THE ACTION OF TISSUE EXTRACTS IN THE COAGULATION OF BLOOD.*

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It has often been demonstrated that blood drawn from a vessel without contact with any injured tissue surface and kept in such a manner as to prevent destruction of the blood corpuscles, exhibits a considerable delay in its coagulation. Bird blood especially will remain fluid almost indefinitely when drawn and kept under such conditions. If, however, the escaping blood flows over an injured tissue surface, or the corpuscles are broken up as by whipping, it is found that the blood exhibits a much more prompt coagulation. The addition of saline extracts of tissues, especially such tissues as the lungs and brain, accelerates the clotting still further, it being possible thus to shorten the coagulation time to a small fraction of a minute.

There is evidently then in tissues and cells in general a substance which possesses the property of quickening the clotting of the blood. This substance has been variously named. Wooldridge (1), who was the first to study the nature and action of it, held that it was a protein-phospholipin compound and that in the process of coagulation it reacted with the fibrinogen to form fibrin, an actual union occurring between the two substances with a loss of phospholipin. Since the tissue material entered into the formation of the fibrin, he termed it tissue fibrinogen. He also showed that there was a substance in the blood itself which was practically identical with the tissue fibrinogen, and this he termed A-fibrinogen. The reaction of this with the ordinary fibrinogen of the blood which he termed B-fibrinogen, resulted in the forma-

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tion of fibrin. Precipitation of this A-fibrinogen by letting the plasma stand in an ice box for some time destroyed the spontaneous coagulability of the plasma. Addition of a new supply of the substance or of the tissue fibrinogen restored the power of clotting to such plasma. Wooldridge was also the first to show that the intravenous injection of the tissue fibrinogen in sufficient amounts would upset the equilibrium existing in the blood between the A-fibrinogen and B-fibrinogen and result in the formation of fibrin in the vessels, causing death from thrombosis. The fluid blood remaining after such intravascular coagulation showed a delayed coagulation time, or absolute non-coagulability. The increased coagulability of the blood he termed the positive phase of coagulation, while the tendency toward non-coagulability was called the negative phase. The negative phase was also produced by sublethal injections of tissue fibrinogen without the formation of solid clots to any great extent, and he considered this to be a protective reaction on the part of the blood itself or of the vascular endothelium. Wright (2), following up the work of Wooldridge, considered the negative phase to be due to the partial digestion or hydrolysis of the tissue fibrinogen in the blood with the liberation of albumoses and peptones, these bodies being more directly responsible for the negative phase reaction on the part of the vascular system. His basis for this conclusion was that the urine always gave a reaction for albumoses after injection of tissue extracts and also that in pneumonia there occurred a delayed coagulation of the blood at the same time that albumoses and peptones were being excreted in the urine, the origin of these bodies being from the resorption of the imperfectly digested exudate in the lungs. Since the direct injection of such albumoses or peptones into the blood was known to produce varying degrees of non-coagulability in the blood of dogs, Wright considered the process here to be of the same order.

It would not be practical to review in detail all the more recent work on the action of tissue extracts, so only a few of the more important articles will be mentioned here. Morawitz (3) and Fuld and Spiro (4) held that the tissue substance acted in the rôle of a kinase to initiate and hasten the formation of thrombin from the calcium and prothrombin of the plasma. Fuld and Spiro termed the substance cytozyme while Morawitz called it thrombokinase. Many workers have since accepted this theory of the action of the tissue material and based upon it their explanations of ex-
experimental observations. Nolf (5) and Howell (6) hold views somewhat different from the above. Nolf believes that fibrinogen is held in solution by being combined with a substance, hepatothrombin, produced by the liver, and that the breaking of this union by removal of the hepatothrombin permits the fibrinogen to precipitate as insoluble fibrin. The hepatothrombin may be removed by a substance derived from the leucocytes and hence called leucothrombin. Howell considers that tissue extracts act by neutralizing the antithrombin present, the antithrombin acting to prevent the interaction of prothrombin and calcium in the formation of thrombin. On neutralizing the antithrombin, then, the thrombin formation proceeds and clotting results. He called the substance thromboplastin or thrombo-plastic substance. Loeb (7) terms the active tissue substance coagulin because he considers it to act in the same manner as thrombin. Thus he finds that it will clot a solution of fibrinogen in the presence of calcium, presumably without the presence of any prothrombin. Mellanby (8), however, has brought forth proof that the prothrombin is very intimately associated with the fibrinogen and probably is not removed in the purification of the fibrinogen.

According to the theories of Morawitz, Fuld and Spiro, Nolf, and Howell, the tissue substance is not concerned in the formation of fibrin except indirectly in the formation of thrombin. That is, it is not a fibrinogen in the sense that it enters directly into the formation of the fibrin. Wooldridge, on the other hand, considered it to be a true tissue fibrinogen, furnishing a part of the material that entered into the fibrin molecule. The present work will be found to support Wooldridge's work in this, as in many other respects, although differing from it in certain important points. Most attention in this work, however, was paid to the effects of tissue extracts on the blood in vivo, especially to determining the nature of the negative phase.

EXPERIMENTAL.

1. Relation of Concentration of Active Material to Time of Clotting.

In a previously published article (9) the author showed that different tissues of the body vary in their content of material active in hastening the clotting of blood. Lung extract was found to be considerably stronger in this respect than extracts of any other tissues made in a similar manner, so it was chosen for use in most of this work. The method found most efficient for testing the activity of these extracts, and that which most nearly approximates the normal conditions of blood coagulation was the use of fresh oxalated blood. Upon recalcification with the optimum amount of CaCl₂, clotting occurred in fresh oxalate plasma in approximately the same time as in normal blood drawn from a vein and let stand. Standing in the ice box causes a gradual
lengthening of the coagulation time of the oxalate plasma, the
time ranging from 3 to 15 minutes during 2 to 4 weeks standing,
the reason for this apparently being a gradual precipitation of the
A-fibrinogen of Wooldridge which is essential for spontaneous
coagulation. Addition of very small amounts of this substance
or of tissue fibrinogen shortens the coagulation time, the shorten-
ing bearing a definite relation to the amount of tissue substance
added.

