precipitation.
II. **METHOD**

Localization of anti-fibrin $^{131}$I on various fibrin substrates

Male and female mongrel dogs weighing between 10 and 20 kilograms were anaesthetized with intravenous nembutol. A thrombus was created in a femoral vein of each dog using the method described by Mersereau. The femoral vein was exposed by an incision over it and dissected free from surrounding structures. A wooden tongue depressor was placed beneath the vein and the vessel emptied of blood. The collapsed vein wall was vigorously traumatized by rubbing it with the handle of a scalpel over the tongue depressor. The vein was dilated digitally and 5 mls. of a one percent polybrene solution instilled around it. The edges of the wound were approximated with a purse string suture and the wound left for two hours. The vein was then opened and its contained thrombus removed and weighed. Two mls. of venous blood in the meantime had been drawn from a forepaw vein and allowed to clot. The clot was incubated at 37°C for two hours to allow for syneresis.

One ml. of an 86 percent clottable solution of fibrinogen was clotted with bovine thrombin and the clot rolled out, washed with saline and weighed. The dogs were then given 5000 units of heparin (Parke-Davis) and the dose was repeated every two hours.

Following the removal of the thrombus an arterio-venous shunt was created between the femoral artery and vein.
of each dog. A siliconized chamber was included in each circuit and the shunt blood flow controlled by a constricting device on the arterial line. The flow was adjusted to approximately 15 mls per minute or 900 mls. per hour. The three fibrin substrates, i.e. the thrombus, clot and fibrin mass were placed in the chambers.

Approximately 2 microcuries of antifibrin $^{131}$I, with a total count of $2 \times 10^6$ c.p.m. using our well type scintillation counter, was given to each dog through a forepaw vein. After four hours of exposure to the circulating radio-active antibody, the three substrates in the chamber were removed and washed with saline. The following samples were also removed and weighed, (a) thyroid gland, (b) limb muscle, (c) urine, (d) whole blood, (e) plasma, (f) traumatized vein wall. All samples were then counted in the counting well.

A four ml. sample of plasma was obtained and the fibrinogen precipitated from it, using the previously described cold ethanol fractionation technique. The fibrinogen precipitate was counted and then redissolved in four mls. of citrate saline prepared as previously described. Five hundred units of bovine thrombin and two mls of a two percent solution of calcium chloride were added, and the fibrin clot which formed homogenized and washed with 0.9% saline. The pH of the solution was adjusted to two with one normal hydrochloric acid to elute the antifibrin $^{131}$I from its complex with fibrinogen. The eluate as well as the fibrinogen precipitate were counted after centrifugation.
One ml. of blood was taken from each animal at the end of the experiment and allowed to clot. The radioactivity of the clot as well as that of the serum was determined, and the percentage of radioactivity removed from a one ml. sample of whole blood by the clotting process calculated.
RESULTS

Results were expressed as the percentage of injected radioactivity removed per gram of sample being measured. Simple multiplication gave the number of counts per minute removed per gram of sample. Because of the variation in weight among the dogs used it was necessary to standardise the results, and these were calculated for a 15 kilogram dog when averages were calculated.

The results for each individual dog appear on the following pages:
DOG 1

Weight: 12 kilograms

Background radiation = 217 c.p.m.
All counts have been corrected for background

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>c.p.m. after four hours</th>
<th>% of injected dose per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombus</td>
<td>55 mgms</td>
<td>2</td>
<td>0.0018</td>
</tr>
<tr>
<td>Clot</td>
<td>925 mgms</td>
<td>83</td>
<td>0.0045</td>
</tr>
<tr>
<td>Fibrin Mass</td>
<td>69 mgms</td>
<td>28</td>
<td>0.0215</td>
</tr>
<tr>
<td>Thyroid</td>
<td>800 mgms</td>
<td>122</td>
<td>0.0076</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>600 mgms</td>
<td>1</td>
<td>0.00083</td>
</tr>
<tr>
<td>Urine</td>
<td>1000 mgms</td>
<td>33</td>
<td>0.0016</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1000 mgms</td>
<td>1345</td>
<td>0.0781</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 mgms</td>
<td>1593</td>
<td>31.8600</td>
</tr>
</tbody>
</table>

One ml. of plasma counts 3023 c.p.m.
Fibrinogen from one ml. of plasma counts 1593 c.p.m.
Percentage of circulating radioactivity associated with fibrinogen \[ \frac{1593}{3023} \times 100 = 53.2\% \]

One ml. of whole blood counts 1563.
Clot from one ml. of whole blood counts 833 c.p.m.
Percentage of radioactivity removed by clotting process \[ \frac{833}{1563} \times 100 = 53.2\% \]
Fibrinogen was precipitated from four mls. of plasma and re-dissolved in four mls. of citrate saline. The solution was clotted with 50 units of bovine thrombin and two mls. of two percent calcium chloride, and the clot homogenized. Elution was carried out as previously described at pH 2.5.

