The Action of "Peptone" and of Nucleic Acids on the Coagulability of the Blood.

By John William Pickering, D.Sc., and James Arthur Hewitt, Ph.D.

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(From the Department of Physiology, University of London, King's College.)

In a previous communication to this Society, the present authors showed that the rapid intravascular injection of "peptone" into cats, deprived of hepatic activity, inhibited coagulation of the blood. It was also shown that when precautions are taken to preserve the surface conditions of the blood of the cat, pronounced retardation of clotting can be obtained *in vitro* by the addition of "peptone" in quantities no greater than are required to produce a like effect *in vivo* (Pickering and Hewitt, 1). Subsequently, Nolf (2) observed that the anti-coagulant action of "peptone" on the blood of the domestic fowl, with the liver extirpated, is actually greater than in the intact animal. Experimenting with the blood of dogs, Doyon (3) found that nucleic acid, prepared by Neumann's method (4) from the thymus and from the mesenteric ganglia of the ox, is anticoagulant *in vivo* and *in vitro*, the former reaction being ascribed to the supposed secretion, by the liver, of an anti-thrombic nucleoprotein.

In this paper the action of Witte's peptone on the coagulability of the blood of the tortoise, rat and dog, and that of thymus and yeast nucleic acids, is described and discussed. In each experiment, except where otherwise stated, the blood was obtained from animals with the liver out of circulation.

I.—Technique and Terms Employed.

The mammals used were anæsthetised with A.C.E., pithed, and artificial respiration was employed for at least 10 minutes prior to injection of either "peptone" or of nucleic acid. The tortoises were anæsthetised with chloroform and decapitated after clamping the vessels of the neck. The mammals were deprived of hepatic activity by ligature of the thoracic aorta and inferior vena cava, the tortoises by ligature of the bulbus arteriosus and sinus venosus.

Blood was shed through evenly paraffined cannulæ into clean glass vessels, in quantities of 1 c.c. or less at a time, as blood shed in small quantities does not exhibit the hyper-coagulability which arises from excessive hæmorrhage.

Wide-mouth vessels were employed, as the present authors (Pickering and Hewitt, 5) have found that the development of clotting may be modified by forces of adhesion. The vessels containing the blood were covered with moist filter paper to avoid concentration of blood by evaporation and consequent acceleration of coagulation.

The Witte's peptone used was dissolved in Ringer in the experiments on tortoises, in Locke-Ringer in those on mammals. The nucleic acids, termed respectively "Doyon" and "Levene," were kindly given to us by Prof. E. Doyon and Dr. P. A. Levene, to whom our cordial thanks are due. The preparations by Dr. Levene are stated by him to be entirely free from protein. The nucleic acids were dissolved in Locke-Ringer and made faintly alkaline to litmus, in accordance with the technique of Doyon.

The terms "commencement" and "completion" of clotting imply respectively the first visible departure from fluidity, other than the appearance of minute filaments on the surface of the blood, and that coagulation has so far advanced that the containing vessel could be inverted without spilling.

Each experiment is representative of a number of concordant results.

II.—The Action of "Peptone" on Tortoises' Blood.

Experiment 1.—Prior to ligature of the sinus venosus and bulbus arteriosus the clotting times of blood shed on to clean glass were :—Commencement, 8 minutes 5 seconds ; completion, 8 minutes 55 seconds. Twelve minutes after ligature the times were 8 minutes 10 seconds and 8 minutes 55 seconds respectively. 0·02 gm. of peptone was injected into the heart. The results are summarised in the subjoined table.

Table I.

Time in minutes after injection of peptone.	Time of commencement of clotting in minutes and seconds.	Time of completion of clotting in minutes and seconds.
	min. sec.	min. sec.
5	23 15	26 0
11	23 0	26 0
18	18 0	22 30
40	18 0	21 30
50	12 30	14 0
60	10 0	12 30
105	6 10	8 15

Notes :—

1. Between 18 minutes and 40 minutes there was but little variation in the coagulation times.
2. At 60 minutes the heart beats were feeble and the blood was distinctly venous.
3. At 80 minutes the heart was beating very slowly and irregularly.
4. At 105 minutes the heart had almost stopped. Blood was withdrawn by suction and was very venous.