The plasma used throughout this work as normal plasma was
obtained from the abattoir by letting the blood flow directly
from the slaughtered animals into a vessel containing sufficient
potassium oxalate to make the whole amount collected about
0.5 per cent. This made the plasma alone, after centrifuging,
about 0.8 per cent oxalate, and this gave too heavy a precipitate
of calcium oxalate on recalcification to make a reliable test fluid
for accurate coagulation time determinations. To such cell-free
plasma CaCl₂ was added in amount just below that necessary
for clotting, and the precipitate of calcium oxalate removed by
sedimentation in the ice box and then by centrifuging out the
last traces. This resulted in a fairly clear plasma which required
only a small amount of CaCl₂ to induce clotting in a normal
manner. It was found necessary to put so much oxalate in at
the time of the drawing of the blood because it was mostly done
by inexperienced hands, and with smaller amounts of oxalate
there frequently occurred clotting due to insufficient mixing of
the oxalate with the blood. The plasma finally used for the test
required usually about 0.2 cc. of 1 per cent CaCl₂ per cc. of plasma
for optimum recalcification. Such a plasma forms a very delicate
medium for detecting the presence of any amount of active
coagulant material.

Table I in the preceding paper (10) giving dilution of lung
extract shows clearly that the shortening of the coagulation time
bears a definite relation to the concentration of the tissue extract.

The lung extract used in these dilution tests was a saturated
solution made by extracting dried ground beef lung tissue with
0.9 per cent NaCl solution (4 cc. of salt solution per gm. of dried
lung tissue). Fresh beef lungs were thoroughly washed in tap
water, hashed in a meat chopper, spread on a glass plate in a
thin layer, and dried in a warm air current for 24 hours. This
drying caused a loss in weight of about 76 per cent, but did not affect the blood-coagulating properties of the active material as was determined by comparative tests. The dried material was now powdered and extracted with 0.9 per cent NaCl solution in the proportion stated above. The use of less salt solution per gm. of tissue did not increase the activity of the solution so saturation was probably complete. Only a few minutes standing was necessary to obtain this saturation, after which all solid tissue particles were removed by centrifuging the solution for 20 minutes at about 3,000 revolutions per minute.

The relation of coagulation time to the dilution of the extract may be represented as shown in Chart 1. The plot of the logarithm of the dilution against the logarithm of the clotting time is nearly a straight line.

Considering the line in Chart 1 to be a straight line, the following formula was very kindly suggested by Dr. Mathews:

\[ \log x = 3.4 \log y - 3.4 \]

where \( x \) represents the dilution of the extract, and \( y \) the coagulation time in seconds. By using this formula it would be possible to express the amount of active material in any extract in terms of lung extract, merely by determining its degree of acceleration of the normal coagulation time, and, letting the observed coagulation time equal \( y \), calculate \( x \). However, it is readily seen that this would only hold good for tests in which the normal clotting time of the plasma used was 250 seconds. If it differed from this figure, which it would do in all likelihood, an approximate value could be arrived at by simple proportion.

Thus, if an extract of unknown strength is tested on oxalate plasma having a normal clotting time of 5 minutes, i.e. 300 seconds, and it is found that 0.04 cc. of the extract (or about 1 drop) accelerates the clotting time to 80 seconds, the strength of the extract as compared to saturated lung extract could be found as follows:

\[ \frac{300}{250} : \frac{80}{y} \]

\[ y = 67 \text{ seconds} \]

Substitute this value of \( y \) in the formula

\[ \log x = 3.4 \log y - 3.4 \]
\[ \log x = (3.4 \times 1.82607) - 3.4 \]
\[ \log x = 2.80864 \]
\[ x = 644 \]
That is, the extract tested is equivalent in strength to saturated lung extract diluted with 643 volumes of 0.9 per cent NaCl solution.

**CHART 1.**

1. Logarithm of the dilution of lung extract = log x
2. Logarithm of the coagulation time in seconds = log y

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**Coagulation of Blood**

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A method of arriving at a quantitative expression of the amount of active substance in any solution would be to define arbitrarily a unit of the substance as that amount present in a certain amount of a given dilution of lung extract, as, for instance, in 0.04 cc. of lung extract diluted 100,000 times, and then express the activity of other solutions in terms of this unit. However, it is hoped that a definite statement can be made very soon as to exactly how much active substance by weight is present in any strength of extract, and thus save establishing any arbitrary unit of measuring the activity. 

Since the preparation of this manuscript, experiments have been carried out to determine the concentration of active tissue substance in solutions of known effect on hastening coagulation of blood. For this determination, about 300 gm. of fresh beef lungs were hashed, ground well with sand, and extracted twice with 0.9 per cent NaCl solution. These two extracts, after removal of all solid particles by sedimentation in the ice box over night, were acidified with 0.5 N H₂SO₄ to make a calculated acidity of 0.002 N. The resulting precipitate of the active substance was washed free of other materials, as described in a later section of this paper. The active material, thus purified, was not dried, but was redissolved in 0.9 per cent NaCl solution, 0.5 N NaOH being added to give a calculated concentration of 0.002 N to aid the resolution of the material. This solution of the active principle diluted to the same volume as the combined original extracts possessed the same amount of activity in hastening coagulation as did the combined extracts before precipitation of the active substance.

Duplicate determinations of the N content of the solution were now made in order to compute the concentration of the active material present. In the preceding paper it is shown that the active substance contains 10.7 per cent N. Using this figure as a basis, the average of the two determinations gave 4.16 mg. of active substance per cc. of solution.

This solution was now tested for its accelerating influence on clotting, and the coagulation time was found to increase almost exactly the same, with dilution, as it did in diluting lung extract, as given in Table I of the preceding paper. Apparently it was about one-fourth as strong, undiluted, as was the saturated lung extract used in the tests recorded in Table I (10).

