**The factors of coagulation in the experimental aplastic anemia of Benzol poisoning : with special reference to the origin of prothrombin / by S.H. Hurwitz, M.D., and C.K. Drinker, M.D.**

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By S. H. HURWITZ, M.D., and C. K. DRINKER, M.D.

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## INTRODUCTION.

In the course of a study (1) on the factors of coagulation in a case of aplastic anemia we were impressed with the analogy existing between the symptom-complex in this disease and that observed by Selling (2, 3) in three cases of benzol poisoning. The chief clinical features presented by Selling's three patients were: first, purpura hemorrhagica, with typical skin lesions, bleeding from the mucous membranes, and retinal hemorrhages; second, a blood picture characterized by a leucopenia of a marked grade, a striking reduction in the blood platelets, and anemia. Pathologically these patients showed well marked aplasia of the bone marrow. A study of his three patients induced Selling to try to produce in animals aplastic anemia with benzol. This he accomplished successfully in rabbits and showed that benzol is a powerful leucotoxic agent, acting chiefly upon the bone marrow, although producing changes of some degree also in other hematopoietic organs.

Workers who have studied the blood in benzol poisoning have concerned themselves thus far chiefly with the formed elements. Selling (3) found both a reduction in the red corpuscles and a striking diminution in the leucocytes, with an almost total disappearance of these elements in the blood of fatally poisoned animals. In addition, Duke (4) found a marked drop in the platelet count. According to Duke, a sufficiently low reduction in blood plates will lead to the prolonged bleeding time noted in benzol intoxication. Duke's studies included also occasional determinations of the blood fibrinogen; but we have been unable to find any complete and systematic studies of the factors of coagulation in experimental conditions associated with aplasia of the bone marrow.

The present series of experiments was undertaken with the hope of learning something about the factors of coagulation in benzol poisoning, but as the work progressed it seemed to give additional information concerning the relation of the bone marrow to the origin of the factors of coagulation studied by us; namely, prothrombin, antithrombin, and fibrinogen.

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Our observations have shown that the clinical symptoms of an animal poisoned with benzol, as far as the hemorrhagic features are concerned, give only slight indication of the profound changes which this myeloid tissue poison produces in the blood of such animals. The latter do not, as a rule, exhibit the usual hemophilic tendency with which we are familiar,—delayed bleeding time, purpura, bleeding from the gums and mucous membranes, etc. Very occasionally one meets with prolonged bleeding from cuts and ear pricks, which, according to the recent observation of Duke (5), occurs only when the plates are reduced to a dangerously low level, 5,000 to 15,000 per cubic millimeter. With the exception of a few instances in which the blood, at autopsy, was found to remain fluid for a long time, no striking departure from normal clotting was noted. A study of the blood showed, however, that striking changes had resulted both in the formed elements and in the prothrombin content, although such changes were not of a degree sufficiently marked to produce the clinical picture of hemorrhagic disease.

As far as the effect of benzol upon the formed elements is concerned, our experiments confirm the work of Selling and of Duke. Following the administration of benzol, there is a rapid disappearance of the white cells from the peripheral circulation. We have observed also an initial rise in some instances (experiments B, F, G, and H). The red blood corpuscles are much less affected than the white. In a few experiments (B, E, and G) the count remained unaltered, whereas in the majority of observations the red cell count was reduced by 50 per cent. or more.

The platelets showed usually the same general reduction in number, though to a less extent. We were impressed with the observation that the blood plates may remain at a high level at a time when the white cells have almost entirely disappeared from the circulation. In only one instance were we able to reduce the number of platelets so low as to reproduce the symptoms of hemorrhagic disease. Such experiments suggest the possibility that the megakaryocytes of the bone marrow either regenerate very rapidly or else are more resistant to the toxic action of benzol than are the forerunners of the polymorphonuclear leucocytes and erythrocytes.[1] This view,

[1] According to Selling's work (*loc. cit.* (3), p. 53), the polymorphonuclear

if correct, would help to explain the difficulty encountered in reproducing by benzol injections the features to which attention has been called.

Besides the formed elements, we have followed the factors of coagulation in the majority of our animals. These studies showed that benzol exerts an important influence in reducing the circulating prothrombin and that this substance is dependent for its production, in part at least, upon bone marrow activity. Two of the other factors of blood clotting, antithrombin and fibrinogen, were also followed in the majority of the experiments. These were found to fluctuate little from the normal.

Our results are in harmony with the recent proof that blood plates contain prothrombin. They give additional evidence in support of the view that fluctuations in the amount of prothrombin may be produced by substances which affect the number of platelets, and that any toxin which produces a reduction in their number will simultaneously cause a diminution in the available prothrombin of the circulating blood. Our knowledge, moreover, of the origin of blood platelets from the megakaryocytes (6) of the bone marrow emphasizes the importance of this tissue in maintaining the normal prothrombin equilibrium of the blood. Although the marrow plays this important part in the elaboration of prothrombin, no definite parallelism could be established between the extent of bone marrow injury, the number of blood plates, and the relative amounts of prothrombin. This would suggest that it is not possible to create a dangerous prothrombin deficiency by a reduction in the number of blood platelets alone, and that some other tissue or tissues play some part in prothrombin formation.

It will be seen, too, that the marrow in no way participates in the production of antithrombin and fibrinogen, for we did not observe any changes in these substances in the blood of animals with aplastic marrow.

## METHODS.

*Experimental Benzol Poisoning.*—The technique employed by us for producing aplasia of the bone marrow was the same as that used

amphophils in the marrow are more resistant than large lymphocytes, myelocytes, erythroblasts, and giant-cells.

by Selling. Because of the knowledge of the pathological lesions produced by benzol in the rabbit, gained from the fundamental observations of this worker, these animals were chosen for our experiments.

Injections were made subcutaneously with chemically pure benzol. The average dose injected was two cubic centimeters of benzol daily per kilogram of body weight, although smaller doses were used when the animals began to show signs of marked intoxication. Sudden death may follow the injection of too large doses. The acute symptoms accompanying such sudden deaths are referable chiefly to the central nervous system, sufficient time not having been allowed for the development of the myelotoxic effect of this poison. It is essential, therefore, to proceed slowly with smaller doses in order to produce the chronic type of poisoning which results in the typical changes in the hematopoietic organs.

We have found certain animals quite resistant to the poison. In some instances, many injections were necessary in order to produce the desired effect. Such resistance, however, is not the usual rule. Most animals succumb after ten doses, and at autopsy show fairly well marked aplasia of the marrow. It was our original plan to bring about an aplasia of the bone marrow, after which regeneration was to be permitted to occur, and to follow the fluctuations of the prothrombin as the animals were returning to a normal state. It was difficult, however, to obtain such an ascending series because repeated injections were necessary to produce demonstrable changes in the prothrombin content of the blood and these usually resulted fatally.

Variations in the white cell count and in the number of blood platelets give a fair index of the condition of the bone marrow, the counts dropping as the aplasia becomes marked. Selling[2] has pointed out that when the number of white cells has reached as low a level as 200 to 800 cells per cubic millimeter, any additional injections usually prove fatal. This has been our experience also.