Experiment 2.—Blood was shed through a paraffined cannula into a glass vessel and was immediately mixed with peptone so that the concentration of the latter was 0·4 per cent. The blood remained completely fluid for three hours and no deposit was found on the walls of the vessel. A portion of this still fluid blood was removed and to it was added one-fourth of its volume of saturated ammonium sulphate. A copious precipitate (fibrinogen) was produced (McLean, 6). The peptone blood clotted spontaneously after 15 hours, a typical gel filling the entire vessel. Normal syneresis occurred after the gel was released from adhesion to the walls of the container. The peptone blood also clotted immediately on dilution with distilled water.

Blood from the same animal when shed through a paraffined cannula on to glass and with no peptone added exhibited clotting times of 8 minutes and 9 minutes.

Experiment 3.—Blood from the animal used in experiment 2 was shed on to its own cut tissues. The clotting times were :—Commencement, 55 seconds ; completion, 1 minute 30 seconds.

Blood which had been in contact for 25 seconds with the lacerated tissue was mixed *in vitro* with peptone so as to contain 0·4 per cent. It commenced to clot in 3 minutes and was completely coagulated in 3 minutes 30 seconds.

The first experiment shows that the intravascular injection of Witte's peptone retards the coagulation of the blood of the tortoise after the animal has been deprived of hepatic activity. Such anti-coagulant action cannot therefore be due to the secretion of anti-thrombin by the liver. As in the cat, inhibition of clotting by peptone is reduced or annulled by increase of carbon dioxide in the blood.

The second experiment demonstrates that moderate concentrations of peptone, when mixed with tortoise blood *in vitro*, cause prolonged delay in clotting without any deposit of material, provided the blood has not been in contact with damaged tissue.

These facts and other data recorded in the protocols of this experiment appear dissonant with the views of Nolf (7) that the delayed coagulation after intravascular injection of peptone is due to the deposition of fibrinogen on the vascular endothelium. They are, however, concordant with the observations of Loeb (8) who was unable to extract any anti-coagulant from the linings of blood vessels.

The blood of the tortoise when mixed with small quantities of peptone after contact with its own damaged tissues clots more slowly than does pure blood under like conditions, but coagulates more rapidly than pure blood shed on to glass surfaces.

In conformity with current theory the last statement would be explained as due to chemical action on the blood of thrombo-plastic material believed to be liberated from the cut surface. An alternative explanation is, however, possible. Contact with damaged tissue sets in motion those changes which are the prelude of clotting more rapidly than does contact with glass, and the condition of the colloids of the plasma may be so far modified as to inhibit union with the peptone. On this view, the specificity of damaged tissues, as excitants of coagulation, would be a matter of variation in the speeds of surface change, rather than an exhibition of specific chemical reactions.

III.—The Action of Witte's Peptone on the Blood of the Rat.

The next table shows the times of completion of clotting of blood shed on to glass from pigmented, partially pigmented, and albino rats, before and after the rapid injection of Witte's peptone into the heart.

Table II illustrates the anti-coagulant action of peptone on the circulating blood of pigmented rats, and shows that decreased coagulability is as marked in animals deprived of hepatic activity as in those with the blood circulating through the liver. In this respect the blood of pigmented rats behaved like that of the cat and tortoise. It differs, however, in that the anti-coagulant action is not annulled by an increase of carbon dioxide in the blood. In these experiments partly pigmented rats were more resistant to the anti-coagulant action of peptone on circulating blood and to its toxic effect on the heart than are animals with completely pigmented fur. A very large amount of peptone (1·3 gm.) failed to inhibit the coagulation of the blood of albino rats weighing from 230 to 250 gm., and this dose appeared not to produce any ill-effect on the hearts of these animals. No such variation in the action of peptone was observed when it was added to the blood of pigmented and albino animals *in vitro*. It is noteworthy that Halliburton and Brodie (9) found that large amounts of nucleo-protein can be injected into the circulation of albino rabbits without causing thrombosis, while one of us (Pickering, 10) found that the intravenous injection of a nucleoprotein into the Arctic Hare (*Lepus variabilis*) causes extensive intravascular clotting during the pigmented condition, fails to induce thrombosis during the unpigmented state, and may either produce or fail to produce thrombi when pigmentation is partial. Hyper-coagulability of shed blood is, however, equally manifested after the intravenous injection of a nucleoprotein into animals with pigmented, partly pigmented and with white fur. The difference in the susceptibility of the circulating blood of coloured and white animals to coagulation by nucleoprotein appears,