The saturated lung extract used contained about 4 × 4.16 = 16.64 mg. of active substance per cc. of solution. Diluted 65,536 times, at which point the last trace of acceleration of clotting was in evidence, there was then 16.64 ÷ 65,536 = 0.000256 mg. of active substance per cc. of the diluted solution. 0.04 cc. of this was added to 1 cc. of oxalate plasma in the test, so that this final concentration in the plasma was 0.000256 ÷ 25 = 0.00001024 mg. per cc., or approximately 1 part of active substance to 100,000,000 parts of plasma.
2. Is the Active Tissue Substance a True Tissue Fibrinogen?

In all the tests with active tissue extracts the clots formed were very much firmer and more resistant to breaking up on shaking than were clots formed from the same plasma without tissue extract addition. This suggested that this active material not only hastened the clotting of the plasma, but also increased the fibrin yield. Schmidt (11), in 1872, described what he termed a "fibrinoplastic substance" which he obtained from blood plasma by dialysis or treatment with weak acid, and which probably corresponded, in part at least, to the active material from the tissues. He claimed to obtain as high as 1 per cent yield from beef plasma, which many times exceeds the amount the writer has been able to demonstrate in such plasma. However, Schmidt demonstrated increases in fibrin yields from given plasmas of from 10 to 30 per cent, under the influence of the addition of different amounts of his "fibrinoplastic substance." Rettger (12), in 1909, showed that from a given fibrinogen solution varying amounts of fibrin could be produced by adding varying amounts of thrombin. However, he says that probably not all the fibrinogen had been changed over to fibrin with the addition of the smaller amounts of thrombin, thus showing that the thrombin acts in a quantitative manner, rather than that it actually increases the amount of fibrin to be obtained from a given amount of blood fibrinogen.

A number of experiments have been carried out to determine the effect of tissue extract addition on increasing the fibrin yield from a given plasma, and results showing increases up to 156 per cent in the fibrin yield have been produced by varying the amount of active tissue substance added. A number of other substances, such as starch paste, boiled dilute egg albumin, and milk, have also been added to plasma in order to see if such inactive solutions or suspensions would increase the weight of the fibrin to be obtained. Thus, a 0.4 per cent starch suspension (boiled) mixed with citrated horse plasma in equal volumes causes only 3.66 per cent increase in the fibrin yield. Out of 1.6 gm. of starch so added, only 0.0868 gm. was retained in the fibrin after thorough washing with 0.9 per cent NaCl solution and distilled water. With the use of lung extract, containing 1.8
per cent of proteins, in the same way, an increase of 152 per cent in the fibrin yield was obtained, 1.641 gm. of the lung material out of 5.448 gm. being bound in the fibrin. Fresh egg white, which is without any marked effect on the clotting time of horse plasma, was diluted with five volumes of distilled water, filtered, and boiled. A milky opalescent suspension resulted, acting like lung extract as far as passing through a filter is concerned; that is, a few cc. pass through readily and then the pores of the filter become blocked and filtration practically ceases. Such a suspension of boiled egg white, containing 2.13 per cent of solids, was added to horse plasma and the fibrin yield observed. An increase of 39.5 per cent in the fibrin occurred, 0.466 gm. of the egg albumin being bound out of 6.366 gm. added.

These experiments, together with many more, will be reported more in detail in the near future. It is greatly to be desired that definite proof be found whether the active coagulant of tissue extracts produces the increase in fibrin by chemically uniting with the latter, or is merely held mechanically due to its coarse state of dispersion in the extracts. Further experiments on this subject are at present being conducted with the aid of Mr. G. M. Guest.

The few results cited above seem to indicate strongly that the active coagulant of lung extract is present in the fibrin in some other form than as mechanically held material. That is, the substance appears to be a true tissue fibrinogen, the name given to it many years ago by Wooldridge. If it does enter chemically into the fibrin formation, such a fact would be difficult to explain under the theories of coagulation that are commonly accepted at present.

3. Tissue Fibrinogen and Blood Fibrinogen Unite only in the Presence of Calcium.

The addition of tissue fibrinogen to oxalate plasma does not result in the formation of fibrin until the plasma has been recalcified, nor does the active material appear to be bound in any way, since its activity persists for days and weeks and it may be precipitated out in active form by making the plasma very weakly acid (10).
Experiments were also tried on the blood of rabbits in vivo to see if the blood could be rendered non-coagulable by decalcification and death from lung extract injection thus be avoided.

Attempts were therefore made to decalcify the blood of rabbits by intravenous injections of potassium oxalate and sodium citrate. The former proved too toxic to permit of even close approach to complete decalcification. After the injection of as much oxalate as the rabbit could withstand, thrombosis resulted when lung extract was injected. The blood of a rabbit completely prostrated by oxalate, clots in the normal time after withdrawal from the vessels, so that this salt is not at all suitable for intravenous use as desired here. Sodium citrate, on the other hand, is much less toxic, as high as 9 cc. of a 5 per cent solution having been injected into an 1,800 gm. rabbit intravenously over a period of 15 minutes, with only spasmodic tremors and twitchings resulting. The following protocol of an experiment will serve to show the effects of lung extract injection after such citrate treatment.

Rabbit, male, 1,800 gm. 9 cc. of 5 per cent sodium citrate injected intravenously over a period of 15 minutes, keeping the rate of injection just below that necessary to produce muscular twitching or evolutions.

This was followed at once by an injection of 1 cc. of lung extract (saturated). Lethal dose of this extract in a normal rabbit was less than 0.1 cc. Spasms occurred 20 seconds after the extract injection, and the rabbit was dead in 1½ minutes.

Immediate examination for clots showed only slight fibrin strings suspended from the chorda tendinae of the right heart. Remainder of blood was fluid, and a sample of it kept in the ice box showed only a slight clot in 24 hours. To another sample was added 1 drop of serum. Clotting occurred in 3 hours. To a third sample were added 2 drops of 1 per cent CaCl₂ which resulted in coagulation in 15 minutes.