Thus far we have been unable to reduce the blood platelets to a dangerously low level. In only three experiments did the number of platelets fall below 100,000, and in only one instance to 30,000. This observation may be of interest in connection with a point to

[2] Selling, *loc. cit.* (3), p. 11.

which attention will be directed later, that it is also impossible by this method to diminish sufficiently the prothrombin of the circulating blood so as to render these animals hemophilic.

*Obtaining Blood.*—The problem of obtaining specimens of blood from rabbits with the precautions which are necessary for this work was solved by resorting to cardiocentesis with a graduated Luer syringe. Before introducing the needle the interior of the syringe was coated with a thin layer of a mixture of white petrolatum 25 parts and ether 300 parts, and the needle was filled with salt solution. With a little practice it is possible to obtain blood in this way after one attempt. This precaution is important, since repeated thrusts of the needle into the heart may traumatize its muscle, setting free neutralizing tissue juices, and frequently producing a hemopericardium which may embarrass the heart's action and cause sudden death. By the exercise of skill it is possible to repeat cardiocentesis many times without endangering the life of the animal.

For our purposes ten cubic centimeters of blood sufficed. This was immediately emptied into a centrifuge tube containing two cubic centimeters of a 1 per cent. solution of sodium oxalate (made up in 0.9 per cent. solution of sodium chloride). After being thoroughly mixed the specimen was centrifugalized at a constant speed (a definite number of revolutions on our centrifuge) for ten minutes. The plasma, which in rabbits is usually watery or opalescent, due to a slight lipemia, was pipetted off and examined for the various factors of coagulation, according to the methods of Howell (7, 8).

*Enumeration of Formed Elements.*—Of all the methods proposed for the enumeration of blood platelets, that of Wright and Kinnicutt (9) appears to have given the most reliable results in the hands of recent workers. As used by Duke (5) this method has given constant results and was the one we used.

*Quantitation of Prothrombin, Antithrombin, and Fibrinogen.*—In the writings of Howell and his coworkers will be found a comprehensive description of the methods introduced by him for determining the amount of prothrombin and antithrombin in blood. In a recent paper the authors have already commented upon the various methods and their usefulness. For purposes of clearness, however, we wish to mention them briefly at this time, particularly with refer-

ence to certain precautions to be followed in carrying out the prothrombin test.

*Prothrombin.*—As yet no good method is available for the isolation and absolute quantitation of prothrombin in blood plasma. An idea of the relative amount or relative strength of the prothrombin in the blood may be obtained, however, by a simple method devised by Howell.[3] This depends upon the observation that the act of oxalating, that is, the decalcifying of the solution, intensifies the activation of prothrombin to thrombin by a subsequent calcification. This results apparently in a greater production of available thrombin for interaction with the fibrinogen of the plasma.

In practice the reaction is carried out as follows: To a series of tubes containing a constant amount of oxalated plasma is added dilute calcium chloride in varying amounts. Coagulation will result and the time of coagulation, as measured by the invertibility of the clot, will be shortest in the tube containing the optimum amount of calcium. Tested by this method, the clotting time of normal rabbit plasma averages about ten minutes.

To insure uniform results, it is necessary to take two precautions: first, the employment of an equal quantity of each reagent; second, centrifugalization of the plasma at a constant speed and for a constant number of minutes in all comparable observations. That the speed of this prothrombin reaction can be influenced by centrifugalization was shown by Lee and Vincent (10) and again emphasized by Howell in a recent publication (11). This was shown to depend upon variations in the thromboplastin content of plasma centrifugalized for different periods of time. Failure to observe these two points may lead to erroneous results in experiments where no wide differences exist between the quantity of prothrombin present in normal and in pathological blood.

*Antithrombin.*—The method of demonstrating the anticoagulating action of normal and pathological plasmas has been so clearly described in recent papers that only brief mention of the technique need be considered here. The test plasma is heated slowly to 60° C. and then centrifugalized to remove the fibrinogen and prothrombin. A drop of this plasma containing the antithrombin is then added to

[3] Howell, *loc. cit.* (7), p. 78.

known amounts of thrombin solution. After a short period of incubation, fibrinogen solution is added. The addition of the antithrombin delays or inhibits completely the action of thrombin on fibrinogen. In tests in which the thrombin is nearly neutralized by the antithrombin, it is very difficult to ascertain when clotting has occurred, since under such circumstances the clot forms in several stages. For purposes of uniformity we have adopted as the end-point of this reaction the first appearance of a delicate or filmy clot. In clinical tests the relative amount of antithrombin in the oxalated plasma of the patient is compared always with a similar specimen from a normal person; in our animal experiments the initial observation made upon the healthy animal always under exactly the same conditions, and with the same thrombin and fibrinogen solutions where possible, served as the control. Reference to one of the protocols given below will help to make clear the method of making the comparisons.

*Fibrinogen.*—The heat coagulation method of determining fibrinogen has also been described many times in recent communications. For details of this method reference should be made to papers by Whipple and Hurwitz (12) and by Whipple (13).

## EXPERIMENTAL DATA.

With the exception of one or two experiments, to which attention will be directed later, the observations may be divided into two main groups: first, those in which the animals showed great susceptibility to the poison, as indicated clinically by a great drop in the formed elements, and pathologically by an aplasia of the bone marrow of varying degree; second, those in which less striking reduction in the formed elements, particularly in the blood platelets, could be produced, and in which autopsy revealed either a less well marked aplasia or in a great many instances a marrow in which the regenerative process was keeping pace with the destructive changes. In the first group a diminution in the circulating prothrombin was demonstrable; whereas in the second group little or no change from the normal was observed.

In all, about fifteen complete experiments were performed. Of these the following two, one from each group, will be summarized,

since they are fairly complete and quite representative of all the other experiments.

TABLE I.

*Rabbit P.*

| Date. | Weight in gm. | Dose of benzol in c.c. | Formed elements. | | | Factors of coagulation. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Platelets. | W. B.C. | R. B. C. | Prothrombin. | | Antithrombin.[4] | | Fibrinogen in gm. per 100 c.c. |
| | | | | | | $CaCl_2$ | Min. | Thrombin. | Min. | |
| Nov. 11 | 1,400 | 2 | 920,000 | 7,800 | 5,200,000 | 1 | 11 | 3 | 15 | 0.719 |
| | | | | | | 2 | 15 | 4 | 5 | |
| | | | | | | 3 | 15 | 5 | 5 | |
| | | | | | | 4 | 15 | | | |
| Nov. 12–15 | | 6 | | | | | | | | |
| Nov. 15 | 1,350 | 2 | 480,000 | 3,200 | 4,700,000 | 1 | 40 | 3 | No clot in 60 | 0.656 |
| | | | | | | 2 | 31 | 4 | 6 | |
| | | | | | | 3 | 30 | 5 | 8 | |
| | | | | | | 4 | 38 | | | |
| Nov. 16–18 | | 4 | | | | | | | | |
| Nov. 19 | 1,175 | 1 | 185,000 | 1,900 | 3,710,000 | 1 | 60 | 3 | 23 | 0.684 |
| | | | | | | 2 | 60 | 4 | 7 | |
| | | | | | | 3 | 60 | 5 | 5 | |
| | | | | | | 4 | 60 | | | |
| Nov. 20–24 | | 8 | | | | | | | | |
| Nov. 25 | 1,100 | | 128,000 | 500 | 1,810,000 | 1 | 50 | 3 | 5 | 0.638 |
| | | | | | | 2 | 55 | 4 | 12 | |
| | | | | | | 3 | 65 | 5 | 5 | |
| | | | | | | 4 | 95 | | | |

PROTOCOL.