Table II.

Number of Experiment.	Colour and weight in grammes.	Hepatic Circulation.	Peptone injected, grammes.	Clotting time before injection.	Clotting time 10 minutes after injection.	Clotting time 15 minutes after injection.	Clotting time 30 minutes after injection.	Condition of heart at end of experiment.
				min. sec.	min. sec.	min. sec.	min. sec.	
4	Black, 210.	In	0·125	5 40	10 50	11 20	12 30	Stopped 20 minutes after injection.
5	Black, 216.	Out	0·125	5 35	12 0	12 30	—	Stopped 16 minutes after injection.
6	Brown, 230.	Out	0·125	5 25	11 20	11 10	12 0	Stopped 20 minutes after injection.
7	Black and white, 220.	In	0·125	5 40	8 0	9 0	10 20	Stopped 30 minutes after injection.
8	White, small black patch, 160.	Out	0·125	6 45	10 30	9 0	7 0	Strong 30 minutes after injection.
9	White, black muzzle, 200.	In	0·125	6 0	9 50	9 30	7 15	Strong 30 minutes after injection.
10	Albino, 232.	In	0·125	6 0	6 0	5 55	5 45	Strong 30 minutes after injection.
11	Albino, 240.	In	0·2	5 50	5 45	5 55	5 55	Strong 30 minutes after injection.
12	Brown, 238.	In	0·2	5 55	16 0	28 0	—	Stopped 12 minutes after injection.
13	Albino, 230.	In	0·3	5 40	5 45	5 35	—	Stopped 20 minutes after injection.
14	Brown, 226.	In	0·3	6 10	19 0	—	—	Stopped after injection.

Note.—In each case the blood shed after the heart stopped was extremely venous.

therefore, to be associated with material which changes when the surface conditions of the blood are altered by contact with foreign surfaces.

Viewing these observations as a whole, there appears that a relationship exists between pigmentation in rodents and their natural resistance to the toxic action of nucleoprotein and of peptone.

The admixture *in vitro* of the blood of pigmented rats with moderate concentrations of peptone (*i.e.*, 0·3 to 0·4 per cent. peptone in the blood) yielded inconstant results. On two occasions slight retardation of clotting occurred but this did not exceed a minute and a half in either instance. With 12 other animals no appreciable inhibition of coagulation was observed. In view of the fact that a well marked increase of coagulation time was invariably found after the rapid intravascular injection of similar amounts of peptone into whole animals and into those deprived of hepatic activity, it may be concluded that the blood of pigmented rats changes more quickly or more markedly when shed than does that of either the cat or the tortoise. Specific differences in the surface conditions of the blood of these animals appear probable, and it is suggested that variations in the surface conditions of the blood of one animal from that of another may account for the toxic effect following the intravascular injection of heterologous blood. Support to this opinion is afforded by the observation of Novy and De Kruif (11), who found that if a quantity of blood from a rabbit, up to 3 c.c. in amount, is injected immediately after withdrawal into the circulation of a guinea-pig it is usually harmless, but if the same amount of blood is retained in a syringe for three minutes and then injected it is fatally toxic, the effects produced being like those of an "anaphylatoxin."

IV.—The Action of Witte's Peptone on the Blood of the Dog.