40 cc. of the blood were oxalated to 0.5 per cent and centrifuged to obtain a clear plasma. The following tests were performed on this plasma:

1 cc. plasma + 2 drops 1 per cent CaCl₂ → clot in 65 sec.
1 cc. normal beef oxalated plasma + 2 drops of 1 per cent CaCl₂ → clot in 6 min.
1 cc. normal beef oxalated plasma + 5 drops of rabbit plasma + 2 drops of 1 per cent CaCl₂ → clot in 65 sec.

The rabbit plasma contained plenty of fibrinogen to form a normal clot, but was lacking in calcium. Therefore the decalcification by the citrate injection was almost complete. The rabbit plasma contained sufficient tissue fibrinogen to accelerate
markedly the clotting of normal beef plasma. Whether the slight clots which were found were sufficient to cause death cannot be stated. There is also the possibility that something else in the tissue extract, other than the tissue fibrinogen, might have caused the death of the animal.

4. Results of Intravenous Injections of Tissue Extracts.

Since the work of Wooldridge and of Wright on intravascular coagulation following injections of tissue fibrinogen little has really been added in this field, except repeated demonstrations that thrombin injections will not cause coagulation. Günemann (13) found that rabbits killed by single injections of rabbit lung extract exhibited the negative phase of coagulation in the portion of the blood remaining fluid, and that this non-coagulability was due to a decrease in fibrinogen, the amount being decreased nine to eleven times. He supposed that this loss was to be accounted for by absorption of the fibrinogen by the clots formed in the vessels. Mellanby (14) showed that certain snake venoms were strongly coagulative for blood and that slow repeated injections of very small amounts rendered the blood of animals non-coagulable (negative phase) by gradual removal of the fibrinogen as fibrin, but without solid clot formation. It will be shown here that tissue extracts possess the same power of defibrinating the blood.

All the lung extracts used in the following injections were of about equal strength, ten parts by weight of 0.9 per cent NaCl solution being used for each gm. of lung tissue taken. This gives a much weaker extract than that used in the preceding section to study the effect of dilution. These extracts possess about one-thirty-second of the activity of the saturated extract.

A. Single Injections of Lethal Doses.—Injections of large doses of lung extract, that is 1 cc. or more, into rabbits intravenously cause respiratory symptoms in 20 seconds, followed about 10 to 15 seconds later by violent spasms and convulsive struggles. Death usually occurs within 1 minute of the time of injection. Defecation and urination usually occur during the struggles. On opening the thorax and abdomen immediately, it is noticed that the intestines are in very active peristaltic motion and that
the heart is usually beating weakly. Clots are always found in
the portal vein, usually in the inferior vena cava and right heart,
and sometimes in the veins above the heart. Only in a very
few cases was the blood in the left ventricle found clotted. Wool-
dridge commented upon the frequency with which the clotting
occurred in the portal vein and stated that it occurred here even
more readily when the animal was in full digestion. Wright.
held that the CO₂ content of the blood was the main factor in
deciding its tendency to coagulate, thus accounting for the prev-
allence of clotting in the venous system but its absence from the
arterial system.

The blood remaining fluid in the vessels, after death from clot-
ting as described above, showed a partial or complete negative
phase of coagulation. Such blood contains no substances which
will inhibit the coagulation of normal blood, but instead it still
contains some of the active tissue fibrinogen so that its action
is to accelerate the clotting of normal blood. It is not deficient
in calcium, but contains an amount sufficient to recalcify normal
oxalate plasma and cause it to clot. The following tests demon-
strate these facts:

1 cc. oxalate plasma + 2 drops 1 per cent CaCl₂ solution → clot in 4
min.
1 cc. oxalate plasma + 1 cc. non-coagulable plasma → clot in 2 min.

Here the 1 cc. of the non-coagulable plasma not only furnishes
the calcium required for the clotting of the oxalate plasma, but
also quickens the clotting time from 4 minutes to 2 minutes.
Fibrinogen tests on the plasma, as by half saturation with NaCl,
usually show some small amounts to be present, although in some
cases there is not the slightest precipitate. Where there is some
fibrinogen present, clotting occurs slowly if the blood is not
disturbed, the time varying from a few hours to several weeks.
These clots are not firm, and are easily broken up by shaking.
The negative phase, or non-coagulability, then seems to be due to
a lack of fibrinogen, either partial or complete. The fact that the
clotting in the vessels occurs while the blood is actively circulating
probably accounts for a portion of the blood not being caught in
the clot. It is well known that agitation of plasma or blood during
clotting will cause the fibrin to precipitate out more in strings
than as a fine network, so that most of the fluid is left free.
B. Negative Phase Production in Rabbits.—Intravenous injection of sublethal amounts of tissue extracts, repeated at intervals of 1 to 2 minutes and in increasing amounts, causes the development of a certain immunity to the material after a few injections, so that about ten times the original lethal dose may be given without symptoms. The following protocol will illustrate this.

Rabbit, 2,500 gm. No anesthesia used. Injections were made into the marginal ear vein.

Lung extract injected.

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<tr>
<td>0.8</td>
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<tr>
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<td>2.6</td>
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<td>9.35</td>
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<td>2.6</td>
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(0.3 cc. of this extract is the lethal dose for a 2,500 gm. rabbit.)

The only symptoms were a slight, but steadily increasing weakness. The animal was killed by a blow on the head 3 minutes after the last injection, and examined for the presence of clots. No trace of clotting was found anywhere. The blood drawn from the inferior vena cava was kept in the ice box for several days without evidence of coagulation occurring. It was very rich in the tissue fibrinogen, as shown by its power to hasten the clotting of normal blood, contained sufficient calcium to clot normal oxalate blood, but was found very poor in fibrinogen. It would not clot with fibrin ferment, CO₂ gas, dilution with water, or the addition of any amount of lung extract.

Thus, although the plasma is itself entirely non-coagulable, it contains sufficient calcium per cc. to recalcify the oxalate plasma and cause it to clot. It must then contain the equivalent of 4 drops of 1 per cent CaCl₂ per cc. Not only does it effectively recalcify the oxalate plasma, but it also quickens the coagulation time to half the normal period. This indicates the presence of some of the tissue fibrinogen still in the plasma. Similar experiments with peptone plasma from a dog show a similar recalcification of the oxalate plasma, but also indicate clearly the presence...
of some powerful inhibitory substance or condition which retards or prevents the coagulation even with sufficient calcium present.

