THE COAGULATION OF BLOOD AND THE EFFECT OF CERTAIN DRUGS AND TOXINS UPON IT

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One of the chief difficulties in experiments on blood coagulation time is the elimination of all variable factors affecting the result of the observations. Before it is justifiable to attribute to any particular drug or condition a definite effect on the time of coagulation it is necessary to take into consideration any possible disease, also the effect of diet and exercise.

Before investigating the influence of drugs on animals, I instituted a series of observations on the coagulability of the blood of a healthy man and found that the diurnal variations were considerable; food especially had an effect in prolonging the time of coagulation. That the condition was due to food was definitely shown as the changes did not occur during fasting.

It is, therefore, difficult to determine a normal coagulation time for a series of observations which holds good for more than a short period. If an experiment extends over twelve hours or more, variations due to food, drink or accidental circumstances will occur; for this reason in drawing conclusions from experiments only marked differences in coagulation time suffice as evidence, as smaller differences do not exclude the natural variations and experimental error.

The term 'coagulation time' requires some notice. Blood in contact with the vessel walls of the body does not clot, but may do so if the condition of these walls be modified by injury or disease. No satisfactory explanation can be given as to why blood in the living body should remain fluid and when shed it should coagulate, but it is known that the more nearly the conditions resemble those obtaining when the blood is in the circulation the longer will the clotting be delayed; thus blood can be kept fluid for a long time in a piece of blood vessel ligatured at both ends and which has been recently

removed from the living body. Contact with a foreign body hastens coagulation, and the larger the relative surface of the foreign body the greater will be the effect. A rise in temperature slightly above that of the normal also hastens coagulation although a low temperature retards it. Hence there is no absolute coagulation time, but only a coagulation time for each set of conditions.

In making experiments on the coagulability of the blood those observations will be the most accurate in which the physical conditions of the blood when drawn are interfered with as little as possible. If the experimental conditions are such that the normal coagulation time is very short, then it is conceivable that those conditions may partly obliterate any differences due to small variations from this normal, and although the observations may be compared relatively, still as the time is shorter the range of experimental error is increased.

The effect of a drug on the coagulation time of the blood may be either direct or indirect. Thus a drug like calcium chloride on entering the circulation may directly affect the coagulability of the blood whereas the administration of another drug may institute a series of metabolic changes which again may modify the time of coagulation. In the latter case, therefore, the effect of the drug is only indirect. The injection of β -naphthylamine into an animal like the rabbit leads to an elevation of the temperature, and as the result of observation I have found that the coagulation time is increased; the question then arises as to whether this increase is the result of abnormal oxidation in the tissues which may accompany the pyrexia, and therefore only indirectly dependent on the drug, or whether it is the direct effect of the drug on the blood tissue.

I give this instance as a sample of the difficulties which have to be overcome in drawing precise conclusions as to the influence of a drug on the coagulation time.

METHOD

Brodie and Russel's coagulometer has been employed in the great majority of the present experiments.

In addition to the precautions usually given it is necessary to

have the same number of drops of water in the central chamber during a series of observations, and each series of experiments should be undertaken by the same observer, the mean of as many observations being taken as possible.

Although there is much to recommend this method, a fallacy may occur which is difficult to remedy. When there is a considerable diminution of the surface tension of the blood, the size of the pendant drop is much altered and therefore the blood is exposed to a relatively larger surface of glass, consequently the observed coagulation time is unduly shortened. This condition was obtained in a particularly pronounced degree when experimenting with diphtheria toxin; it is, therefore, difficult to compare the later observations with the normal coagulation time taken at the beginning of the experiment. In such conditions better results may be obtained by employing Wright's method.¹ Under ordinary conditions Wright's method allows the blood to clot much quicker than is desirable in consequence of the relatively large area of the surface of the capillary tubes to the contained blood.

It is of interest to contrast the results obtained by the two methods, and I give below, therefore, an observation on two samples of blood drawn from a man in normal health and taken at the same time, thus:—Coagulation time taken by Brodie and Russel's instrument (mean of three experiments), three minutes ten seconds. Coagulation time taken by Wright's instrument, two minutes thirty seconds (mean of three experiments). There is a difference of forty seconds between the two determinations, and I prefer the former method because, as the time of clotting is longer, the percentage of error must be smaller.

For the purpose of these experiments rabbits were found to be by far the most suitable animals. It is easy to shave and cleanse the ear and obtain a specimen of blood by pricking the marginal vein. Drugs were injected subcutaneously with a hypodermic syringe. The experiments were so conducted that the animal received just a

1. Wright and Paramore, *Lancet*, 1905, p. 1096.

sufficiency of food during an extended experiment, and none at all in a short one of a few hours duration, and by this means the experimental error due to natural variations after food, &c., was reduced. When it was found necessary to obtain counts of the number of white corpuscles synchronously with the coagulation time, the Thoma-Zeiss method was employed; the blood issuing from a puncture of the marginal vein served for both observations.