*Rabbit P.*—(Table I.) Nov. 11. Young brown male; weight 1,400 gm. Red blood count 5,200,000; white blood count 7,800; platelets 920,000; fibrinogen 0.719 gm. per 100 c.c. of plasma.

*Prothrombin Test.*

| Oxalated plasma. | $CaCl_2$, 1 per cent. | Coagulation. |
|---|---|---|
| 5 drops | 1 drop | 11 min. |
| 5 drops | 2 drops | 15 min. |
| 5 drops | 3 drops | 15 min. |
| 5 drops | 4 drops | 15 min. |

*Antithrombin Test.*[5]

| Heated plasma. | Thrombin. | Time interval. | Fibrinogen solution. | Coagulation. |
|---|---|---|---|---|
| 1 drop | 3 drops | 15 min. | 7 drops | 15 min. |
| 1 drop | 4 drops | 15 min. | 7 drops | 5 min. |
| 1 drop | 5 drops | 15 min. | 7 drops | 5 min. |

Benzol 2 c.c. given subcutaneously.

[4] Control solutions of fibrinogen and thrombin clotted in from two to four minutes.

[5] Control 2.5 to 3 min.

Nov. 12, 13, and 14. On each of these dates 2 c.c. of benzol were injected. Following the injections the animal became dull, drowsy, and inactive.

Nov. 15. Weight 1,350 gm.; red blood count 4,700,000; white blood count 3,200; platelets 480,000; fibrinogen 0.656 gm. per 100 c.c. of plasma.

*Prothrombin Test.*

| Oxalated plasma. | $CaCl_2$, 1 per cent. | Coagulation. |
|---|---|---|
| 5 drops | 1 drop | 40 min. |
| 5 drops | 2 drops | 31 min. |
| 5 drops | 3 drops | 30 min. |
| 5 drops | 4 drops | 38 min. |

*Antithrombin Test.*[6]

| Heated plasma. | Thrombin. | Time interval. | Fibrinogen solution. | Coagulation. |
|---|---|---|---|---|
| 1 drop | 3 drops | 15 min. | 7 drops | No clot in 1 hr. |
| 1 drop | 4 drops | 15 min. | 7 drops | 6 min. |
| 1 drop | 5 drops | 15 min. | 7 drops | 8 min. |

Nov. 16, 17, and 18. Daily injections of benzol amounting to 4 c.c. Animal is beginning to look very ill; very weak; does not eat. No paralysis of legs noted.

Nov. 19. Weight 1,175 gm. Red blood count 3,710,000; white blood count 1,900; platelets 185,000; fibrinogen 0.684 gm. per 100 c.c. of plasma.

*Prothrombin Test.*

| Oxalated plasma. | $CaCl_2$, 1 per cent. | Coagulation. |
|---|---|---|
| 5 drops | 1 drop | 60 min. |
| 5 drops | 2 drops | 60 min. |
| 5 drops | 3 drops | 60 min. |
| 5 drops | 4 drops | 60 min. |

*Antithrombin Test.*[7]

| Heated plasma. | Thrombin. | Time interval. | Fibrinogen solution. | Coagulation. |
|---|---|---|---|---|
| 1 drop | 3 drops | 15 min. | 7 drops | 23 min. |
| 1 drop | 4 drops | 15 min. | 7 drops | 7 min. |
| 1 drop | 5 drops | 15 min. | 7 drops | 7 min. |

Benzol 1 c.c. given subcutaneously.

Nov. 20, 21, 22, 23, and 24. Benzol 8 c.c. injected during these five days. Animal becoming very weak and emaciated.

Nov. 25. Weight 1,100 gm. Red blood count 1,810,000; white blood count 500; platelets 128,000; fibrinogen 0.638 gm. per 100 c.c. of plasma.

*Prothrombin Test.*

| Oxalated plasma. | $CaCl_2$, 1 per cent. | Coagulation. |
|---|---|---|
| 5 drops | 1 drop | 50 min. |
| 5 drops | 2 drops | 55 min. |
| 5 drops | 3 drops | 65 min. |
| 5 drops | 4 drops | 95 min. |

[6] Control 2.5 min.

[7] Control 3.5 min.

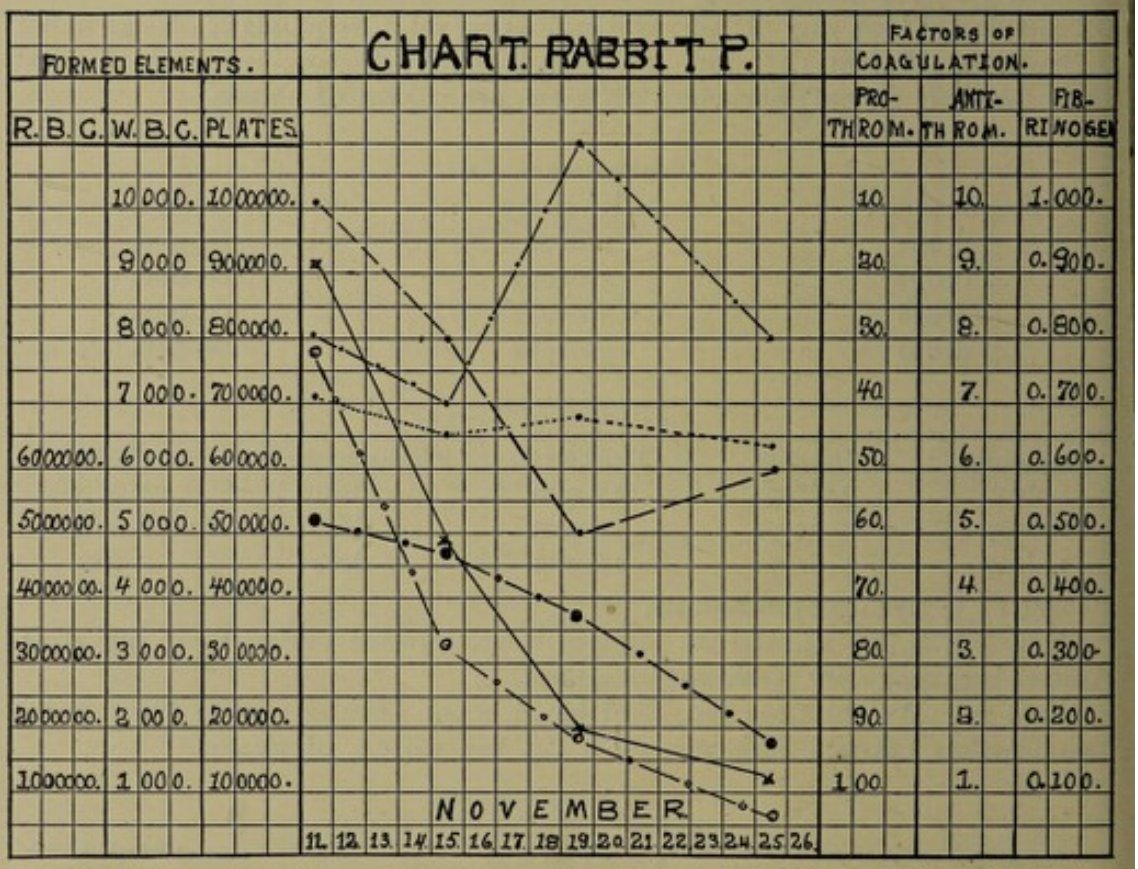
*Antithrombin Test.*[8]