Fibrin clot counted 5336 c.p.m.
Eluate from homogenized clot counted 3196 c.p.m.
Percentage of radioactivity eluted (presumably antibody radioactivity)

\[
\frac{3195}{5336} \times 100 = 59.8\% 
\]
DOG 2

Weight: 10 kilograms

Background radiation = 217 c.p.m.

All counts have been corrected for background

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>c.p.m. after four hours</th>
<th>% of injected dose per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombus</td>
<td>12 mgms</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clot</td>
<td>830 mgms</td>
<td>114</td>
<td>0.0069</td>
</tr>
<tr>
<td>Fibrin Mass</td>
<td>72 mgms</td>
<td>58</td>
<td>0.019</td>
</tr>
<tr>
<td>Thyroid</td>
<td>850 mgms</td>
<td>192</td>
<td>0.013</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>650 mgms</td>
<td>1</td>
<td>0.00077</td>
</tr>
<tr>
<td>Urine</td>
<td>1000 mgms</td>
<td>501</td>
<td>0.0250</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1000 mgms</td>
<td>1972</td>
<td>0.1094</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 mgms</td>
<td>2297</td>
<td>45.90</td>
</tr>
</tbody>
</table>

One ml. of plasma counts 4133 c.p.m.

Fibrinogen from one ml. of plasma counts 2297.

Percentage of circulating radioactivity associated with fibrinogen

\[
\frac{2297}{4133} \times 100 = 55.5\%
\]

One ml. of whole blood counts 2189 c.p.m.

Clot from one ml. of whole blood counts 1311 c.p.m.

Percentage of radioactivity removed by clotting process

\[
\frac{1311}{2189} \times 100 = 59.8\%
\]
Fibrinogen was precipitated from 3.5 mls. of plasma and re-dissolved in four mls. of citrate saline. The solution was clotted with 50 units of bovine thrombin and two mls. of two percent calcium chloride, and the clot was homogenized. Elution was carried out as previously described at pH 2.5.

Fibrin clot counted 7694
Eluate from homogenized fibrin clot counted 4283 c.p.m.
Percentage of radioactivity eluted (presumably antibody)

\[
\frac{4283}{7694} \times 100 = 55.6\%
\]
DOG 3  
Weight: 19 kilograms

Background radiation = 215 c.p.m.

All counts have been corrected for background.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>c.p.m. after four hours</th>
<th>% of injected dose per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombus</td>
<td>76 mgms</td>
<td>12</td>
<td>0.0079</td>
</tr>
<tr>
<td>Clot</td>
<td>716 mgms</td>
<td>158</td>
<td>0.0110</td>
</tr>
<tr>
<td>Fibrin Mass</td>
<td>97 mgms</td>
<td>76</td>
<td>0.0390</td>
</tr>
<tr>
<td>Thyroid</td>
<td>970 mgms</td>
<td>144</td>
<td>0.0075</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>284 mgms</td>
<td>25</td>
<td>0.0043</td>
</tr>
<tr>
<td>Urine</td>
<td>1000 mgms</td>
<td>585</td>
<td>0.0292</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1000 mgms</td>
<td>1030</td>
<td>0.0515</td>
</tr>
<tr>
<td>Vein Wall</td>
<td>560 mgms</td>
<td>630</td>
<td>0.0566</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 mgms</td>
<td>1262</td>
<td>25.24</td>
</tr>
</tbody>
</table>

One ml of plasma counts 2290

Fibrinogen from one ml. of plasma counts 1262

Percentage of circulating radioactivity associated with fibrinogen

\[
\frac{1262}{2290} \times 100 = 55\%
\]

One ml of whole blood counts 1030

Clot from one ml. of whole blood counts 584

Percentage of radioactivity removed by clotting process.

\[
\frac{584}{1030} \times 100 = 56.6\%
\]
DOG 4  
Weight: 17 kilograms

Background radiation = 215 c.p.m.