In the experiments with β -naphthylamine the variations of the body temperature of the animal were taken by means of a flat-bulbed rectal thermometer, the rabbit being secured by the hind and fore-legs during the process.

EXPERIMENTS

It is convenient first to discuss some of the results of the observations on the variations of the coagulation time in a healthy man particularly with respect to food, rest and exercise.

Experiment I.—Man, age 31, in normal health. The time of meals and other details are given in relation to the observations on the coagulation time.

9 a.m.	...	...	Breakfast.
11 a.m.	...	...	Coag. time, 3 minutes 10 seconds.
11 a.m. to 1.30 p.m.			Walking exercise.
1.30 p.m.	...	...	Dinner.
2.30 p.m.	...	...	Coag. time, 5 minutes 30 seconds.
3 to 5 p.m.	...	...	Walking exercise.
5.30 p.m.	...	...	One cup of tea.
7.30 p.m.	...	...	Coag. time, 3 minutes 50 seconds.
8.15 p.m.	...	...	Supper.
10 p.m.	...	...	Coag. time, 4 minutes 30 seconds.
11 p.m. to 7 a.m.	...	...	Rest in bed.
8 a.m.	...	...	Coag. time, 2 minutes 30 seconds.
(Fasting before breakfast)			

Experiment II.—Healthy man, age 26.

6.30 p.m.	...	...	Coag. time, 3 minutes 30 seconds.
7.15 p.m.	...	...	Dinner.
8.30 p.m.	...	...	Coag. time, 5 minutes 10 seconds

Experiment III.—Effect of exercise. Subject same as in Experiment I.

11 a.m.	...	...	Coag. time, 3 minutes 23 seconds.
11 a.m. to 11.20 a.m.	Fairly severe muscular exercise with dumbbells and Sandow Exerciser.		
11.25 a.m.	...	...	Coag. time, 3 minutes 10 seconds.

Experiment IV.

5 p.m.	...	...	Coag. time, 3 minutes 40 seconds.
5 to 5.15 p.m.	...	...	Severe muscular exercise with dumbbells.
5.20 p.m.	...	...	Coag. time, 3 minutes 25 seconds.

All these observations were made in duplicate.

On examining the observations in Experiments I and II, it is seen that the longest coagulation time occurs one hour after the principal meal of the day, the blood becoming less coagulable as absorption becomes established after the meal. Conversely, the shortest time is recorded when the subject is fasting before breakfast.

As regards the effect of exercise, Experiments III and IV show a certain amount of shortening in the time of coagulation after fifteen or twenty minutes severe physical exertion. It is possible that the specific gravity of the blood may be slightly increased after loss of fluid from the plasma of perspiration, but this was not very great during the experiments and, therefore, it is probable that the slight increase in coagulability was independent of any alteration of the specific gravity. Food and drink, therefore, are the more potent factors in modifying the time of coagulation of the blood. These experiments are typical examples taken from a large series.

The different experiments on animals are classed together in groups, and the conclusions which have been arrived at in each group of experiments are discussed separately.

Group I. Deals with the action of calcium chloride, citric acid and milk on the coagulation time of the blood.

Group II. Includes drugs which are capable of producing leucocytosis after hypodermic injection, such as colchicine and nuclein; the effect of such drugs on the coagulation time are contrasted with the effect of certain pathological conditions accompanied by leucocytosis such as catarrhal pneumonia and appendicitis.

Group III. Deals with agents which dilute the blood, such as normal saline solution.

Group IV. Deals with the effect of β -naphthylamine and the influence of temperature.

Group V. Includes experiments with diphtheria toxin.

GROUP I

Experiment I.—(Citric acid). Weight of rabbit, 3.2 kilos. Normal coagulation time taken at the beginning of the experiment 2 minutes 35 seconds. .5 gram. of citric acid was injected subcutaneously at 4 p.m.

	Time of observation			Coagulation time
Commencement	4 p.m.	...	...	2 min. 35 secs.
	4.15 p.m.	...	...	2 min. 40 secs.
	4.40 p.m.	...	...	3 min. 5 secs.
	5.15 p.m.	...	...	4 min. 10 secs.

Experiment II. Weight of rabbit, 2.1 kilos. Normal coagulation time, 3 minutes. .5 grams. citric acid injected subcutaneously at 3.20 p.m.