1 cc. oxalate plasma + 1 drop lung extract + 1 drop 1 per cent CaCl₂ → clot in 12 sec.
1 cc. oxalate plasma + 0.25 cc. peptone plasma + 4 drops lung extract → clot in 15 sec.
1 cc. oxalate plasma + 0.5 cc. peptone plasma + 4 drops lung extract → clot in 60 sec.
1 cc. oxalate plasma + 1.0 cc. peptone plasma + 4 drops lung extract → no clot.

The peptone plasma used here had stood several weeks in the ice box without clotting. It is evident from the above tests that 1 cc. of this plasma contains more than enough of the inhibitory material to counterbalance the accelerating effect of the 4 drops of the lung extract. The first test shows that the lung extract is very strong also, 1 drop of it causing 1 cc. of oxalate plasma to clot in 12 seconds when recalcified.

A further experiment was tried, using blood freshly drawn from a dog and mixing with it the peptone plasma and non-coagulable plasma used above.

1 cc. fresh dog blood drawn and let stand → clot in 4 min.
1 cc. fresh dog blood drawn into 1 cc. non-coagulable plasma → clot in 1 min.
1 cc. fresh dog blood drawn into 1 cc. lung extract → clot in 40 sec.
1 cc. fresh dog blood drawn into 1 cc. 0.9 per cent NaCl solution → clot in 4½ min.
1 cc. fresh dog blood drawn into 1 cc. peptone plasma → very slight clot in 18 hrs.

Here it is evident that, while peptone plasma does strongly inhibit the clotting of normal blood, the non-coagulable plasma obtained by tissue extract injection is not only free from such action, but contains some of the tissue material in active form so that its action is to hasten normal clotting markedly. Since the decrease in fibrinogen in this plasma was demonstrated by half saturation with NaCl and comparison with normal plasma similarly treated, the non-coagulability was probably due to such fibrinogen deficiency.

This condition was induced in four rabbits, with complete non-coagulability of the blood resulting. In many other cases
the immunization was not so well accomplished, the animal being killed by too sudden an increase in the dosage. In such a case there were usually found slight clots either in the portal vein or on the chordae tendineae of the right heart. The fluid blood presented the characteristics described above, however. In no experiment was there any substance present in the non-coagulable blood which would inhibit the clotting of normal blood.

C. Negative Phase Production in Dogs.—This same immunization was carried out in seven different dogs with the animals under anesthesia. Blood pressure records were made and blood samples drawn at frequent intervals to study the blood changes. The following protocol will serve as a typical example of these experiments.

Dog, male, 9 kilos. Anesthetized with ether. Tracheal cannula. Blood pressure from right carotid artery. Injections from a burette into left femoral vein. Cannula in right femoral artery for blood samples. Table I shows the injections made and the results obtained.

In Table I, by lung extract ($\times \frac{1}{4}$) is meant lung extract diluted with three volumes of 0.9 per cent NaCl solution. Lung extract ($\times 1$) is the undiluted lung extract. The coagulation time was taken as the time when the tube containing the blood could be carefully inverted without loss of its contents. By “activity” of the blood samples is meant their accelerating effect on the coagulation of normal oxalate beef plasma. All the samples drawn for these tests and for the alkali reserve were oxalated to about 0.5 per cent to prevent clotting, and the corpuscles centrifuged out to obtain clear plasma for the tests. Degree of activity of the plasma samples was tested as follows:

1 cc. oxalate beef plasma + 2 drops 1 per cent $\text{CaCl}_2$ → clot in 4½ min.
1 " " " " + 5 " Plasma 1 + 2 drops 1 per cent $\text{CaCl}_2$ → clot in 2 min., 40 sec.

It is shown in the table of dilution of lung extract (Table I (10)) that the hastening of the coagulation time bears a very definite relation to the concentration of the active tissue fibrinogen present, so that, by testing the amount of shortening of the coagulation time induced by a given amount of the fluid to be tested, one may rather accurately estimate the amount of the active substance present. Now, by reference to the results listed in Table I of the
### Coagulation of Blood

**TABLE I.**

**Negative Phase in a Dog.**

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<td></td>
<td>cc.</td>
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<td>Coagulation time.</td>
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<tr>
<td>2.34 p.m.</td>
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<td>1</td>
<td>4 min.</td>
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<tr>
<td>2.50</td>
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<td>2</td>
<td>4 min., 10 sec.</td>
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<tr>
<td>2.51</td>
<td>0.5 (X1)</td>
<td></td>
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</tr>
<tr>
<td>2.52</td>
<td>1.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.53</td>
<td>1.2 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.54</td>
<td>1.5 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.54</td>
<td>2.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.55</td>
<td>3.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.56</td>
<td></td>
<td>3</td>
<td>34 min.</td>
</tr>
<tr>
<td>2.56</td>
<td>4.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.57</td>
<td>5.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.58</td>
<td>6.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.59</td>
<td>7.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00</td>
<td>8.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.01</td>
<td>9.0 (X1)</td>
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<td></td>
</tr>
<tr>
<td>3.02</td>
<td>10.5 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.03</td>
<td>12.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.05</td>
<td>15.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.09</td>
<td>1.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.11</td>
<td>2.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.12</td>
<td>2.5 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.13</td>
<td>3.5 (X1)</td>
<td>4</td>
<td>60 min.</td>
</tr>
<tr>
<td>3.14</td>
<td>4.5 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.16</td>
<td>6.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.18</td>
<td>8.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.21</td>
<td>10.0 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.25</td>
<td></td>
<td>5</td>
<td>Slight clot, 5 min.</td>
</tr>
<tr>
<td>3.27</td>
<td>12 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.31</td>
<td>15 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.33</td>
<td>25 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.40</td>
<td>35 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.42</td>
<td>50 (X1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.44</td>
<td>50 (1 per cent NaHCO₃)</td>
<td>6</td>
<td>Slight clot, 15 min.</td>
</tr>
<tr>
<td>4.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C. A. Mills

TABLE I—Concluded.