All counts have been corrected for background

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>c.p.m. after four hours</th>
<th>% of injected dose per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombus</td>
<td>50 mgms</td>
<td>8</td>
<td>0.008</td>
</tr>
<tr>
<td>Clot</td>
<td>468 mgms</td>
<td>134</td>
<td>0.014</td>
</tr>
<tr>
<td>Fibrin Mass</td>
<td>62 mgms</td>
<td>20</td>
<td>0.016</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1155 mgms</td>
<td>92</td>
<td>0.004</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>432 mgms</td>
<td>56</td>
<td>0.0065</td>
</tr>
<tr>
<td>Urine</td>
<td>1000 mgms</td>
<td>1308</td>
<td>0.0654</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1000 mgms</td>
<td>1112</td>
<td>0.0556</td>
</tr>
<tr>
<td>Vein Wall</td>
<td>972 mgms</td>
<td>1080</td>
<td>0.0555</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 mgms</td>
<td>1184</td>
<td>23.68</td>
</tr>
</tbody>
</table>

One ml. of plasma counts 2230

Fibrinogen from one ml. of plasma counts 1184

Percentage of circulating radioactivity associated with fibrinogen.
\[
\frac{1184}{2230} \times 100 = 53.1\%
\]

One ml. of whole blood counts 1112

Clot from one ml. of whole blood counts 602

Percentage of radioactivity removed by the clotting process
\[
\frac{602}{1112} \times 100 = 54.1\%
\]
**DOG 5**  
Weight: 20 kilograms

Background radiation = 215 c.p.m.

All counts have been corrected for background

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>c.p.m. after four hours</th>
<th>% of injected dose per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>853 mgms</td>
<td>184</td>
<td>0.0108</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>1062 mgms</td>
<td>112</td>
<td>0.00526</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1000 mgms</td>
<td>952</td>
<td>0.0476</td>
</tr>
<tr>
<td>Vein Wall</td>
<td>302 mgms</td>
<td>200</td>
<td>0.0331</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 mgms</td>
<td>905</td>
<td>18.1</td>
</tr>
</tbody>
</table>

One ml of plasma counts 1704

Fibrinogen from one ml. of plasma counts 905

Percentage of circulating radioactivity associated with fibrinogen

\[
\frac{905}{1704} \times 100 = 53\%
\]
**Background radiation = 215 c.p.m.**

**All counts have been corrected for background**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>c.p.m. after four hours</th>
<th>% of injected dose per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>698 mgms</td>
<td>96</td>
<td>0.00690</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>772 mgms</td>
<td>16</td>
<td>0.00103</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1000 mgms</td>
<td>960</td>
<td>0.04800</td>
</tr>
<tr>
<td>Vein Wall</td>
<td>127 mgms</td>
<td>220</td>
<td>0.08650</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 mgms</td>
<td>1080</td>
<td>21.60000</td>
</tr>
</tbody>
</table>

One ml. of plasma counts 1986

Fibrinogen from one ml. of plasma counts 1080

Percentage of circulating radioactivity associated with fibrinogen

\[
\frac{1080}{1986} \times 100 = 54.5\% 
\]
<table>
<thead>
<tr>
<th>Dog</th>
<th>Limb Dog Muscle</th>
<th>Thyroid</th>
<th>Urine</th>
<th>Fibrinogen</th>
<th>Clot</th>
<th>Thrombus</th>
<th>Blood Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000664</td>
<td>0.00608</td>
<td>0.00128</td>
<td>0.0182</td>
<td>0.00360</td>
<td>0.00144</td>
<td>0.0781</td>
</tr>
<tr>
<td>2</td>
<td>0.000505</td>
<td>0.00850</td>
<td>0.01660</td>
<td>0.0125</td>
<td>0.00460</td>
<td>0.0000</td>
<td>0.0730</td>
</tr>
<tr>
<td>3</td>
<td>0.00500</td>
<td>0.00950</td>
<td>0.03700</td>
<td>0.0495</td>
<td>0.01390</td>
<td>0.01000</td>
<td>0.0650</td>
</tr>
<tr>
<td>4</td>
<td>0.00735</td>
<td>0.00450</td>
<td>0.07400</td>
<td>0.0180</td>
<td>0.01580</td>
<td>0.00910</td>
<td>0.0630</td>
</tr>
<tr>
<td>5</td>
<td>0.00700</td>
<td>0.01440</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0635</td>
</tr>
<tr>
<td>6</td>
<td>0.00137</td>
<td>0.00920</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0640</td>
</tr>
</tbody>
</table>

UPTAKE OF RADIOACTIVITY EXPRESSED AS THE PERCENTAGE OF INJECTED DOSE REMOVED PER GRAM OF SAMPLE AND STANDARDIZED FOR A 15 KILOGRAM DOG.
### TABLE II