	Time of observation			Coagulation time
Commencement	3.20 p.m.	...	...	3 mins.
	4 p.m.	...	...	3 min. 10 secs.
	4.20 p.m.	...	...	5 min. 45 secs.
	5 p.m.	...	...	5 min. 50 secs.

Both these experiments show very considerable lengthening of the coagulation time within one hour from the injection of the drug. We have reason to believe that citric acid combines with some of the calcium in the blood to form calcium citrate, if, therefore, by this means the calcium were removed from the circulation it is quite conceivable that the coagulation would be modified, but calcium citrate is soluble in the blood so that we might expect the calcium to exert its specific effect on the coagulation. The fact that this is not so may be explained by the views of Sabbatani¹ and C. J. Martin,² who have shown independently, and at the same time, that citrates act by removing the calcium ions and so preventing the formation of fibrin ferment. C. J. Martin has carried the matter a step further by demonstrating that this is brought about by the formation of a complex body containing the calcium

This substance ionises into (Na ion -) and (Ca Cit. ion +) so that on electrolysis the calcium passes to the anode.

1. Sabbatani, *Arch. Ital. de Biol.* XXXIX, 333, 1903.

2. Martin, C. J. A communication to the Physiol. Soc. at the Lister Instit., 1904.

Dr. Dixon has given me the records of the blood pressure of a cat ; the animal was receiving intravenous injections of sodium citrate, in doses of 5 c.c. of a 5 per cent. solution, at intervals during the experiment, and the coagulation time of the blood was being determined about seven minutes after each fresh injection. It was found that although the coagulability of the blood was at first decreased, as when the drug is given under the skin, yet ultimately, after continued injections of sodium citrate, a reverse effect was produced, the blood taking as long as seven and ten minutes to clot.

Experiment III.—Weight of rabbit, 3·2 kilos. Normal coagulation time, 3 minutes 5 seconds. ·1 gram calcium chloride in 1 c.c. normal saline injected, subcutaneously, at 3·55 p.m.

	Time of observation				Coagulation time
Commencement	3·55 p.m.	...	...	...	3 min. 5 secs.
	4·25 p.m.	...	...	...	1 min. 45 secs.
	4·55 p.m.	...	...	...	1 min. 15 secs.

Experiment IV.—Weight of rabbit, 2·6 kilos. Normal coagulation time, 3 min. 10 secs. ·1 gram of calcium chloride injected at 12·5 p.m.

	Time of observation				Coagulation time
Commencement	12·5 p.m.	...	...	...	3 min. 10 secs.
	12·35 p.m.	...	...	...	2 min. 5 secs.
	1 p.m.	...	...	...	1 min. 55 secs.
	1·15 p.m.	...	...	...	1 min. 20 secs.
	3·0 p.m.	...	...	...	1 min. 15 secs.

The coagulation time is very much reduced in the short space of one hour and, as seen in the last experiment, the condition remains constant after three hours.

In order to contrast the effect of calcium chloride on the coagulation time when injected hypodermically, with the effect produced when given by the mouth, ·5 gram $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ was dissolved in 5 c.c. of water and the whole solution absorbed by soft bread. This was given to a fasting rabbit and entirely devoured, the whole of the CaCl_2 being thus ingested. The following two protocols show the results :—

Experiment IV (a).—Weight of rabbit, 2·8 kilos. Normal coagulation time, 3 mins., 5 secs. .5 gram. of calcium chloride given by the mouth at 2 p.m.

Time of observation			Coagulation time
Commencement	2 p.m.	...	3 min. 5 secs.
	2.30 p.m.	...	3 min. 10 secs.
	3.30 p.m.	...	2 min. 55 secs.
	4.30 p.m.	...	2 min. 58 secs.
	5.30 p.m.	...	2 min. 55 secs.

Experiment IV (b).—Weight of rabbit, 2·6 kilos. Normal coagulation time, 2 min. 50 secs. .5 gram. of calcium chloride given by mouth at 6 p.m.

Time of observation			Coagulation time
Commencement	6 p.m.	...	2 min. 50 secs.
	6.30 p.m.	...	2 min. 55 secs.
	7 p.m.	...	2 min. 52 secs.
	8 p.m.	...	2 min. 45 secs.
	10 p.m.	...	2 min. 45 secs.

On examining the last two experiments it is obvious that the time of coagulation is not appreciably affected four hours after the .5 gram. of calcium chloride had been given by the mouth, whereas .1 gram. given hypodermically very considerably shortened the coagulation time within less than an hour from the administration. I am, therefore, driven to conclude that absorption of the calcium from the stomach and intestinal canal is either not appreciable or much delayed. If this be so, it is clearly of little use to expect any considerable alteration of the coagulability of the blood in man after the administration of the drug by the mouth in ordinary doses.