| Heated plasma. | Thrombin. | Time interval. | Fibrinogen solution. | Coagulation. |
|---|---|---|---|---|
| 1 drop | 3 drops | 15 min. | 7 drops | 5 min. |
| 1 drop | 4 drops | 15 min. | 7 drops | 12 min. |
| 1 drop | 5 drops | 15 min. | 7 drops | 5 min. |

Nov. 26. Animal killed and autopsied at once. Serous cavities were all normal except for a small organized clot in the pericardial sac. The heart showed many small scars covered with fibrin at the points where the cardiac muscle was pierced in obtaining blood. No gross changes were demonstrable in any of the other organs. Bone marrow obtained from the middle of the femur was mottled brown, of fair consistency, with small pin-point hemorrhages.

Microscopical sections showed an aplastic marrow in the stage of fairly advanced regeneration. In some parts of the section the aplasia was found to be fairly complete, but superimposed upon the edematous aplastic reticulum there were groups and clumps of parenchymal cells; these consisted of granulocytes and normoblasts. Megakaryocytes were present in fairly large numbers.

Reference to the protocol and to table I and text-figure 1 will




TEXT-FIG. 1. Formed elements: heavy dots and dashes = red blood corpuscles; open circles and dashes = white blood corpuscles; solid line = blood platelets. Factors of coagulation: broken line = prothrombin; small dots and dashes = antithrombin; dotted line = fibrinogen.

[8] Control 3 min.

show the effect of benzol poisoning upon both the formed elements and the factors of coagulation in this particular animal. The observations, in this instance, were carried on over a period of two weeks. At the outset the animal was healthy, active, and weighed about 1.5 kilograms. Examinations of the formed elements and the factors of coagulation made at the beginning of the experiment (November 11) showed normal figures. It may be said, however, that the platelet count represents the high limit of normal, and that this is true also of the blood fibrinogen. Benzol injections were given daily, the average dose being two cubic centimeters. Only after four such injections were given did the animal begin to manifest any signs of intoxication. Studies of the blood at this time showed about 50 per cent. reduction in the blood platelets, a similar reduction in the white cells, a moderate grade of anemia, a prothrombin content (as measured by the coagulation time of the recalcified plasma) one-third of the control, an unaltered antithrombin content, and a very slight reduction in the blood fibrinogen.

As the benzol injections were continued, the animal began to exhibit more marked loss of weight, emaciation, anorexia, and dullness. No tendency to bleed was observed, however; but, as indicated in the protocol, both the formed elements and the prothrombin showed a still greater reduction at this time. At the end of two weeks, after twenty-three cubic centimeters of benzol had been injected, a final study of the blood was made. The platelets had fallen to 13 per cent. of the normal, and the prothrombin diminished to such an extent that the coagulation time was six times the control. The antithrombin and fibrinogen values were found to show normal variations.

For purposes of brevity the protocol for experiment G is not reproduced here. The results obtained on this animal are recorded in table II. In this experiment, an example of the second group, determinations of the formed elements and coagulation factors were made over a period of six weeks (September 1 to October 11). At the beginning of the experiment all the formed elements and the prothrombin showed normal values. Unfortunately the antithrombin and fibrinogen were not followed until several weeks after the benzol injections were commenced. Throughout the course of the experi-

TABLE II.

*Rabbit G.*

| Date. | Weight in gm. | Dose of benzol in c.c. | Formed elements. | | | Factors of coagulation. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Platelets. | W. B.C. | R. B. C. | Prothrombin. | | Antithrombin. | | Fibrinogen in gm. per 100 c.c. |
| | | | | | | $CaCl_2$ | Min. | Thrombin. | Min. | |
| Sept. 1 | 2,200 | | | 4,500 | 4,400,000 | 1 | 0 | | | |
| | | | | | | 2 | 9 | | | |
| | | | | | | 3 | 10 | | | |
| | | | | | | 4 | 11 | | | |
| Sept. 2–5 | | 6 | 840,000 Sept. 2 | | | | | | | |
| Sept. 5 | 2,040 | | 520,000 | 2,400 | 5,640,000 | 1 | 0 | | | |
| | | | | | | 2 | 17 | | | |
| | | | | | | 3 | 14 | | | |
| | | | | | | 4 | 45 | | | |
| Sept. 7–9 | | 4 | | | | | | | | |
| Sept. 9 | 1,800 | | 612,000 | 5,600 | 4,430,000 | 1 | 25 | | | |
| | | | | | | 2 | 22 | | | |
| | | | | | | 3 | 25 | | | |
| | | | | | | 4 | 17 | | | |
| Sept. 11 | | 2 | | | | | | | | |
| Sept. 13 | 1,640 | | 216,000 | 200 | 5,670,000 | 1 | 18 | | | 0.925 |
| | | | | | | 2 | 15 | | | |
| | | | | | | 3 | 19 | | | |
| | | | | | | 4 | 19 | | | |
| Sept. 14 | | 2 | | | | | | | | |
| Sept. 16 | 2,085 | 2 | 240,000 | 1,500 | 5,100,000 | 1 | 12 | 3 | 45 | |
| | | | | | | 2 | 9 | 4 | 10 | |
| | | | | | | 3 | 18 | 5 | 5 | |
| | | | | | | 4 | 9 | | | |
| Sept. 17–18 | 2,400 | 2 | 144,800 | 1,800 | 3,470,000 | | | | | |
| Sept. 19 | 2,085 | | 180,000 | 1,200 | 2,880,000 | 1 | Lost | 3 | 25 | 0.900 |
| | | | | | | 2 | 18 | 4 | 7 | |
| | | | | | | 3 | 18 | 5 | 7 | |
| | | | | | | 4 | 18 | | | |
| Sept. 22–26 | 1,890 | 4 | | | | | | | | |
| Oct. 2 | 1,830 | 2 | | 1,400 | 3,730,000 | 1 | 13 | 3 | 40 | 0.252 |
| | | | | | | 2 | 13 | 4 | 27 | |
| | | | | | | 3 | 12 | 5 | 10 | |
| | | | | | | 4 | 13 | | | |
| Oct. 3–4 | | 4 | | | | | | | | |
| Oct. 5 | 1,795 | 2 | 196,000 | 1,460 | 3,780,000 | 1 | 8 | 3 | No observation | 0.273 |
| | | | | | | 2 | 6 | 4 | 20 | |
| | | | | | | 3 | 8 | 5 | 20 | |
| | | | | | | 4 | 8 | | | |
| Oct. 6–11 | 1,425 | 16 | 276,000 | 1,400 | 4,230,000 | | | | | |

