STUDIES IN BLOOD COAGULATION.

II. BLOOD COAGULABILITY IN MALIGNANT TUMOR AND OTHER DISEASES OF THE RABBIT.

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Alterations in the coagulability of the blood will be described which occur in various stages of the growth and extension of a malignant tumor of the rabbit, and, as a basis of interpretation, there is explained also the behavior of coagulation in other diseases and abnormal states of this animal. The methods of test employed have been described (1), and include estimation of clot initiation and clot formation rate and the rate and extent of clot retraction.

I. Disease in General.

Relation of Blood Coagulability to Presence and Extent of Disease Lesions.

Experimental.—100 adult male rabbits, taken from stock as received from the breeders, were placed under observation for 1 or more weeks. All grades of physical condition were presented, although the great majority were free from external evidences of disease. The blood coagulability of each animal was then tested, and autopsy examination was made within 24 hours afterward. A comparison of the readings with the anatomical findings follows.

Results.—As regards clot formation rate, the readings in almost every instance were within the limits of normal variation, i.e., between 4 and 8 minutes. Five exceptions to this presented, and all of these were in extreme stages of disease: Two gave clot formation time of 10 minutes, and showed at autopsy fatty degeneration of the kidneys and profound cachexia, which changes were probably secondary to an occult disease process. One rabbit with extensive coccidiosis of the

1 These rabbits are part of a larger group being used for other experiments in the study of normal and abnormal organ weight variations.
liver, another with snuffles complicated by septicemia and a third marked emaciation but without discernible lesions gave delayed clot initiation and an irregular clot formation allowing of no distinct reading.

The extent of clot retraction was also normal in the majority of the animals, i.e., reading between 87 and 100 per cent. In the last

<table>
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<tr>
<th>Autopsy finding</th>
<th>Analysis of groups on a basis of clot retraction rate</th>
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<td>Rabbits grouped on a basis of stress of disease</td>
<td>[40-60] [30-40] [20-30] [0-20] No reading obtainable</td>
</tr>
<tr>
<td>11, without lesions and in good physical condition</td>
<td>91 9</td>
</tr>
<tr>
<td>10, with healed lesions of various types</td>
<td>80 20</td>
</tr>
<tr>
<td>24, with coccidial lesions, small and of questionable activity</td>
<td>25 33 29 13</td>
</tr>
<tr>
<td>13, in process of recovery from various types of infections</td>
<td>32 60 8</td>
</tr>
<tr>
<td>18, with purulent infections, snuffles, mastoiditis, etc.</td>
<td>5½ 11 78 5½</td>
</tr>
<tr>
<td>8, with coccidial lesions, numerous and obviously active</td>
<td>40 50 10</td>
</tr>
<tr>
<td>4, emaciated, with fatty degenerative lesions of kidneys</td>
<td>25 75</td>
</tr>
<tr>
<td>2, emaciated, with active parasitic lesions of kidneys</td>
<td>100</td>
</tr>
<tr>
<td>2, emaciated, with focal lesions of appendix</td>
<td>100</td>
</tr>
<tr>
<td>3, emaciated, but without discoverable disease process</td>
<td>67 33</td>
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three rabbits above mentioned occurred the only abnormal values, 85, 85½ and 81 per cent, respectively.

Clot retraction rate, on the other hand, was extremely variable, both in rabbits obviously ill and those without symptoms. The autopsy findings are correlated statistically in Table I with the retraction rate values. On the left, the rabbits are grouped according
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[...]

to the type of disease found and the groups are arranged in order on a basis of degree of physical deterioration (disease stress) evident. On the right, analysis is made of each group from a standpoint of the clot retraction rates, and for this purpose four divisions are used. It will be seen that in the highest rate division, 40 to 60, fall 91 per cent of animals without lesions and in good physical condition, also, 80 per cent of those with healed lesions only and 25 per cent with insignificant coccidial lesions. As analysis proceeds thus from group to group dealing with disease of increasing severity, the distribution of rate values plainly shifts across the scale, and the last five groups lie entirely within the lowest rate division, 0 to 20. The animals which fall principally in the intermediate divisions, 20 to 30 to 40, are those with moderately active disease or in process of recovery from severe infections. Retraction rate could not be measured in three rabbits, those last referred to above, because of the fragmentary nature of the coagulative process.