Before leaving the subject of calcium chloride, it is necessary to add a word of warning on the hypodermic injection of the drug. Recently, on Dr. Dixon's suggestion, calcium chloride was injected under the skin of a patient in order to arrest an hæmoptysis which had continued for two days. The hæmorrhage stopped completely within half an hour, but a small patch of gangrene about the size of a shilling formed on the skin at the site of the injection. If calcium chloride be injected hypodermically, the solution must not be too concentrated, nor contain more than two or three grains of the salt.

As of late years many statements have appeared as to the effect of milk (indirectly through its calcium) in shortening the time of

clotting of the blood, some experiments were performed to test the validity of the statements.

Experiment V.—Weight of rabbit, 1·18 kilos. Normal coagulation time, 3 min. 10 secs. Ten cubic centimetres of milk were injected subcutaneously at 4.30 p.m.

Time of observation				Coagulation time
Commencement	4.30 p.m.	...	...	3 min. 10 secs.
	5 p.m.	...	...	3 min. 5 secs.
	5.30 p.m.	...	...	3 min.
	6 p.m.	...	...	3 min. 20 secs.
	9.30 a.m. (next day)			2 min. 50 secs.

Experiment VI.—Weight of rabbit, 3 kilos. Normal coagulation time, 3 min. 50 secs. Ten cubic centimetres of milk injected at 4 p.m.

Time of observation				Coagulation time
Commencement	4 p.m.	...	...	3 min. 50 secs.
	4.30 p.m.	...	...	3 min. 55 secs.
	5 p.m.	...	...	3 min. 30 secs.
	6 p.m.	...	...	3 min. 45 secs.

These results clearly show that milk has no decided action in increasing the coagulability of the blood. It is true that there is a slight shortening of the coagulation time at the termination of each experiment, but this is so small as to fall even within the limits of experimental error. It is also possible that, in view of the length of time occupied for the first experiment, other factors may have been introduced which would influence the later observations. It will be noticed that the weight of the rabbit used in the former experiment was 1·18 kilos.; if the weight of the blood be taken as one-thirteenth of the body weight, it may be assumed to be roughly about 81 grammes, and, therefore, its volume is about 80 cubic centimetres. Ten cubic centimetres of milk were injected, which is, therefore, a very considerable ratio when compared to the volume of the blood.

The effect of maternal milk on the coagulation time of suckling mammals was next determined.

Experiment VII.—A suckling rabbit was used for this experiment (weight 155 grammes). The mean of three observations gave 3 minutes 5 seconds as the coagulation time).

Experiment VIII.—Another suckling rabbit was taken (weight 140 grammes). The mean of three observations on the coagulation time was 2 minutes 50 seconds.

I therefore conclude that the coagulability of the blood varies but little from that of the average normal for adult rabbits.

These results on animals are not concordant with the experiments on the coagulation time of the blood of infants, as mentioned by Wright and Paramore. These observers noted the time of coagulation of the blood of six infants ranging from six weeks to six months in age; two were fed on human milk, one on human milk supplemented by cows' milk, and three on cows' milk alone. In all the six cases the maximum coagulation time did not exceed thirty-seven seconds. It must be remembered, however, that the method of capillary tubes employed by Wright and Paramore gives a relatively short coagulation time.

EXPERIMENTS ON THE EFFECT OF MILK DIET IN MAN

Case I.—M. C., woman, age 26. Health normal. The diet consisted of two pints of milk and a milk pudding for the twenty-four hours. At commencement of experiment on January 20, 6 p.m., the coag. time was 3 minutes, 10 seconds; on January 21, at 5 p.m., 3 minutes, 5 seconds.

Case II.—H. C., man, age 28. Health normal. Two pints of milk were added to the ordinary dietary for the twenty-four hours.

February 1.—12.30 p.m., the coag. time was 3 minutes, 25 seconds.

„ 2.—9 p.m., „ „ „ 4 „ 3 „

It does not appear from these results that milk as a diet increases the coagulability of the blood.

It is mentioned in Schafer's 'Text Book of Physiology'¹ that part of the calcium present in milk is united more or less firmly to the caseinogen and the excess of this base over the mineral acids to organic acids such as citric acid. The negative effect of calcium citrate on the coagulation time of the blood has already been discussed, when it was shown how it differed from salts composed of calcium and the mineral acids.

It would not, therefore, be surprising if the administration of milk produced no definite result on the coagulation time of the blood, as much of the calcium present cannot be available as a factor in controlling the coagulation.