ment, this animal showed unusual resistance to benzol poisoning, and in spite of the development of transient palsy of the right leg, remained fairly healthy. As the intoxication progressed, the plate-

lets reached as low a level as 180,000, the white cells 1,200, and the red cells 2,800,000. Determinations of the coagulation time of the recalcified plasma during this period showed variations between nine and eighteen minutes, the time becoming less than normal (six minutes) toward the end of the experiment. This shows that the prothrombin content of the blood remained normal in spite of the long period of intoxication and the quite well marked drop in the platelets.

The first antithrombin determinations were made two weeks after the experiment began; this factor was followed subsequently, and at the completion of the experiment no change was noted from the initial determination.

The fibrinogen in the blood fell precipitously during the last three weeks of the experiment. From an initial high level of 0.900 of a gram it fell to 0.273 of a gram. At the time of the last determination, the animal was much emaciated, having lost about 400 grams in weight. This is the only instance in which we have observed such an extensive reduction in fibrinogen following benzol poisoning. We are not prepared to explain this, unless it is due to the gradual wasting and emaciation which developed during the long period of intoxication.

At autopsy we were interested to find that this animal showed evidences of a wide-spread regeneration of the bone marrow. Zones of aplasia still were present, but these were small. Parenchymal elements were found scattered diffusely in long strands throughout the edematous reticulum. Granulocytes and normoblasts were present in abundance, and the megakaryocytes were also found in considerable number. The relatively high platelet count, in spite of repeated benzol injections, is in keeping with such a regenerating marrow.

## SUMMARY OF RESULTS.

*Formed Elements.*—By referring to tables III and IV, it will be seen that all the formed elements are reduced materially in the blood of benzolized rabbits, although the leucotoxic action is the most pronounced. In normal rabbits the average leucocyte count is 7,820 with variations between 11,000 and 4,200. With few exceptions benzol injections cause a striking drop in the count. In our series the white cells dropped to an average level of 1,390, with varia-

TABLE III.

*Summary. Prothrombin Diminished. Antithrombin and Fibrinogen Normal.*

| Date. | Rabbit. | Weight in gm. | Total dose of benzol in c.c. | Formed elements. | | | Factors of coagulation.[9] | | | Bone marrow. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Platelets. | W. B.C. | R. B. C. | Prothrombin per min. | Antithrombin per min. | Fibrinogen in gm. per 100 c.c. | |
| Aug. 15 | B | | 18 | | 4,200 | 5,400,000 | 6 | | | Well marked aplasia. Reticulum aplastic and hyperemic; small nests of erythroblasts; megakaryocytes fairly numerous. |
| Aug. 24 | | | | | 0 | 5,480,000 | 35 | | | |
| Aug. 31 | E | 1,460 | 8 | 760,000 | 8,400 | 4,960,000 | 9 | | | Advanced aplasia. Edema and hyperemia of reticulum; practically no parenchymal elements remaining, excepting fixed tissue cells, lymphocytes, and polyblasts. |
| Sept. 5 | | 1,420 | | 520,000 | 3,100 | 4,430,000 | 14 | | | |
| Sept. 10 | | 1,200 | | 148,000 | 900 | 3,930,000 | 310 | | | |
| Oct. 2 | K | 1,380 | 12 | | 8,400 | 5,980,000 | 8 | 27 | 0.587 | Well marked aplasia (compare rabbit B). |
| Oct. 5 | | 1,305 | | 408,000 | 5,200 | 3,980,000 | 8 | | 0.679 | |
| Oct. 7 | | 1,300 | | 502,000 | 11,200 | 4,200,000 | 18 | 23 | 0.639 | |
| Oct. 10 | | 1,160 | | 336,000 | 20,800 | 2,130,000 | 34 | 15 | 0.899 | |
| Oct. 2 | L | 1,620 | 18 | | 6,400 | 4,200,000 | 13 | 31 | | Advanced aplasia. Extreme hyperemia (compare rabbit E). |
| Oct. 5 | | 1,550 | | 720,000 | 4,600 | 3,800,000 | 10 | 35 | 0.543 | |
| Oct. 11 | | 1,205 | | 364,000 | 2,200 | 3,000,000 | 24 | 19 | 0.702 | |
| Nov. 11 | N | 1,300 | 23 | 780,000 | 10,400 | 4,100,000 | 10 | 12 | Lost | Advanced aplasia. Almost complete disappearance of parenchymal elements (compare rabbits E and L). |
| Nov. 15 | | 1,300 | | 320,000 | 3,000 | 4,070,000 | 19 | 9 | 0.532 | |
| Nov. 19 | | 1,122 | | 234,000 | 1,400 | 2,800,000 | 39 | 11 | 0.524 | |
| Nov. 25 | | 910 | | 64,800 | 800 | 2,300,000 | 70 | 17 | 1.200 | |
| Nov. 11 | O | 1,460 | 23 | 620,000 | 9,200 | 5,800,000 | 15 | 8 | 0.510 | Advanced aplasia. Microscopic picture identical with that of rabbits L and N. |
| Nov. 19 | | 1,345 | | 242,800 | 3,200 | 3,980,000 | 39 | 14 | 0.438 | |
| Nov. 25 | | 1,170 | | 31,200 | 900 | 2,100,000 | 45 | 8 | 0.422 | |
| Nov. 11 | P | 1,400 | 23 | 920,000 | 7,800 | 5,200,000 | 11 | 8 | 0.719 | Aplastic marrow with early regeneration. Areas of complete aplasia with groups and islands of parenchymal cells, erythroblasts, and granulocytes. Megakaryocytes numerous but poorly preserved. |
| Nov. 15 | | 1,350 | | 480,000 | 3,200 | 4,700,000 | 30 | 7 | 0.656 | |
| Nov. 19 | | 1,175 | | 185,000 | 1,900 | 3,710,000 | 60 | 11 | 0.684 | |
| Nov. 25 | | 1,100 | | 128,000 | 500 | 1,810,000 | 50 | 8 | 0.638 | |
| Nov. 11 | Q | 1,640 | 23 | 530,000 | 8,800 | 5,400,000 | 12 | 8 | | Aplasia with well advanced regeneration. Long strands of parenchymal cells; megakaryocytes, both young and adult forms, in large numbers. |
| Nov. 15 | | 1,510 | | 524,000 | 4,700 | 4,200,000 | 16 | 9 | 0.226 | |
| Nov. 19 | | 1,440 | | 120,000 | 4,000 | 4,200,000 | 30 | 11 | 0.368 | |
| Nov. 25 | | 1,470 | | 76,000 | 980 | 2,800,000 | 50 | 9 | 0.321 | |
| Dec. 2 | R | 1,930 | 14 | 700,000 | 11,000 | 4,200,000 | 9 | 19 | 0.852 | Advanced aplasia. Only fixed tissue cells, lymphocytes, and polyblasts present. |
| Dec. 10 | | 1,600 | | 280,000 | 2,100 | 4,400,000 | 25 | 16 | 0.708 | |
| Dec. 12[10] | | | | | | | 75 | 7 | | |

[9] The figures in the prothrombin columns represent the coagulation time in minutes of the tube containing the

TABLE IV.