<table>
<thead>
<tr>
<th>Time</th>
<th>Lung extract injected</th>
<th>Blood samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>No.</td>
</tr>
<tr>
<td>4.14 ½ p.m.</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>4.20 “</td>
<td>50 (1 per cent NaHCO₃)</td>
<td>8</td>
</tr>
<tr>
<td>4.22 “</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>4.35 ½ “</td>
<td>50 (1 per cent NaHCO₃)</td>
<td>10</td>
</tr>
<tr>
<td>4.38 ½ “</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>4.51 “</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00 “</td>
<td>Death, Bleed from heart</td>
<td></td>
</tr>
</tbody>
</table>

Present paper under “Activity,” it is seen that the blood normally contained a quite definite amount of the material, since the clotting time of oxalate plasma was shortened from 4½ minutes to 2 minutes, 40 seconds, by 5 drops of the dog plasma. During the injections of the lung extract, this accelerating effect of the samples of dog plasma on normal clotting of beef oxalated plasma became more and more marked, so that it must be concluded that the concentration of the active substance in the blood of the dog was steadily increasing. Sample 5, taken 2½ minutes after the last lung extract injection, was able to shorten the clotting time of normal oxalate blood to 45 seconds, while Sample 6, taken 24½ minutes after the last injection, only shortens the clotting time to 50 seconds. Thus during that 22 minute period there occurred a noticeable decrease in the concentration of the tissue fibrinogen in the dog’s blood. This decrease is probably to be accounted for by an excretion of the material into the urine, since the urine collected at the end of the experiment contained almost as much of the substance as the blood. 5 drops of the urine were able to
accelerate the clotting time of 1 cc. of normal oxalate plasma to 60 seconds, whereas the same quantity of blood plasma drawn at death gave a clotting time of 50 seconds to normal plasma. This excretion of the material in active form by the kidney is discussed later in this paper.

The coagulation time of the blood of the dog is seen to be unaffected by the injections until injections of 2 to 3 cc. of undiluted lung extract are used. (About 3 cc. of the extract are the fatal dose for a dog this size when only a single injection is given.) More frequent drawing of blood samples before this point always shows a quickening of the clotting time to $1\frac{1}{2}$ to 2 minutes, this then being followed by the negative phase. Samples 5 to 11 formed only slight clots which were readily broken up by the slightest agitation. Several days standing in the ice box failed to show any further clotting. Tests for fibrinogen in the corresponding oxalated samples showed the presence of a small quantity of fibrinogen, but nothing at all to be compared to the normal amount. The negative phase seems, then, to be due to a loss of fibrinogen from the blood. It is not due to any inhibiting substance present, or to lack of calcium, since this plasma will recalcify normal oxalate plasma and cause it to clot in a shorter time than will the addition of CaCl₂ alone. Tests similar to those given for the non-coagulable rabbit blood were carried out on this plasma and the results were so similar that they need not be given here. The fate of this lost fibrinogen is not known, since it is not present as visible clots anywhere in the vascular system. Sometimes slight fibrin strings were to be found attached to the chordae tendineae of the right ventricle, but no clots elsewhere. Furthermore the dog gave no symptoms of intravascular clotting during the injections. A possible explanation for the fate of the lost fibrinogen will be set forth in the discussion at the end of this paper.

The alkali reserve was determined with the Van Slyke CO₂ apparatus, following the method of Van Slyke and Cullen (15). There is a very sudden fall in the alkali reserve just at the time of the development of the negative phase in the blood. This occurred in each such experiment performed on dogs. It is not due to the anesthesia, for dogs kept under anesthesia, as controls, for 2 hours show a very much smaller fall. The signifi-
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The occurrence of such a sudden fall is unknown. It is probably due to negative phase production, since it is so constantly associated with it, but it is not the cause of the loss of coagulability, since bicarbonate injections later in the experiment show no effect on the coagulation time.

D. Effect of Acid and Alkali Injections. Asphyxia.—In order to see whether or not the fall in alkali reserve was a possible causative factor in the negative phase production, weak acid was injected into a dog until the alkali reserve reached an extremely low level. Instead of a lessened coagulability of the blood, there was found a greatly shortened clotting time. The protocol of the experiment follows.


The procedure followed and the results obtained are shown in Table II.

This experiment is inserted here merely to show that the great fall in alkali reserve during the production of the negative phase by tissue extract injections probably has in itself nothing to do with the non-coagulability. In this experiment it is to be noted that the CO₂-combining power of the plasma falls rapidly as the coagulation time of the blood markedly shortens, and again that it rises as the coagulation time lengthens.

Although this experiment has little bearing on the mode of action of tissue extracts on the blood in vivo, it merits attention as regards the action of acids and alkalies on the blood and its coagulability.

In text-books on physiology it is often found stated that death from asphyxia leaves the blood non-coagulable. In such a death the hydrogen ion concentration of the blood probably increases after the CO₂-combining power of the plasma has been exhausted, and so a preliminary experiment on asphyxia was carried out on this dog. Complete occlusion of the air supply for 2½ minutes was all the dog could stand and still be able to recover. The blood became almost black in color, but the coagulation time was not affected. An increase of over 40 per cent in the CO₂-combining power of the plasma was found. Such an increase has been found by others in the past and has been taken to mean that the
corpuscles have contributed material to the plasma for binding the CO₂. It has been shown that the alkali reserve of the whole blood increases much less than does the plasma alone. It is doubtful whether in this experiment, the CO₂-combining power