1. Vol. I, page 130.

GROUP II

Dixon¹ has shown recently that the injection of colchicine subcutaneously induces a very considerable leucocytosis, probably greater than that which can be produced by any known substance. The first effect, which lasts for about an hour after the injection of the drug, is a hypo-leucocytosis, the leucocytes being expelled from the circulation; afterwards this is followed by hyper-leucocytosis, which may attain a maximum in about twelve hours; the augmentation consists nearly entirely of the poly-morphonuclear cells. Stockman has confirmed these results in man. A similar effect, though it is slower in appearing, is obtained by the injection of small quantities of nuclein and allied bodies.

It was my purpose in the following experiments to investigate the possible influence of leucocytosis on the coagulation time of the blood, and to contrast these results with observations on clinical cases in which leucocytosis was a prominent feature, as in acute appendicitic and catarrhal pneumonia.

As a rule, no differential count of the white corpuscles has been undertaken.

Colchicine

Experiment IX.—Weight of rabbit, 2.5 kilos. Normal coagulation time, 3 minutes 30 secs. Number of white blood corpuscles per cubic millimetre, 7,200. (*Thoma-Zeiss Method.*) Two c.c. of .5 per cent. colchicine injected at 12 a.m.

Time		Coagulation time	No. of W.B.C. per cubic millimetre
Commencement			
12 a.m.	Aug. 4th	3 min. 30 secs.	7,200
12.30 p.m.	„	3 min. 35 secs.	5,600
1 p.m.	„	3 min. 40 secs.	3,800
2.15 p.m.	„	3 min. 50 secs.	2,600
3.15 p.m.	„	4 min. 30 secs.	4,160
4.30 p.m.	„	4 min. 20 secs.	10,600
6 p.m.	„	5 min. 0 secs.	15,000
10 a.m.	Aug. 5th	8 min. 33 secs.	11,000
5.15 p.m.	„	3 min. 35 secs.	5,120

(See Fig. I.)

1. *Manual of Pharmacology*, 1906.

FIG. I.—EFFECT OF COLCHICINE

Two cubic centimetres of .5 per cent. Colchicine injected hypodermically at 12 noon. Black lines show coagulation time of blood. Dotted lines show number of white corpuscles per cubic millimetre.

NOTE.—The portion of the diagram included in brackets as an interval of 16 hours is not drawn to scale as regards time interval as otherwise the length of the diagram would be unwieldy for publication. The same conditions is shown in Figs. IV and V.

Experiment X.—Weight of rabbit, 2.7 kilos. Normal coagulation time, 3 min. 5 secs. Number of white blood corpuscles, 6,700 per cubic millimetre. Two c.c. of .5 per cent. colchicine injected at 9.30 a.m.

	Time	Coagulation time	No. of W.B.C. per cubic millimetre
Commencement	9.30 a.m.	3 min. 5 secs.	6,700
	10.30 a.m.	4 min. 20 secs.	2,900
	11.30 a.m.	3 min. 40 secs.	5,360
	12.30 p.m.	3 min. 55 secs.	8,000
	2 p.m.	4 min. 10 secs.	11,200
	3.30 p.m.	5 min. 0 secs.	16,200
	5.30 p.m.	5 min. 20 secs.	31,500
	8 p.m.	6 min. 10 secs.	31,000

(See Fig. II.)

FIG. II.—EFFECT OF COLCHICINE

Two cubic centimetres of .5 per cent. Colchicine injected hypodermically at 9.30 a.m.

Experiment XI.—Weight of rabbit, 2.3 kilos. Normal coagulation time, 3 min. 20 secs. Number of white blood corpuscles, 7,040 per cubic millimetre. Two c.c. of .5 per cent. colchicine injected at 9.30 a.m.

	Time of observation	Coagulation time	No. of W.B.C. per cubic millimetre
Commencement	9.30 a.m.	3 min. 20 secs.	7,040
	10 a.m.	3 min. 30 secs.	5,500
	10.45 a.m.	4 min. 10 secs.	3,200
	11.30 a.m.	4 min. 30 secs.	3,584
	12.30 p.m.	5 min. 10 secs.	8,320
	2 p.m.	6 min. 0 secs.	12,800
	3.30 p.m.	9 min. 40 secs.	15,800
	5.10 p.m.	12 min. (approx.)	25,600

(See Fig. III.)

FIG. III.—EFFECT OF COLCHICINE

Two cubic centimetres of .5 per cent. Colchicine injected hypodermically at 9.30 a.m

The results of these experiments are contrasted as curves in Figures I, II and III, the black lines representing the coagulation time, and the dotted lines the number of white blood corpuscles per cubic millimetre. It is seen on examining these diagrams that during the preliminary stage, in which the number of white blood corpuscles is diminished, that the coagulation time of the blood is already somewhat lengthened. In Fig. I this is shortly followed by a reverse effect, but afterwards as leucocytosis becomes well established, in Figs. I, II and III, the coagulation time lengthens to a very considerable degree.