*Summary. Prothrombin, Antithrombin, and Fibrinogen Normal.*

| Date. | Rabbit. | Weight in gm. | Dose of benzol in c.c. | Formed elements. | | | Factors of coagulation. | | | Bone marrow. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Platelets. | W.B.C. | R.B.C. | Prothrombin per min. | Antithrombin per min. | Fibrinogen in gm. per 100 c.c. | |
| Sept. 1 | F | 2,125 | 15 | 600,000 | 6,600 | 5,110,000 | 8 | | | Aplasia.[11] Marked edema of reticulum; capillaries filled with red cells; clumps of erythroblasts and polyblasts; megakaryocytes numerous. |
| Sept. 5 | | 2,110 | | 560,000 | 3,300 | 5,640,000 | 17 | | | |
| Sept. 11 | | 2,010 | | 312,000 | 266 | 3,760,000 | 8 | | | |
| Sept. 1 | G | 2,200 | 46 | 840,000 | 4,500 | 4,480,000 | 9 | | | Marrow in state of active regeneration. Large islands of granulocytes separated by small areas of aplastic reticulum; numerous islands of normoblasts inside and outside of capillaries; megakaryocytes fairly abundant. |
| Sept. 5 | | 2,040 | | 520,000 | 2,400 | 5,640,000 | 14 | | | |
| Sept. 9 | | 1,800 | | 612,000 | 5,600 | 4,430,000 | 17 | | | |
| Sept. 13 | | 1,640 | | 216,000 | 200 | 5,670,000 | 18 | | 0.925 | |
| Sept. 16 | | 2,080 | | 240,000 | 1,500 | 5,100,000 | 9 | 20 | | |
| Sept. 19 | | 2,025 | | 180,000 | 1,200 | 2,800,000 | 18 | 13 | 0.900 | |
| Oct. 2 | | 1,830 | | | 1,400 | 3,730,000 | 13 | 25 | 0.252 | |
| Oct. 5 | | 1,795 | | 196,000 | 1,460 | 3,780,000 | 6 | 20 | 0.273 | |
| Sept. 14 | H | 1,600 | 4 | 744,000 | 6,600 | 6,400,000 | 11 | 10 | 0.716 | Hyperplasia of marrow. Parenchymal cells in dense masses and well preserved. |
| Sept. 16 | | 1,500 | | 254,000 | 10,200 | 4,550,000 | 15 | 12 | | |
| Sept. 14 | I | 2,375 | 6 | 460,000 | 7,800 | 5,410,000 | 13 | 9 | 0.123 | No sections. |
| Sept. 19 | | 2,205 | | 280,000 | 2,800 | 5,090,000 | 16 | 15 | 0.240 | |
| Dec. 2 | U | 1,510 | 14 | 650,000 | 8,000 | 4,900,000 | 10 | 7 | 0.432 | Regenerating marrow (compare rabbit G). |
| Dec. 10 | | 1,200 | | 570,000 | 3,800 | 3,700,000 | 20 | 11 | 0.768 | |
| Dec. 2 | T | 1,730 | 42 | 840,000 | 9,200 | 5,600,000 | 9 | 8 | 0.252 | Hyperplasia of marrow (compare rabbit H). Normal architecture preserved; no areas of aplasia; cellular elements increased in number; granulocytes and erythroblasts well preserved; megakaryocytes numerous, and of young and old type. |
| Dec. 10 | | 1,600 | | 412,000 | 6,300 | 4,900,000 | 20 | 6 | 0.211 | |
| Dec. 14 | | 1,550 | | 253,000 | 7,200 | 3,940,000 | 20 | 6 | 0.260 | |
| Jan. 8 | | 1,470 | | 328,000 | 2,000 | 2,800,000 | 9 | 5 | 0.728 | |

[11] Death occurred five days after the last prothrombin determination. On the day before death platelets were 208,000; white blood count 600; and red blood count 2,730,000.

tions between 3,800 and the complete disappearance of leucocytic elements from the circulating stream. The extent of reduction depends upon the susceptibility of the animal. We have records of only two instances in which the intoxication was associated with a leucocytosis. In one of these (experiment K), after an initial fall, the white cells rose rapidly to 20,800; autopsy gave no satisfactory explanation of this leucocytosis. In the second observation (experiment H) the leucocyte count rose forty-eight hours after the first injection. This may be explained by the initial stimulating action of the benzol.

Some reduction in the number of platelets of the circulating blood is invariably the rule. The fall in count, however, is not usually so pronounced, in most instances, as that of the leucocytes. The average platelet count in normal rabbits was found to be 683,400, with variations between 920,000 and 408,000. These averages approximate those recorded by Duke (4) (average 757,000, with variations between 1,200,000 and 380,000). Following benzol inoculations wide variations in the extent of platelet reduction occur. In our series the average number of platelets following benzol injections was 233,800. Only once were we successful in lowering the count to 31,000. This fact explains our inability to produce in these animals hemorrhagic symptoms, such as occur only after more extreme lowering of the platelet count.

Where the platelets and leucocytes were found much diminished in number, the marrow usually showed fairly well marked aplasia. It should be emphasized, however, that destructive changes rarely were noted without signs of regeneration. In some specimens megakaryocytes could hardly be found; whereas in others giant-cells were present in enormous numbers (experiment Q). In the latter case it was apparent that regeneration was going on at a very rapid rate. It is not unlikely that such rapid regenerative phenomena offer some explanation for the difficulty encountered in reducing the blood platelets in rabbits to a dangerously low level.

Although the erythroblastic tissue of the bone marrow suffers, the circulating erythrocytes are injured relatively little. From an average cell count of 5,100,000 the erythrocytes in our series fell to an average level of 3,500,000 following benzol injections. The lowest

reduction in number was obtained in experiment P, in which the red cells were reduced to 1,800,000. As marked an anemia as Selling noted in his clinical cases of benzol poisoning (640,000 and 1,150,000 red cells) apparently cannot be reproduced experimentally in this way.