### TABLE II.

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment and injections</th>
<th>Blood samples.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.10 p.m.</td>
<td>15 min. after etherization.</td>
<td>1</td>
</tr>
<tr>
<td>3.16 &quot;</td>
<td>Trachea clamped to cut off air.</td>
<td>2</td>
</tr>
<tr>
<td>3.18½ &quot;</td>
<td>Air supply restored.</td>
<td>3</td>
</tr>
<tr>
<td>3.26 &quot;</td>
<td>10 cc. 0.1 N HCl injected.</td>
<td></td>
</tr>
<tr>
<td>3.27½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.30½ &quot;</td>
<td>25 cc. 0.1 N HCl injected. (No more ether given.)</td>
<td>4</td>
</tr>
<tr>
<td>3.33½ &quot;</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>3.36½ &quot;</td>
<td>50 cc. 0.1 N HCl injected.</td>
<td>6</td>
</tr>
<tr>
<td>3.41½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.43½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.50½ &quot;</td>
<td>50 cc. 0.1 N HCl injected.</td>
<td>7</td>
</tr>
<tr>
<td>3.56½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.59½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.06½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.13 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.10 &quot;</td>
<td>50 cc. 0.1 N NaHCO₃.</td>
<td>8</td>
</tr>
<tr>
<td>4.20 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.27 &quot;</td>
<td>50 cc. 0.1 N NaHCO₃.</td>
<td>9</td>
</tr>
<tr>
<td>4.31 &quot;</td>
<td>(Ether given again.)</td>
<td></td>
</tr>
<tr>
<td>4.32½ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.38 &quot;</td>
<td>35 cc. 0.1 N NaHCO₃.</td>
<td>10</td>
</tr>
<tr>
<td>4.49 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.50 &quot;</td>
<td>12 cc. 1 per cent CaCl₂. (Death during injection, intravascular clotting.)</td>
<td>11</td>
</tr>
<tr>
<td>5.02 &quot;</td>
<td>Blood taken from left heart.</td>
<td></td>
</tr>
</tbody>
</table>

of the blood was exhausted, and, if it was not, the rise in H⁺ concentration would have been negligible.

Injection of 0.1 N HCl was without marked effect until after 85 cc. had been given, when the blood in the vessels became so-
viscid that it would scarcely issue from a medium sized cannula in the femoral artery. Breathing was very labored and no ether was necessary until during the alkali injections. Blood pressure fell to a very low level although the heart action was apparently well maintained. All these effects probably came as a result of the greatly increased viscosity of the blood. Injection of another 50 cc. of the 0.1 N HCl only increased the above effects. The blood would issue from the cannula so slowly that it would clot before it could be mixed with oxalate. It was with difficulty that enough could be oxalated, unclotted, for the alkali reserve determination. From the very great decrease in the CO₂-combining power of the plasma here it is probable that the H⁺ concentration had risen considerably.

The first 50 cc. injection of 0.1 N bicarbonate restored the blood to its normal fluidity, caused a cessation of the labored respiration, and a marked elevation of the blood pressure. Further bicarbonate injections restored the dog to apparently normal condition so that it was necessary to restore the ether administration. The most noticeable peculiarity to be observed in the results is the marked slowing of the coagulation time as the proper bicarbonate concentration is restored to the blood. However, the injection of CaCl₂ that followed, with the resulting solid intravascular clotting in the heart and all large veins examined, showed that the delayed coagulation during the bicarbonate injections was probably due to a partial and increasing decalcification of the blood by precipitation of the calcium as CaCO₃. The CaCl₂ injection seemed to restore the coagulability to the blood through recalcification, and the intravascular clotting probably was caused by the presence throughout the blood of CaCO₃ particles which acted as centers for the initiation of the clotting process.

The action of the acid in so markedly increasing the viscosity of the blood suggested that the affair was probably a matter of increasing the hydration capacity of the blood proteins, and, since the coagulation time of the fibrinogen was affected, this protein must have been one of those affected. To see if a similar action could be found in vitro some fibrinogen was precipitated from oxalate plasma by half saturation with NaCl, and redissolved in 0.9 per cent NaCl solution. This fibrinogen solution
was placed in a series of tubes and acid added to give a calculated concentration ranging from 0.0002 to 0.5 N. No change in the contents of the tubes occurred until the acid concentration reached 0.01 N when a distinct opalescence was to be seen. At 0.02 N acidity this opalescence was more marked and at 0.1 N there was almost complete precipitation of the fibrinogen. Now the first effect of the acid injection on the dog occurred after 85 cc. of 0.1 N HCl had been injected. This 5 kilo dog had probably 400 cc. of blood, figuring one-tenth to one-thirteenth of the body weight as blood, and of this blood about 60 per cent, or 240 cc., would be fluid plasma. 85 cc. of 0.1 N HCl added to 240 cc. of plasma would make approximately a N/40 HCl solution, if no acid was lost. However, the blood proteins would bind considerable of the acid, and it would be lost rapidly into the tissue lymph and secretions. At any rate, it is seen that the acid concentration in the blood probably fell within the limits of concentration which cause an opalescence to appear in a fibrinogen solution in vitro.

E. Effect of Tissue Extracts on Blood Pressure.—One further point of interest in regard to the intravenous injection of tissue extracts is the effect on blood pressure. With the injection of amounts sufficient to cause extensive intravascular clotting, the blood pressure rises abruptly at the time of the clotting. With sublethal doses, however, when no clotting occurs, the blood pressure effects are almost identical with those of histamine (ergamine, β-imidazolylethylamine). There occurs the same abrupt fall in pressure followed by the same more gradual rise back to normal. Like histamine, also, lung extract will cause such an effect time after time, at intervals of 1 to 3 minutes; that is, the sensitivity of the vascular system to its effects is not diminished except very slowly. Besides this similarity to histamine in its vasodilator effect, there is also its stimulating action on other smooth musculature. Urination and defecation nearly always occur following the injection of a moderately large or lethal dose into rabbits while with much smaller doses the increased peristaltic movements of the intestines can be readily observed even without opening the abdomen. Also the gurgling of the contents becomes very marked as a result of such peristalsis.

As has been mentioned before, it is considered by some that tissue extracts contain a mother substance capable of freeing
histamine when injected into the blood, so that it is supposed to be the effects of the histamine that give the above described results. In another place the writer has mentioned that the purified tissue fibrinogen gives a negative test for the presence of imidazole ring compounds when tested by Ehrlich's diazo reaction, although the crude tissue extract gives a distinctly positive test when thus tested. Whether the positive test in the crude extract is due to histidine or to histamine has not been determined.