In Experiment XI (Fig. III), during the maximum height of the leucocytosis the coagulation time has actually reached twelve minutes; it is worth noting that in this experiment the curve representing the coagulation time ascended from the very beginning. The important point in all these experiments is the decided rise of this curve as leucocytosis increases, and it would, therefore, appear that there is some connection between them.

Although the time of coagulation increases synchronously with the leucocytosis this increase is not strictly proportional, nor do the two maxima exactly correspond. A large number of experiments would be necessary to investigate the exact relation between the two phenomena.

Nuclein

When small quantities of nuclein are injected hypodermically into animals a hypo-leucocytosis occurs, which is followed by a hyper-leucocytosis. This is similar to what takes place after the injection of colchicine, except that the stage of the experiment during which the number of leucocytes is diminished is of longer duration with the nucleins. The results obtained in the two following experiments are concordant with the observations on colchicine; in both, as the time of coagulation lengthens, the leucocytosis increases.

Experiment XII.—Weight of rabbit, 3.1 kilos. Normal coagulation time, 4 min. 25 secs. Number of white blood corpuscles, 6,400 per cubic millimetre. Two c.c. of .5 per cent. nuclein injected subcutaneously at 1 p.m.

	Time	Coagulation time	No. of W.B.C. per cubic millimetre
Commencement	1 p.m.	4 min. 25 secs.	6,400
	2 p.m.	2 min. 10 secs.	3,200
	3 p.m.	2 min. 20 secs.	2,560
	4.15 p.m.	3 min. 20 secs.	4,480
	5.45 p.m.	3 min. 50 secs.	6,600
	9.15 a.m.	6 min. 30 secs.	16,200
	11 a.m.	6 min. 20 secs.	15,100

(See Fig. IV.)

FIG. IV.—EFFECT OF 5 PER CENT. NUCLEIN

Two cubic centimetres of 5 per cent. Nuclein injected hypodermically at 1 p.m.

Note the very considerable shortening of the coagulation time during the first few hours: this was not a feature in the following experiment.

Experiment XIII.—Weight of rabbit, 2.5 kilos. Normal coagulation time, 3 min. 10 secs. Number of white blood corpuscles per cubic millimetre, 8,000. Two c.c. of .5 per cent. nuclein injected at 9.45 a.m.

	Time	Coagulation time	No. of W.B.C. per cubic millimetre
Commencement	9.45 a.m.	3 min. 10 secs.	8,000
	11 a.m.	3 min. 0 secs.	4,000
	12.30 p.m.	4 min. 20 secs.	3,200
	2 p.m.	6 min. 0 secs.	8,960
	4.30 p.m.	7 min. 10 secs.	10,100
	6 p.m.	9 min. 0 secs.	14,000
	9.30 a.m.	7 min. 0 secs.	12,000

(See Fig. V.)

FIG. V.—EFFECT OF 5 PER CENT. NUCLEIN

Two cubic centimetres of 5 per cent. Nuclein injected hypodermically at 9.45 a.m.

I propose to contrast these experiments on animals with observations on the coagulation time of a few clinical cases of pneumonia and appendicitis in which a considerable leucocytosis was present. An increase in the number of leucocytes is nearly always one of the pathological phenomena of pneumonia. Osler, in his 'Practice of Medicine,'¹ points out that in most cases of pneumonia a leucocytosis appears early, persists, and ultimately disappears with the crisis, the number of leucocytes varying from 12,000 to 40,000, and even 100,000 per cubic millimetre.

Acute Pneumonia

Case I.—T. F. P., man, age 45. Third day of disease. Respirations, 40 per minute. Temperature, 103°4. Leucocyte count shows 15,600 white blood corpuscles per cubic millimetre. Coagulation time, 6 mins. 10 secs.

Case II.—F. M., man, age 34. Fourth day of disease. Respiration, 35 per minute; some dyspnoea. Temperature, 102°8. Leucocytes, 25,000 per c.mm. Coagulation time, 6 mins. 30 secs.

Case III.—J. K., woman, age 28. Second day of disease. Respiration, 38 per minute; fine crepitations over right base. Temperature, 104°2. Leucocytes, 18,000 per c.mm. Coagulation time, 5 mins. 40 secs.

Appendicitis

Case I.—J. P., boy, age 15. Illness began six days ago. Vomiting frequent until to-day, when it ceased. Great tenderness over lower part of abdomen. Localised dulness and swelling in right iliac fossa. Constipation during last three days. Tongue dry and furred. Temperature, 101·8 F. Leucocytes, 15,600 W.B.C. per c.mm. Coagulation time, 6 mins. 15 secs. An appendical abscess was opened the day following.