*Antithrombin.*—Determinations of the amount of antithrombin in the blood were made in twelve of the fifteen experiments. Great care was exercised to keep the technique uniform throughout the series, and in seven of the experiments the same strengths of thrombin and fibrinogen solutions were used, so that these latter are comparable among themselves. In none of the examinations was the variation from the control found to be large. Of the twelve complete records, about six (experiments G, H, O, P, Q, and T) showed an unaltered antithrombin content following benzol inoculations; three (experiments K, L, R) showed a slightly diminished, and three (experiments N, I, and U) a slightly increased amount. The slight diminution or excess observed in some instances depends probably upon the period during which the observation was made. It will be seen from text-figure 1 (rabbit P) that there was an interval during the experiment (November 19) when the antithrombin of the blood was present in excess (compare also experiments G, L, and O). Such fluctuations in the relative amount of antithrombin are due most likely to variations in the supply of tissue juices (thromboplastin), for it will be recalled that during the normal circulation, platelets are continually undergoing dissolution, and that the thromboplastin which they furnish is capable of neutralizing antithrombin.

*Prothrombin.*—The relative amounts of prothrombin, before and after benzol injections, were determined by the method already described. It may be said, in general, that the time of coagulation of the recalcified plasma of normal rabbits was fairly constant, averaging about ten minutes, with variations between six and fifteen minutes. The average coagulation time of such plasma of benzolized rabbits was found to be about fifty minutes, although, as will appear from table III, wide variations of between twenty-four and three hundred and ten minutes were observed. It is of interest that in the latter experiment, which represents an isolated instance where the coagulation time was so markedly prolonged, the marrow

at autopsy showed the most complete aplasia observed in the entire series.

The delayed coagulation of the recalcified plasma of benzolized rabbits may be explained theoretically in one of two ways: it may be due, in the first place, to the presence of an excessive amount of antithrombin; or, in the second place, to a deficiency of prothrombin. In the preceding paragraphs it has been shown, however, that the amount of antithrombin is not altered materially by benzol injections; in fact, there may be relative diminution in the amount, so that the prothrombin deficiency may be even greater than the recorded coagulation time indicates. It is fair to assume, therefore, that the delay in the coagulation time of the recalcified plasma is due to an actual deficiency of prothrombin, and not to an excess of antithrombin.

An examination of table III will show, furthermore, that this prothrombin deficiency does not parallel absolutely the reduction in blood platelets. For instance, certain animals with a much lower count may show a less marked diminution in prothrombin than animals with a higher count (compare experiments E, P, and U with O and Q). Such findings are readily explainable, however, if it is kept in mind that regeneration of the myeloid tissue usually goes hand in hand with aplasia, and that the number of platelets at any given time will depend largely upon the degree of regeneration. This fact helps also to explain why such difficulty is encountered in lowering both the platelets and the prothrombin of the circulating blood to a dangerous point.

No definite parallelism was demonstrable between the prothrombin deficiency and the reduction in leucocytes. The white cells may be much reduced or absent at a time when the prothrombin content is little altered (experiments F and G), or there may be some deficiency in prothrombin associated with a leucocytosis (experiment K). In general, however, a prothrombin deficiency has been found associated with a reduction in the number both of platelets and leucocytes.

*Fibrinogen.*—All the fibrinogen determinations were made by the method of heat coagulation. In every instance the reading was corrected for the oxalate solution used in receiving the blood, so that

the results represent the quantity of fibrinogen in 100 cubic centimeters of actual plasma. Wide fluctuations in the blood fibrinogen of normal rabbits were observed. The average was 0.560 of a gram per 100 cubic centimeters of plasma, but variations between a minimum of 0.123 of a gram, and a maximum of 0.925 of a gram were noted. This is in harmony with Whipple's figures for normal healthy dogs (average 0.466 of a gram; minimum 0.198 of a gram; maximum 0.867 of a gram) (13). In almost all instances repeated injections of benzol (four to forty-two cubic centimeters) caused a rise in the blood fibrinogen. The average of the final readings made from two to thirty days after the initial determination was found to be 0.617 of a gram per 100 cubic centimeters of plasma, but in some individual observations enormous rises were observed (experiments I, K, L, N, Q, T, and U). In about three experiments (O, P, and R) little variation from the normal occurred, and in only one instance (experiment G) was there a striking reduction in the blood fibrinogen. A possible explanation for this drop has been found in the marked loss of weight and extreme cachexia produced in this animal by the benzol inoculations. Similar reductions in the quantity of fibrinogen have been reported by Whipple in human cases of cachexia. The low values in such cases are difficult to explain.

The rise in blood fibrinogen observed in the majority of animals following benzol injections has been observed also in other conditions associated with intoxication.[12] It is possible that benzol may stimulate the fibrinogen-forming organs to over-functional activity and that the fibrinogen may rise above normal at these times. Such an increase is not out of keeping with the gross anatomical and the histological appearance of the liver in benzol poisoning. Aside from a slight degree of parenchymatous change, no other pathological lesion was demonstrable in the liver parenchyma.

### THE RELATION OF THE BONE MARROW TO THE ORIGIN OF PROTHROMBIN, ANTITHROMBIN, AND FIBRINOGEN.

The experiments and observations already detailed throw some additional light upon this more complex problem of coagulation. In-

[12] Whipple, *loc. cit.* (13), p. 57.

terest in the origin of the various substances concerned in the clotting of blood has stimulated much work among investigators in this field. From time to time different tissues and organs have been made responsible for the elaboration of the factors participating in the process; and, although there are still some workers (14, 15, 16) who ascribe to the liver the important function of forming all the fibrin factors, the majority are agreed that certain of the formed elements of the blood play an important part in the origin of some of these substances. Without entering upon a discussion of the experimental basis for their views, we wish to review briefly some of the work of different observers which is or is not in harmony with our deductions.[13]

*Origin of Prothrombin.*—With the exception of Wooldridge[14] all workers since Alexander Schmidt (17) have recognized the importance of the formed elements of the blood in the elaboration of prothrombin. The view, which originated with Schmidt, that the leucocytes give rise to this fibrin factor has been discredited largely by later work (11, 18). Bizzozero (19) was the first to suggest the platelets as the possible prothrombin formers. To him, too, we owe the knowledge that these elements are independent morphological structures and not degeneration products of leucocytes or erythrocytes. The work of Morawitz (18) has furnished the necessary experimental evidence that the theory of Bizzozero and others was correct. Both Morawitz and more recently Bayne-Jones (20), working in Howell's laboratory, have demonstrated conclusively that aqueous extracts of platelets obtained by differential centrifugalization contain a substance which will clot fibrinogen solutions in the presence of calcium. They have shown further that platelets contain another substance, thromboplastin, so that the disintegration and solution of platelets, when blood is shed, facilitate clotting in two ways: first, by setting free prothrombin; second, by liberating a thromboplastic substance which hastens coagulation by neutralizing the antithrombin present normally in circulating blood.

*Origin of Antithrombin.*—It has long been known that the intravenous injection of peptone solutions into animals gives rise in the blood of these animals to a substance which is capable of inhibiting coagulation (Schmidt-Mülheim, 1880). The French School, in particular, has done much experimental work to learn in what organ and by what mechanism this anticoagulation substance is formed. The work of Contjean (21), Delezenne (22), and others has established with certainty that the liver is essential for the formation of this substance, and that antithrombin is not formed if the liver be excluded. There is no direct evidence, however, that this coagulation-inhibiting substance is of the same nature as the antithrombin present normally, in greater or less amount, in

[13] Excellent discussions of this phase of the subject will be found in the papers by Morawitz (prothrombin), Delezenne (antithrombin), and Whipple and Hurwitz and Meek (fibrinogen).