Five rabbits are excluded from the table for special mention: Two had been inoculated intratesticularly with the spirochete of yaws and had an early, active orchitis. At autopsy no other lesions were found. The retraction rates were normal, 42 and 48 per cent. Two rabbits had well developed goiter, were otherwise in good condition and gave readings, 45 and 56 per cent, within the normal range. One rabbit although a female was included in the series to illustrate a point of interest. She was abundantly fat and sleek and presented nothing pathological beyond a small, healing cuniculi papule on the genitalia. Nevertheless, the clot had an extremely low retraction rate, 9 per cent; at autopsy pregnancy at about the 10th day was discovered.

Relation of Blood Coagulability to Course of Disease.

Experimental.—The behavior of blood coagulation was investigated during the course of various abnormal and diseased states, including instances of spontaneous infections, non-bacterial lesions and artificially induced affections of the blood coagulative mechanism. In each case, periodic tests were made and symptoms recorded, with autopsy examination at the termination of the experiment.

Findings.—Brief protocols of typical examples of each disease are given:
Snuffles.—Text-fig. 1 is the chart of a rabbit which had been followed for some time because of chronic snuffles. The infection persisted for about 2 months, gradually cleared, and the nasal discharge had ceased, when abruptly a relapse set in with profuse discharge and sneezing and the animal passed through a second typical course of the disease, this time lasting 3 weeks. At autopsy, the nasal passages were free from inflammation; the right middle ear spaces contained a small quantity of inspissated pus. There was no other disease. Evidently the rabbit had had chronic otitis in addition to the attacks of snuffles. The period of snuffles remission only is included in the chart of blood findings. The rate of
retraction had been distinctly below normal and dropped still farther with the redevelopment of rhinitis. Later, as the infection cleared, the curve rose to its previous level. The readings of retraction extent and clot formation rate showed throughout no variations beyond the normal range.

**Auditory Canal Abscess.**—The charts are given in Text-fig. 2 of three rabbits from a group of fifteen which were being followed as controls for other purposes. At this time xylol was being used routinely painted on the inner surface of the ear to produce vessel dilatation for the collection of blood specimens, and in two instances, Rabbits 1 and 2, the xylol extended accidentally into the auditory meatus. The applications were discontinued immediately in favor of other methods, but the inflammation in the canal persisted and an abscess developed in its recesses. Rupture occurred in 2 weeks, with drainage of pus for another week. Rabbit 3 is given for comparison, for here no irritation arose, aside from a slight erythema of the pinna. From the charts, it is to be seen that in the cases of the first two rabbits clot retraction rate fell after xylol application to a low level on the 15th and 9th days respectively, and with rupture of the abscess the reading began to rise and reached normal with the healing of the lesion. Retraction extent in the first rabbit was elevated in the early stage of inflammation, but in the second, where the rate drop had been more sudden and pronounced, the extent reading also fell temporarily. Both rate and extent of clot retraction in the third rabbit remained during this period within the normal limits. Clot formation showed no abnormalities in any of the animals.

**Subcutaneous (Sterile) Abscess.**—Six rabbits were injected either subcutaneously or intrapleurally with 0.5 cc. of a 10 per cent suspension of aleuronat. In each, pus accumulated at the site of injection, and in two of the animals which were allowed to live for 3 weeks the abscess was absorbed and no trace remained except
slight local increased vascularity. The record of one of the latter is represented in Text-fig. 3, the two being similar in all respects. The curve of retraction rate fell sharply within 24 hours after injection and remained at a low level for 6 days during the period of abscess development. Then the curve began to rise and by the 15th day was at normal, when the lesion was healed. The extent of retraction did not leave the normal range. Clot initiation and formation were delayed slightly at the height of the inflammatory reaction.

Operative Incision.—Four rabbits were operated upon under ether anesthesia with sterile precautions for the purposes of other work. In two the spleen was removed and in the others laparotomy only was done, as control. The wounds healed per primam. The coagulation curves of one of the control rabbits, which were typical of all, are given in Text-fig. 4, No. 1. Immediately after the operation the rate of retraction dropped and reached a low point on the 3rd day, when it began to return and attained its original level on the 16th day. The extent of retraction remained stationary throughout. No clot formation readings were made.