Average percentage of injected dose and average c.p.m. removed per gram of sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of injected dose removed per gram</th>
<th>C.p.m. per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>27.93000</td>
<td>558,600</td>
</tr>
<tr>
<td>Vein Wall</td>
<td>0.07320</td>
<td>1464</td>
</tr>
<tr>
<td>Blood</td>
<td>0.06770</td>
<td>1354</td>
</tr>
<tr>
<td>Urine</td>
<td>0.03220</td>
<td>644</td>
</tr>
<tr>
<td>Fibrin Mass</td>
<td>0.02430</td>
<td>486</td>
</tr>
<tr>
<td>Blood clot</td>
<td>0.00950</td>
<td>190</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.00869</td>
<td>174</td>
</tr>
<tr>
<td>Thrombus</td>
<td>0.00513</td>
<td>103</td>
</tr>
<tr>
<td>Limb Muscle</td>
<td>0.00365</td>
<td>73</td>
</tr>
</tbody>
</table>
TABLE III

Percentage of plasma radioactivity associated with circulating fibrinogen.

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.2</td>
</tr>
<tr>
<td>2</td>
<td>55.5</td>
</tr>
<tr>
<td>3</td>
<td>55.0</td>
</tr>
<tr>
<td>4</td>
<td>53.1</td>
</tr>
<tr>
<td>5</td>
<td>53.0</td>
</tr>
<tr>
<td>6</td>
<td>54.5</td>
</tr>
</tbody>
</table>

AVERAGE = 54.05 percent
TABLE IV

Percentage of whole blood radioactively removed by the clotting process.

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.2</td>
</tr>
<tr>
<td>2</td>
<td>59.8</td>
</tr>
<tr>
<td>3</td>
<td>56.6</td>
</tr>
<tr>
<td>4</td>
<td>54.1</td>
</tr>
</tbody>
</table>

AVERAGE = 55.92 percent
DISCUSSION

From the results obtained it appears that the in vivo localization of a rabbit anti-dogfibrin antibody does not occur on a fibrin substrate (Tables I and II). This observation is not at all unexpected for the following reasons. Fibrinogen is a normal plasma protein with a concentration which varies between 200 and 500 mgm. per 100 mls. of dog plasma. Fibrin monomer is formed by the splitting off of two fibrinopeptides from the end and side of the fibrinogen molecule. The specific antigenic properties of a protein molecule are related to its size, geometric configuration and the presence of polar or determinant groups. In order for specific antigenic difference between fibrinogen and fibrin to occur, determinant groups present on the fibrin molecule but not present on the fibrinogen molecule would have to be exposed by the initial hydrolysis of the fibrinopeptides.

Since fibrin (polymer) is insoluble it must be phagocytosed or lysed into soluble components by the reticulo-endothelial system of the recipient animal before antibodies can be formed against it. This process could involve lysis at hydrogen or disulphide bonds as well as possible transmolecular breakdown. The resulting proteins might be made up of a combination of fibrin monomer plus moieties of lower molecular weight, but still similar antigenically to groups on the original fibrinogen molecule.
The problem thus resolves itself into one of crossed antigenicity between a monomer and its polymer. It appears from our results that in the case of fibrinogen versus fibrin specificity does not occur to any degree, and the cross reaction is such that almost all of the antifibrin $^{131}$I reacts with circulating fibrinogen which is present in a gross amount compared to the fibrin substrate used as a challenge.

Although only 57 percent of the radioactivity in the antifibrin $^{131}$I solution was associated with active antibody, the finding that 53 to 55 percent of the circulating radioactivity precipitated with the fibrinogen fraction suggests that almost all the antifibrin $^{131}$I reacted with circulating fibrinogen (Table III). That this was not a non-specific effect may be ruled out by the observation that in vitro studies showed that only five percent of the radioactivity in the antifibrin $^{131}$I solution precipitated when the cold ethanol method for fibrinogen precipitation was applied to the solution. Also since fibrinogen amounts to only four percent of plasma protein, it is fair to assume that an antigen-antibody system is responsible for its association with 54 percent of the plasma radioactivity when antifibrin $^{131}$I is injected.

Further evidence to support the hypothesis that an antifibrin $^{131}$I fibrinogen complex forms is the observation that radioactive protein could be eluted from a homogenized clot formed from the redissolved fibrinogen-antifibrin $^{131}$I precipitate. The fact that only 55.6 to 59.8 percent of the radioactivity was recoverable probably means that some antibody was trapped in the
interstices of the clot particles and was not exposed to the forces of the elution procedure.