Since the purified material gave a negative test for histamine, it is interesting to note that intravenous injections of it into rabbits produced death in exactly the same fashion as did the crude lung extracts. It has not yet been tried on dogs, nor has its effect directly on smooth musculature been observed. This would make it seem as though the presence of histamine is not necessary for the characteristic effects of the substance, but rather that the effects probably depend on changes induced in the blood during the transformation of the fibrinogen to fibrin.

5. Excretion of Tissue Fibrinogen in Urine.—Tissue fibrinogen injected intravenously into dogs or rabbits so as to produce a non-coagulability of the blood always results in the excretion of some of the material unchanged in the urine. It so happened that all the urine samples collected from the animals were just about neutral to litmus. What would happen if the urine were acid cannot be stated, although it is likely the material excreted might lose some of its activity. The urine collected at the end of the dog experiment described on page 181, showed the presence of almost as much tissue fibrinogen as did the blood plasma. By reference to the protocol, it will be noted that the dog was not killed until 1 hour and 20 minutes after the last injection of lung extract, and that during this time the concentration of the material in the blood decreased, as shown by the activity tests. This active tissue fibrinogen in the urine retained all the properties of the material injected, the most characteristic of which was its activity on the clotting of blood. It could readily be salted out of the urine by half saturation with (NH₄)₂SO₄, just as it could from the lung extract.

It has been shown by the author in the preceding paper (10) that a presumably slight change in the protein-phospholipin compound,
Coagulation of Blood

which is here termed tissue fibrinogen, results in a partial or complete loss of its activity on coagulation. Since the size of the molecules of the substance must be very great (as judged by present molecular weight figures, the substance would probably consist of 1 protein molecule to which are united about 12 phospholipin molecules) it is extremely difficult to imagine any process by which it must have passed intact from the blood stream into the urine, unless the two living membranes through which it passed possess pores through which it might pass, just as the white corpuscles pass through the capillary walls.

6. Specificity of Tissue Fibrinogen.—Loeb (16) has shown that a substance derived from the tissues, or blood cells, is necessary for the completion of the coagulation process in the blood of various lower forms of animal life. He termed this substance "tissue coagulin." It is very likely identical with what is here termed tissue fibrinogen. Loeb (17) also states that he found a definite class specificity to exist for these tissue coagulins; that is, the extracts of the tissues of one species of animal will hasten the clotting of the blood of other animals of the same class, but will be without effect on the blood of animals of a different class. Thus the tissue extracts made from any mammalian tissue would hasten the clotting of the blood of any mammal but would be ineffective on bird, reptilian, amphibian, or fish blood.

The writer has not gone deeply into this question, but has found that extracts of the lungs of cattle, dogs, rabbits, rats, and guinea pigs all show marked activity in the coagulation of the blood of any of the animals mentioned, either in vitro, or when injected intravenously. The intravenous injections were made mostly into rabbits, although rats and dogs were used to some extent. The only animal outside the mammalian class so far tested was the frog. It was found that extracts of frog lungs would very readily clot frog blood in vitro, or cause solid intravascular clotting, but such extracts were entirely without effect on the coagulation of mammalian plasma. Mammalian lung extract, however strong, would not cause intravascular clotting of frog blood, although it would hasten the clotting of such blood in vitro to some extent.
SUMMARY.

1. Tissue extracts accelerate the clotting of blood in a very definite manner, the coagulation time of the blood increasing from a minimum of about 10 seconds up to the normal time as the tissue extract added is diluted. The logarithm of the coagulation time in seconds plotted against the logarithm of the dilution of the extract gives almost a straight line. It is possible to get a noticeable quickening of the clotting process with the active tissue substance added to blood plasma in the proportion of 1 part of active substance to 100,000,000 parts of plasma.

2. The active tissue substance will not react with the blood fibrinogen to form fibrin, either in vitro or in vivo, except in the presence of soluble calcium salts.

3. Injected intravenously, rapidly, and in sufficient amounts, tissue extracts, or the purified active substance, cause intravascular clotting and death in a very definite manner. Injected slowly and in smaller amounts the blood is rendered non-coagulable, partially or completely, the non-coagulability apparently depending on a gradual removal of the greater part of the fibrinogen from the blood stream. A marked decrease in the alkali reserve of the plasma develops along with the development of the negative phase of coagulation, but is apparently not the cause of it.

4. The injection of active tissue extracts into the blood stream so as to produce non-coagulability without clot formation, is followed by an excretion of the active tissue substance apparently unchanged in the urine (dogs and rabbit).

5. As observed by Loeb, there is present a class specificity in regard to the action of the tissue extracts on blood clotting. The specificity, however, is not absolute, since mammalian lung extract will accelerate the clotting of frog blood.

6. There is a latent period in the coagulation process which is remarkably constant under similar conditions. Upon this depended the success of showing accurately the result of dilution of the tissue extracts upon their coagulative action. But it may be demonstrated also for intravascular clotting of the blood. Thus, after the injection of a fatal dose of lung extract into the ear vein of a rabbit, the time that elapsed before the onset of the
convulsive struggles did not vary more than 10 seconds in different rabbits, being 15 to 25 seconds after the injection. This gave time for at least a partial distribution of the active substance through the blood with the resulting great dilution of it. Just as plasma \textit{in vitro} cannot be made to clot much quicker than 10 seconds, regardless of the concentration of the tissue material, so it was also found to be with the blood in the vessels. Always, if coagulation occurred in any degree in the vessels following tissue extract injection, it occurred within 30 seconds after the injection. If the amount injected was not sufficient to give results in that time, then no solid clots formed, but a negative phase of coagulation set in. Further injections into the same animal of like or gradually increasing amounts, only served to increase the negative phase, until finally the blood was totally non-coagulable. The diminution, or lack of fibrinogen in such blood appears to be the cause of its non-coagulability.

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THE ACTION OF TISSUE EXTRACTS IN
THE COAGULATION OF BLOOD
C. A. Mills


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