Ether Anesthesia.—In order to ascertain the part played by ether in the preceding results, two animals were anesthetized for a period of ½ hour. The subsequent behavior of clot retraction in both was the same; the curves of one are given in
Text-fig. 4, No. 2. The rate became retarded in a manner similar to that in the chart above, but returned much more rapidly to normal.

Nephritis.—The chart is given in Text-fig. 5 of a normal rabbit which developed nephritis spontaneously while under observation. The animal had been in good health and gained steadily in weight until the 4th week of observation, when it began to grow thinner without other symptoms. 1000 gm. were lost before death 3 weeks later. At autopsy, the body tissues were found dehydrated and wasted; the only lesion presenting was with reference to the kidneys. These organs were markedly swollen, pitted and intensely yellow, with a fine surface stippling. On section, the cortices were found widened, of an opaque yellow color and without architectural markings. The medullae were pale and presented radial grey streaks. From the chart, it will be seen that clot retraction rate

![Clot retraction rate graph](image)

Text-Fig. 5.

(estimated by a 1 hour serum reading in comparison with the final serum output, a less sensitive index) showed marked retardation on the 25th and 36th days, a return toward normal on the 43rd day, and another drop before death. Retraction extent and formation rate showed no abnormalities until the final reading, when the latter was delayed.

Specific Affections of the Blood-Coagulative Mechanism.—Effort was made to reproduce experimentally conditions of blood coagulability imitating those in the hemorrhagic diatheses, hemophilia and thrombocytopenic purpura.

The properties of the blood in hemophilia are said to be obtained temporarily in animals by the injection of heparin (2), a phosphatide extracted from the liver by the process of Howell, that is, clotting time is prolonged but, when coagulation ensues, the clot retraction appears normal. In the present study seventeen rabbits
were used and heparin, at a concentration of 0.5 per cent in physiological saline solution, was injected intravenously in amounts from 5 to 40 mg. per kilo body weight. Both single and series of doses extending over long periods were employed. In none of the animals appeared symptoms of any sort referable to the drug, nor did microscopic examination of the bone marrow, spleen, liver and other organs reveal changes. Marked alterations in blood coagulability were presented however, shown typically in Text-fig. 6. Here a single dose of 17 mg. per kilo was injected. The blood, examined immediately and again after 1 hour, failed to coagulate. 2 hours after treatment a specimen gave moderately delayed initiation of coagulation and formation time of 23 minutes. At 2 hours, 40 minutes, these two functions were still tardy but became normal 1 hour later. Although clot retraction was observed to occur in portions of specimens as early as 2 hours after injection, the process was so imperfect as to exclude estimation. At 3 hours, 40 minutes, the first measurement was obtainable and gave a considerably reduced rate and a normal extent. Further depression in rate was found after 24 hours, and then this function returned gradually to its original level by the 11th day. The other factors of coagulation were normal after the initial interference. These alterations in the blood varied in degree among the animals with the dose employed. After repeated treatments (20 in 60 days, of 40 mg. per kilo) rabbits appeared to have become somewhat resistant to the drug, although this was not consistent.

The pneumococcus extract of Avery and Neill (3) is known to have a specific lytic effect upon the blood platelets and to produce in mice a transitory purpuric condition. I am indebted to Dr. Avery and Dr. Julianelle of The Rockefeller Institute Hospital for a supply of the extract. This was first warmed to remove the hemolysin, and amounts from 0.25 to 3.50 cc. per kilo body weight were