It was observed that between 53.2 and 59.8 percent of the radioactivity of whole blood was removed by the clotting process (Table IV). Since the protein content of serum is similar to that of plasma except for the loss of fibrinogen, further suggestive evidence of the existence of an anti-fibrin $^{131}$I-fibrinogen complex becomes apparent.

The presence of an antibody complexed with a fibrinogen molecule did not seem to interfere with the polymerization of fibrinogen during the clotting process. However, the concentration of antibody in our experiment, compared to that of fibrinogen, was indeed small and larger concentrations of antifibrin associated with fibrinogen do prolong the clotting time$^2$.

The localization of an antibody to rat fibrin in certain rat tumors, probably is non-specific and results from the inflammation with increased blood vessel permeability engendered by the tumor. It does not appear that antigen antibody forces between fibrin and antifibrin play a very significant role. The fact that only tumors which elicit an appreciable inflammatory reaction in the surrounding host tissues, and within themselves show the localization phenomenon is further evidence that inflammation is the key to the localization phenomenon.

The explanation of the sequence of events is probably as follows. Antifibrin $^{131}$I when injected into the circulation
of a tumor bearing rat, forms a complex with circulating fibrinogen. Fibrinogen is an important component of an inflammatory exudate having a short half life of 62 to 65 hours in the normal animal. The half life is probably reduced in the experimental animal which is continuously forming a fibrinous exudate. The antifibrin $\text{I}^{131}$ - fibrinogen complex leaves the circulation at the tumor site because of increased vascular permeability and hemorrhage, and a local build up of radioactivity occurs. Because of the rapid turn-over of fibrinogen in a relatively short period of time, most of the antifibrin $\text{I}^{131}$-fibrinogen complex appears in the tumor.

If this hypothesis is true fibrinogen labelled with $\text{I}^{131}$ should, on injection, localize to almost the same degree as an antifibrin $\text{I}^{131}$-fibrinogen complex. It has been shown by Bale that fibrinogen $\text{I}^{131}$ does in fact localize in Murphy Sturm lymphosarcoma in rats, and its localization is doubled if epsilon amino caproic acid is administered simultaneously to prevent the lysis of fibrinogen $\text{I}^{131}$ once it appears in the tumor.

For our purpose it would appear that a radioactive antibody to fibrin would not be of value for the in vivo detection and localization of blood clots or thrombi because of the presence of antigenically similar circulating fibrinogen.
CONCLUSIONS

1. Antibodies to dog fibrin were readily produced in rabbits.
2. Five percent of the total protein in the rabbit antisera was antifibrin globulin.
3. Because of the insolubility of fibrin, extraction of antifibrin from the antisera and its subsequent purification is a simple procedure using an elution technique.
4. In our hands radio-iodination of the antifibrin globulin was a difficult procedure, probably due to the small quantity of protein available.
5. Antifibrin $^1{\text{I}}_{31}$ when given to dogs cross reacted with circulating fibrinogen.
6. With small quantities of antifibrin there is no impairment to the polymerization of fibrinogen.
7. Whole blood carried approximately twenty times as much radioactivity per unit weight as a performed thrombus after a four hour exposure to circulating antifibrin $^1{\text{I}}_{31}$.
8. Whole blood carried approximately seven times as much radioactivity per unit weight as a preformed blood clot after a four hour exposure to circulating antifibrin $^1{\text{I}}_{31}$.
9. Antifibrin $^1{\text{I}}_{31}$ appears to localize in an area of inflammation by virtue of its association with circulating fibrinogen, and not because of an antifibrin-fibrin reaction at the site of a fibrin substrate.
10. Specific localization of an $^1{\text{I}}_{31}$ labelled fibrin antibody does not occur on a thrombus to a sufficient degree to allow its detection by surface scanning techniques.
SUMMARY

Antibodies to dog fibrin were produced in rabbits. Antifibrin was extracted by elution at an acid pH from an antifibrin-fibrin complex. Radio-iodination of antifibrin was carried out by a modification of the jet iodination technique. It was found that antifibrin on injection cross reacted with circulating fibrinogen, and there was no selective localization on a fibrin substrate when the latter was exposed to the circulating blood for a period of four hours. Significant localization did occur in traumatized vein wall presumably by virtue of the inflammatory reaction induced. It was concluded that the localization of antifibrin $^{131}$I is specific only in so far as it becomes part of an inflammatory exudate along with circulating fibrinogen. Antifibrin $^{131}$I therefore is not of clinical use for the detection of intravascular thrombosis.
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