[14] Cited by Morawitz (18).

he blood of animals and man. Their identity is assumed because they both act n a similar way. In this connection it is of interest to note that Whipple (23), n a study of clinical cases, observed fluctuations in antithrombin in the blood of atients suffering from liver disease and in one patient with aplastic anemia. n the latter instance antithrombin was present in excess, although the marrow vas completely aplastic. It would appear, therefore, that the reaction of the one marrow is not a factor in the production of antithrombin. This observaion is in harmony with the conclusion deduced from our experiments; namely, hat aplasia of the marrow in rabbits produces no appreciable change in the antihrombin of the blood.

*Origin of Fibrinogen.*—An extensive discussion concerning the origin of blood ibrinogen is not needed, as this phase of the subject has been treated fully in everal recent papers (13, 24). Suffice it to say that various workers have dduced evidence in support of one or other of the following theories regarding ts origin: first, that fibrinogen is formed by the intestine (Mathews (25)); econd, that fibrinogen is either produced in the liver or is wholly dependent pon that organ for its production (Doyon (26) and his coworkers, Nolf (27), Whipple and Hurwitz (12), and Meek (24)); third, that fibrinogen is formed by the bone marrow and is perhaps dependent upon the white cells (Müller (28), Morawitz and Rehn (29)).

It would appear that the best experimental evidence is in favor of the second view, although recent studies (Goodpasture (30)) on the regeneration of fibrinogen show that normal fibrinogen production is a result of the combined activity of the liver and the intestine, the latter organ being an important contributing factor in the rapid formation of fibrinogen.

As far as the authors are aware, no direct experimental proof has been produced for or against the view of Müller and of Morawitz and Rehn that the bone marrow is a fibrinogen former. Only a few casual observations are recorded by recent workers upon this function of the marrow. Whipple and Hurwitz[15] noted that with the rapid drop in fibrinogen following chloroform poisoning, there may be a leucocytosis caused by the liver necrosis and repair, and that the bone marrow at such a time may be hyperplastic. Conversely, Whipple,[16] in a study of a clinical case of aplastic anemia, has shown the blood fibrinogen to be normal (0.4032 of a gram), whereas the bone marrow at autopsy was found to be completely aplastic. Similarly in our observations upon experimental aplastic anemia in benzolized rabbits, we have seldom noted low fibrinogen values associated with aplasia of the bone marrow. In almost all our observations the fibrinogen was found either little changed from the normal or somewhat higher than normal. In only one experiment (G) was there any appreciable drop in fibrinogen, and we believe that this has been satisfactorily explained. It seems fair to conclude from such evidence that the bone marrow does not participate in the production of blood fibrinogen.

[15] Whipple and Hurwitz, *loc. cit.*, p. 138.

[16] Whipple, *loc. cit.* (13), p. 59.

DISCUSSION.

The experimental observations recorded have shown that injur to the myeloid tissue by benzol causes a diminution in the prothrom bin content of the blood, and that this tissue must be intimately asso ciated with the elaboration of this substance. Our experiments offe no convincing evidence, however, as to the relative importance o the various formed elements of the marrow in prothrombin produc tion; but the more direct proof given by other workers makes it fairly certain that the platelets and not the leucocytes are the essential ele ments. Neither Morawitz (18) nor Howell (11) have been able to obtain satisfactory proof that the white cells contain any appre ciable amount of prothrombin, whereas both workers have prepared active prothrombin solutions from aqueous extracts of blood plate lets. In view of these observations the conclusion appears justified that the blood platelets play more than a mere mechanical part in the process of coagulation. Presumably, during the normal circulation, these elements undergo more or less gradual dissolution and thus help to maintain the prothrombin equilibrium of circulating blood.

Already attention has been directed to the lack of parallelism be tween the drop in prothrombin and the extent of destruction of myeloid tissue. This finding is in keeping with the experimental and clinical observations of other workers. Pratt (31), for in stance, has shown that there is no direct interdependence between the number of platelets and the coagulation time. More recently Duke has emphasized this point by showing that blood in which the plate lets are reduced to 10 per cent. of normal by repeated benzol injec tions may clot at a normal rate. Undoubtedly in such extreme cases the delayed bleeding time must depend upon the mechanical impor tance of these elements, for it is well known that agglutination of platelets is essential to effective clotting. It follows, therefore, that the platelet count and the circulating prothrombin may be con siderably diminished without causing symptoms of hemophilia.

On the whole, the conservative conclusion would seem to be that the maintenance of the prothrombin equilibrium of the blood de pends only in part upon the blood platelets. Besides the facts al ready mentioned, there is other evidence in support of this view. It is well established, for instance, that the platelet count may be

erfectly normal in hemophilia in which the coagulation time may e markedly prolonged in consequence of a deficiency in the amount f the contained prothrombin (7). Furthermore, in a recent pub-cation, Howell (11) has shown that prothrombin is present in solu-on in the circulating lymph, although blood platelets do not con-itute a normal element of this fluid. The delayed clotting of mph depends rather upon a deficiency in the contained thrombo-lastin, and consequently upon a relative excess of antithrombin. uch evidence suggests the possibility that some tissue in addition to ne bone marrow is concerned with prothrombin formation. This iew is supported also by the knowledge, to which reference has lready been made, that the elaboration of fibrinogen depends upon ne combined activity of at least two organs,—liver and intestine. The maintenance of the prothrombin equilibrium of the blood is bviously so important for the organism that the assumption that ther tissues or organs participate in this process harmonizes better vith our present conception of the factors of safety in physiological rocesses emphasized by Meltzer.

Our experiments afford no support for or against the view of Nolf and others that liver cell activity is essential for prothrombin roduction, nor do they make it clear what tissues besides the narrow play a part in its elaboration.

## CONCLUSIONS.

1. Subcutaneous injections of benzol in rabbits produce marked destructive changes in the hematopoietic organs, especially in the myeloid tissue.

2. Benzol poisoning registers a change not only in the formed elements of the blood, but also in the factors of coagulation.

3. The circulating prothrombin is considerably reduced in amount and in most instances animals in which such a diminution occurs show aplasia of the bone marrow.

4. The association of extreme aplasia of the marrow without a fatal diminution in the circulating prothrombin suggests one of two possibilities: either other tissues and organs in addition to the bone marrow are concerned with prothrombin formation; or a minimum amount of myeloid tissue suffices to maintain the quantity of pro-thrombin above a dangerous level.

5. The myeloid tissue plays no part in the production of antithrombin.

6. Bone marrow activity is not essential for the production of fibrinogen.

In conclusion we desire to express our appreciation to Dr. Katherine R. Drinker for numerous fibrinogen determinations, and to the Pathological Department of the Peter Bent Brigham Hospital for many courtesies.

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