\begin{center}
\textbf{Text-Fig. 6.}
\end{center}

2 Heparin may be obtained from Hynson, Westcott and Dunning, of 150 Nassau Street, New York City, and Baltimore, Md.
injected intravenously in twelve rabbits. No symptoms were observed, although one rabbit receiving the highest dose was found dead after 18 hours. No purpura or tendency to bleed (as indicated by the incisions in the ear) was evident, even when the platelets were practically absent from the blood stream. Microscopic study of the bone marrow, spleen, liver, lymph nodes and other organs and tissues discovered no changes. Charts of two of the rabbits are given (Text-figs. 7 and 8). Curves have been added representing the platelet content of the blood; these values
were obtained with the thrombocytocrit, an instrument recently described (4), and give estimations of platelets volumetrically instead of numerically as customary. It will be seen that platelet lysis commenced immediately after injection of the extract and reached an extreme degree on the 2nd day. The platelets then reaccumulated rapidly and in great excess, attaining a maximum between the 7th and 9th days, after which their volume returned to normal. This behavior is similar to that described in the mouse by Julianelle and Reimann (5). In Text-fig. 7, following the injection of 1.25 cc. extract per kilo, the rate and extent of clot retraction began to fall within 1 hour, and continued to descend until the 2nd day, when the clot, although of firm consistency, failed to retract. The next day retraction had returned to normal in extent but took place at a very slow rate. The rate returned to its original level by the 7th day. Clot formation showed no departure from normal throughout. In Text-fig. 8, following the injection of a smaller dose, the retraction rate fell as before but its extent remained unaffected. By the 5th day the rate had begun to ascend, but once more was depressed coincidently with the occurrence of a slight accidental burn of the rabbit's ear while warming it to secure blood, and the typical reaction to the extract was thus altered. Clot formation became slightly retarded on the 2nd day. These changes in blood coagulation were found to vary in degree among the rabbits approximately with the size of dose employed. Even with as small an amount as 0.25 cc. per kilo retraction rate was markedly reduced, but the extent of retraction was affected only with the larger amounts of the extract. A close parallelism was demonstrated between retraction rate and the platelet content of the blood, even to the extent that thrombocytosis was associated with a slightly hypernormal retraction rate. The relation held, however, only in animals free from infections, for, as in Text-fig. 8, depression in rate took place under conditions of disease without regard for the platelet reading. The extent of retraction was interfered with only when the platelets reached an exceedingly low level.

II. Tumor.

The tumor which forms the basis of these experiments is an epithelioma of variable malignancy (6, 7). It arose spontaneously in a rabbit and has been carried in this species for purposes of study by successive transplantation. Testicular inoculation of tumor tissue, as routine, gives rise invariably to local neoplastic growth. Metastases develop in a high proportion of instances, often very profuse and resulting fatally within a period of from 20 to 80 days. In others, after a certain amount of growth, the neoplasm becomes necrotic and is absorbed, with survival of the animal.

3 Obtainable from Arthur H. Thomas Co., Philadelphia, and from Firma Arno Haak, Jena, Germany.
Examination of the blood coagulability has been carried out in 58 rabbits inoculated with tumor, tests being made at regular intervals throughout the course of the disease. In many cases the results have been difficult to interpret because of complication of the picture by the effects of spontaneous infections, most frequently appearing where the tumor ran a long and severe course and resulted in physical depletion of the animal. However, uncomplicated cases are abundantly at hand, and typical examples will be described.

A very malignant form of the tumor is illustrated by an animal whose chart is given in Text-fig. 9. The primary tumor grew rapidly and on the 18th day a metastasis was discerned in the right iris. This developed vigorously and 3 days later the left eye was similarly involved. During this period the rabbit lost in strength and ate little. The hind quarters were found paralyzed on the 24th day and the experiment was terminated. At postmortem examination the general state of nourishment was found reduced but not depleted. The primary tumor measured 1.5 by 1.5 by 5.0 cm., had completely destroyed the testicle and invaded the spermatic cord. Extensive involvement by metastases of the abdominal viscera was presented, also, of both eyes, lymph nodes of the neck, femoral bone marrow on both sides and spinal column. The blood readings obtained are shown in the chart, a curve being added to represent the rate of growth of the primary
tumor as estimated by measurements during life (8). Shortly after inoculation, at a time when the initial tumor could be felt in the testicle as a pea-sized nodule, a distinct depression was present in clot retraction rate, and this value continued to fall still farther. On the 18th day the reading tended to return somewhat toward normal but soon fell again and reached a very low level at the end of the experiment. The curve of retraction rate, thus, is seen to represent practically an inversion of the growth curve of the tumor. Retraction extent remained within the normal range throughout. Clot initiation and formation were normal until the last 2 days when marked delay occurred in these functions.