Case II.—S. W., woman, age 23. Temperature, 102·3. Clinical signs of appendical abscess. Leucocytosis, 17,600 W.B.C. per c.mm. Coagulation time, 5 mins. 45 secs. An abscess was opened on the same day.

Case III.—E. C., man, age 33. Third day of present illness. Bowels confined. Tongue furred. Vomited twice yesterday. Tenderness in right iliac fossa and also near umbilicus. No obvious swelling or localised dulness. Temperature, 99·2. Leucocytosis, 15,600 per c.mm. Coagulation time, 5 mins. 30 secs. An operation was undertaken the same day the appendix was covered with inflammatory lymph and had perforated at the apex. The feature in this case was the asthenic type of the disease, although there was a definite leucocytic reaction.

These observations, as far as they go, certainly show a coagulation time which is longer than the normal average, and are, therefore, not discordant with my experiments on animals as regards a connection between leucocytosis and a decrease in the coagulability of the blood. Pye-Smith, in his article on pneumonia appearing in 'Allbutt's System of Medicine' (Vol. V), calls attention to the fact that the blood in pneumonia clots slowly, the red corpuscles subsiding before they are entangled in the meshes of fibrin; he mentions also that this condition is present in many other inflammatory diseases. Muir, in his contribution on Leucocythaemia in the same volume, points out that the blood in this disease usually coagulates less readily than normal blood.

GROUP III

In the following experiments the normal saline solution injected subcutaneously into rabbits was found to be rapidly absorbed, and to account for a great increase in the length of the time of coagulation.

Experiment XIV.—Weight of rabbit, 2·5 kilos. Normal coagulation time, 3 mins. 10 secs. Eighty cubic centimetres of normal saline at 39° C. were injected subcutaneously at 3.30 p.m.

	Time of observation		Coagulation time
Commencement	3.30 p.m.	...	3 min. 10 secs.
	4 p.m.	...	10 min.
	4.30 p.m.	...	7 min.

Experiment XV.—Weight of rabbit, 2·1 kilos. Normal coagulation time, 3 min. 20 secs. One hundred cubic centimetres of normal saline at 39° C. were injected at 9.30 a.m.

	Time of observation		Coagulation time
Commencement	9.30 a.m.	...	3 min. 20 secs.
	10 a.m.	...	7 min. 10 secs.
	10.30 a.m.	...	8 min. 30 secs.
	11 a.m.	...	7 min. 30 secs.

The result of the two experiments are quite concordant. It is important to realise the effect of the injection of normal saline on the coagulation time, as this is a frequent procedure during severe operations or after great loss of blood.

GROUP IV

The administration of β -naphthylamine has been shown by Stern,¹ and afterwards by Fawcett and White², to raise the temperature of such animals as the dog, cat and rabbit. Dixon³ has verified these results. The subcutaneous injection of the drug produces great muscular weakness, and the respirations become shallow and frequent; the latter effect is especially seen in the cat. The rise of temperature may be from 3° to 4° C., although it does not invariably occur.⁴

It was suggested to me that an artificial febrile condition so induced might show the effects on the coagulability of the blood. It is true that objection can be brought forward that any effect on

1. Stern, *Virchow's Arch.*, CXV, p. 34, and CXXI, p. 376.

2. Fawcett and Hale White, *Journ. Physiol.*, XXI, p. 435.

3. Dixon, in Hale White's *Textbook of Pharmacology*, p. 745.

4. Dixon, *Ibid.*

the blood is not necessarily due to the pyrexia, but may depend directly on the drug. This point will be discussed later. The result of three experiments on rabbits showed that the temperature was raised about 2° C. in two cases, and in these the coagulation time became lengthened also.

The temperature was taken by a rectal thermometer.

Experiment XVI.—Weight of rabbit, 2.6 kilos. Normal coagulation time, 3 min. 35 secs. Temperature taken per rectum, 39.6° C. Two c.c. of 3 per cent. β -naphthylamine were injected at 10.45 a.m.

	Time	Coagulation time	Temperature	Remarks
Commencement	10.45 a.m.	3 min. 35 secs.	39.6	
	11.15 a.m.	3 min. 20 secs.	37.5	
	11.45 a.m.	3 min. 15 secs.	39.6	
	12.30 p.m.	3 min. 30 secs.	39.8	Respirations increased in frequency.
	2.30 p.m.	4 min. 15 secs.	40.1	
	4 p.m.	4 min. 45 secs.	41.6	Paresis of hind limbs.
	5 p.m.	5 min. 10 secs.	42.2	
	6 p.m.	5 min. 15 secs.	40.8	Great muscular weakness.