An instance of more gradually extending but severe neoplastic disease is given in Text-fig. 10. The primary tumor developed steadily to large size. On the 19th day the first metastasis was discovered and others followed. From this time on the animal lost in weight and by the 35th day had become emaciated and weak, when it was killed and autopsied. The body tissues were wasted. The primary tumor completely distended the scrotal sac, and tumor nodules were found in the spermatic cord and retroperitoneal lymphatics. The adrenals were largely replaced by metastases; the omentum contained clusters of them. Nodules were present also in the mediastinum, posterior lymphatics of the neck and right iris, and the nasal cavities and sinuses were filled with neoplastic growth. A nodule had entirely destroyed the hypophysis, eroding the sella turcica. In the chart it will be seen that clot retraction rate began to diminish at an early stage of tumor
development and dropped progressively with its further extension, to a low value at death. Here again, the curve of this function parallels inversely the tumor curve. Fluctuations which occurred in the extent of retraction and in clot formation rate were minor and lay within the limits of normal.

A comparatively benign course of the disease is illustrated in the cases of Text-figs. 11 and 12. The primary tumors grew to moderate (Text-fig. 11) or large (Text-fig. 12) size, and then receded without giving rise to discoverable metastases. The charts show a progressive depression of clot retraction rate during the early stages of the disease, and a return to normal as the tumors become absorbed. Retraction extent and formation rate showed no definite changes.

The rabbits whose charts are given in Text-figs. 13 and 14 are presented as types with complications. In the first, the testicular tumor developed to moderate proportions and metastases were felt in the abdominal wall on the 19th day. The iris of both eyes became involved. After the 25th day the tumors ceased to grow and were eventually absorbed. 48 days after inoculation an attack of snuffles set in with sneezing and profuse nasal discharge. A few days later the experiment was terminated. At postmortem examination the nasal cavities were found filled with pus; other than this and the scar of the healed primary tumor no lesions were present. The chart (Text-fig. 13) indicates that during the period of tumor activity retraction rate was depressed, and showed a secondary depression while the necrotic tumor tissue was being absorbed. After returning finally to a normal level, it dropped a third time at the onset of upper respiratory infection. Other factors in the blood tests were normal throughout.

The rabbit of Text-fig. 14 pursued an extended and rather severe form of the disease. The testicle containing the primary tumor was withdrawn into the abdomen soon after inoculation and the growth of the tumor could not be followed. On the 28th day appeared a metastasis in the right iris and others followed in great numbers,—with masses of tumor to be felt in the deep tissues of the neck and in the abdomen. Growths developed at the gingival margins of the upper molar teeth, protruding into the mouth to such an extent as to interfere with eating. From the 30th day on the rabbit became thinner and ultimately cachectic, although it remained vigorous at all times. The experiment was terminated 58 days after inoculation. Autopsy: The tissues were everywhere wasted. An enormous primary tumor lay within the peritoneal cavity, and massive collections of metastases involved the retroperitoneal lymphatics, adrenals, kidneys, spleen, lungs, mediastinum, thymus, neck muscles and lymphatics, hypophysis, both eyes and the maxillary antrum, with infiltration of the alveolar processes and gums. All tumors, however, were thickly encapsulated and entirely necrotic. Evidently the neoplastic process had been arrested for some time, and the cachexia was the combined result of absorption of necrotic tumor tissue and the inability of the animal to take food properly. In the chart, retraction rate is indicated by a 1 hour serum reading in comparison with the final, and this value is seen to have fallen slightly during the period of metastasis (30th to 43rd days), and to have risen to a
TEXT-FIG. 11.

TEXT-FIG. 12.
high position the last week of the experiment. Other factors in the tests showed no alterations of note.

DISCUSSION.

The reports in the literature are very inconsistent with regard to blood coagulability in disease in general, excluding the hemorrhagic diatheses, but where authors have used carefully controlled methods of testing the results indicate that coagulation rate and the retraction of the clot as observed qualitatively remain quite normal. Addis (9), in examining 112 patients, all of whom were seriously ill and presenting various sorts of disease both bacterial and non-bacterial in nature, found changes in coagulation rate in 30 per cent, and these were in an extremely critical condition when the blood was tested. Lee and White (10) conducting a similar investigation also obtained alterations only in the critically ill, and then not constantly. Pneumonia is an exception, for Dochez (11) and others have demonstrated consistent delay in clotting rate during its progressive stages; this is probably to be accounted for in the presence of the pneumococcus toxin (Avery) and its specific action upon elements of the blood connected with coagulation. Imperfectly retracting or non-retractile blood clot has been reported to occur occasionally in certain infections, i.e., diphtheria, pneumonia, miliary tuberculosis, nephritis, ulcerative colitis, smallpox, etc., and these, too, have been cases of unusual severity. Thrombocytopenic purpura hemorrhagica is the only condition which presents non-retractile blood clot with any regularity. The results of the present work in diseases of the rabbit agree with these views, i.e., alterations in the rate of clot formation and extent of clot retraction were found confined to rapidly progressing pathological conditions, at the height of the disease and at terminal stages, and here inconstantly. They failed entirely to appear in gradually developing maladies, even where profound emaciation and weakness were reached.

One factor of the blood coagulative process, however, the rate with which the clot retracts, was found very sensitive to the presence of disease whether local or general in nature. Lesions without apparent constitutional effects, a small blistered burn, a sterile incision, a tumor of pin-head size, produced distinct fluctuations of this value. The
onset of rhinitis was indicated before the appearance of symptoms. The relation of clot retraction rate to the course of disease could be traced with particular assurlty in the case of the tumor where a measure was also afforded of the progress of the lesion. Here, not only was the curve of retraction rate, in uncomplicated instances, nearly an exact reflection of the curve of tumor growth, but metastases were detected during or immediately following periods in the disease characterized by very low retraction rate readings. That reduction in the rate of clot retraction has to do with disease activity rather than with a poor state of bodily nutrition per se, is demonstrated by the fact that body weight was found to run parallel to retraction rate only in so far as it also reflected the stress of disease. Examples from the cases cited illustrate this: While in Text-fig. 11 body weight and clot retraction rate were simultaneously reduced during the active stages of tumor growth, in Text-figs. 9 and 10 the animals gained steadily in weight with the first inroads of the tumor, and retraction rate fell; in Text-fig. 14, when disease activity had totally ceased and cachexia was extreme, the rate of retraction remained at a normal level. There is also no consistent correspondence between anemia (hematocrit values) and defective clot retraction.

The alterations in blood composition that are responsible for changes in clot retraction have not been entirely explained. Hayem (12) showed that blood plasma deprived of all cellular elements formed a firm clot on standing and that the clot failed to retract. On the other hand, when he allowed the platelets alone to remain in the plasma, the clot which formed retracted normally. It was pointed out by Duke (13) that in purpura hemorrhagica with a reduction in the number of platelets in the blood below about 50,000 per c.mm., specimens of blood formed non-retractile clots, and le Sourd and Pagniez (14), among others, have been able to induce clot retraction in such blood specimens by adding normal platelets. However, the latter authors (15) came to the conclusion, after both clinical and experimental observation, that, while the presence of blood platelets seems indispensable for normal retractility of the blood clot, retraction occasionally fails to take place even when platelets are present in abundance. This has been our experience, also, with regard to the relationship of both rate and extent of clot retraction to the blood
platelet content. It is possible also that the quality (fragility or chemical nature) of the platelets, as well as the quantity, may vary and play a rôle in clot retraction. The fact is well known, moreover, that the addition to the blood of minute traces of acid changes markedly the consistency of the clot, and the hydrogen ion concentration of the blood may readily be a determining factor in clot retractility.

SUMMARY.

Description is given of changes in blood coagulability found in diseases of the rabbit, including malignant tumor, spontaneous infections, non-bacterial diseases and lesions, and hemorrhagic states specifically induced. The changes involved variously the time of onset of blood coagulation, clot formation rate and the rate and extent of clot retraction.

BIBLIOGRAPHY.