The rise of temperature was considerably delayed in this experiment, when compared with the figures given by Dixon.

Experiment XVII.—Weight of rabbit, 3.65 kilos. Coagulation time, 3 min. 50 secs. Temperature taken per rectum, 38.7° C. Five c.c. of 3 per cent. β -naphthylamine injected at 3.15 p.m.

	Time	Coagulation time	Temperature	Remarks
Commencement	3.15 p.m.	3 min. 50 secs.	38.7	
	3.45 p.m.	4 min.	38.9	
	4.15 p.m.	4 min. 30 secs.	39.4	Muscular weakness and paresis.
	5 p.m.	5 min. 10 secs.	39.8	
	5.30 p.m.	6 min. 30 secs.	41.1	A peculiar noise is occasionally made by animal.
	6 p.m.	6 min. 10 secs.	40.8	

The rise of coagulation time is coincident with the rise in temperature in both these experiments.

Experiment XVIII.—Weight of rabbit, 1.35 kilos. Normal coagulation time, 4 min. 15 secs. Temperature, 39°5. Three c.c. of 2 per cent. β -naphthylamine injected at 11.45.

	Time	Coagulation time	Temperature	Remarks
Commencement	11.45 a.m.	4 min. 15 secs.	39°5	
	12.15 p.m.	4 min.	38°9	
	12.45 p.m.	3 min. 50 secs.	38°8	
	1.15 p.m.	4 min. 10 secs.	38°8	Muscular paresis.
At 2.15, 2 c.c. of 2 per cent. β -naphthylamine were injected in addition.				
	3 p.m.	3 min. 55 secs.	38°7	Marked paresis of hind limbs.

In Experiment XVIII, although the effect of the drug as regards muscular weakness and increased frequency of respiration was quite apparent, yet no rise in temperature was observed; on the contrary, a slight fall occurred. There was also no alteration of importance in the coagulation time. It has already been mentioned that in those experiments in which an elevation of temperature was noted the coagulation time became lengthened, whether as a result of the pyrexia or the direct effect of the drug.

As the two phenomena occurred synchronously, and were both absent from the last experiment, it is probable that there is either a connection between them or they are both the result of some common condition.

On referring to the experiments on leucocytosis and coagulation it will be noticed that the clinical cases of pneumonia and appendicitis showed varying degrees of fever.

GROUP VI

The diphtheria toxin employed in the next experiment was of such a strength that $\frac{1}{10}$ c.c. sufficed to kill a guinea-pig in four days.

Experiment XIX.—Weight of rabbit, 2 kilos. Normal coagulation time, 3 min. 10 secs. One c.c. of diphtheria toxin injected at 5 p.m.

	Time of observation			Coagulation time
Commencement	...	5 p.m.	...	3 min. 10 secs.
Next day	...	11 a.m.	...	5 min. 40 secs.

Death occurred at 12.20 a.m.

A correct determination of the coagulation time was difficult in consequence of an alteration in the viscosity of the blood. The

final observation is unduly shortened, for as the surface tension of the blood is reduced, the size of the drop is less, and the relative surface of glass to which it is exposed is greater.

SUMMARY

1. It is difficult to determine a normal coagulation time for a series of observations on the same animal which shall hold good for more than a short period. When an experiment is prolonged for twelve hours or more variations due to food, drink or accidental circumstances occur. For this reason, only marked differences in coagulation time are regarded as significant.

2. The administration of citric acid lengthens the time of coagulation by forming sodium citrate, a body which is incapable of ionization so as to form the Ca ion (C. J. Martin).

The hypodermic injection of calcium chloride shortens the time of coagulation. If the drug be administered by the mouth absorption is slow and incomplete, and, therefore, the effect of the drug is less.

The effect of milk, whether used as a diet or injected subcutaneously, is negative. My observations are opposed to those of Wright and Paramore.

3. When leucocytosis occurs, either as the result of some pathological condition or of the hypodermic injection of a drug, there is a lengthening of the time of coagulation of the blood.

4. Solutions of normal saline injected subcutaneously have a pronounced effect in diminishing the coagulability of the blood.

5. The experiments with β -naphthylamine show that after the hypodermic injection of this drug there is a lengthening of the time of coagulation.

This seems to be due to the accompanying pyrexia, because occasionally the pyretic phenomena are absent whilst the other effects of the drug on the central nervous system, heart respiration, etc., are typical; in these cases the time for coagulation remains unchanged.

6. Diphtheria toxin increases the time of coagulation.