Experiment 15.—After the injection of 0·5 gm. of Witte's peptone into a 10-kilo. dog respiring air, with the liver out of the circulation, retardation of coagulation of 5 minutes 30 seconds in commencement and 8 minutes 40 seconds in completion was observed. As in the cat the inhibition of coagulation was annulled by partial asphyxiation of the animal.

Taking into account that about only one-fourth of the animal's blood was circulating, these retardations of clotting were not nearly so marked as has been observed in intact animals. Unfortunately, our observations on the dog are too few in number to be conclusive, lack of a supply of active Witte's peptone suspending the work.* It is, however, noteworthy that Gley (12)

* Witte's peptone, procured this year (1923) from the makers, has proved singularly ineffective as an anti-coagulant.

found wide variation in the action of both Witte's and Charponteux's peptones on intact dogs, normal coagulability of the blood being found in some animals after the injection of even 0·5 gm. per kilo. of either preparation. Nolf (13) found a retardation of 11 minutes in the coagulation of the blood of a dog into which 1·1 gm. of Witte's peptone had been injected after the occlusion of the thoracic aorta and inferior vena cava. Release of these vessels, however, increased the inhibition of clotting. The same observer injected peptone into dogs with the liver extirpated and found a preliminary hyper-coagulability followed by inhibition of coagulation, sometimes by complete suspension of that process. The injection of ox serum into sensitised dogs with the liver extirpated also inhibited clotting. Doyon (14) states that although the speed of coagulation of the blood after the injection of peptone into dogs deprived of hepatic activity is usually normal, yet he has observed cases in which incoagulability has been produced under these conditions.

It thus appears that great differences exist in the susceptibility of the blood of dogs to the anti-coagulant action of peptone, and this occurs both in animals with the liver in circulation and in those deprived of hepatic activity.

The injection of peptone into these animals may cause retarded coagulability of the blood or may have no demonstrable effect. So far no explanation has been advanced to account for this variation, yet the thrombin theories are based on the positive experiments and the negative results are ignored.

V.—The Action of Nucleic Acids on the Blood of the Cat and the Rat.

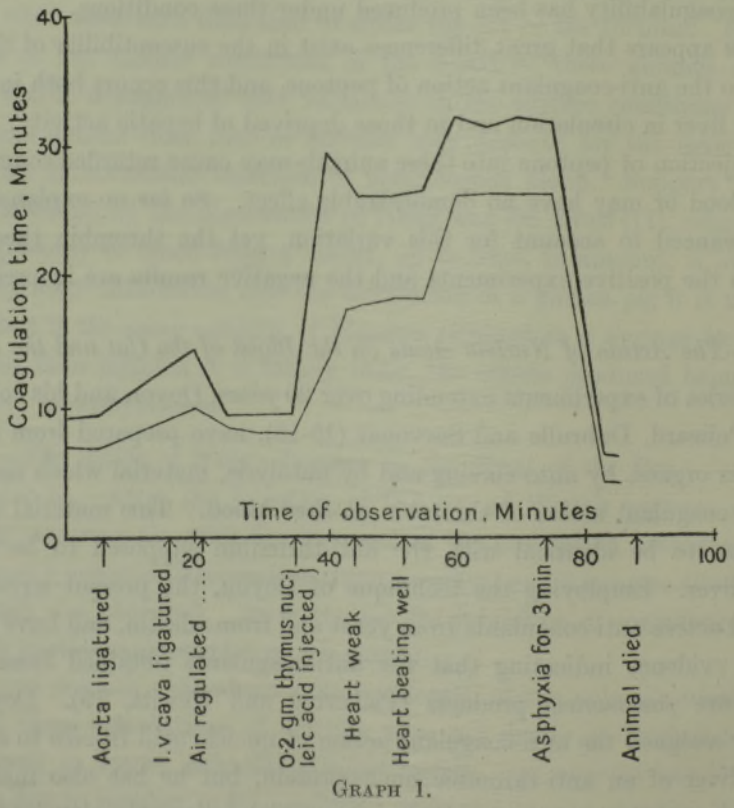
In a series of experiments extending over 20 years, Doyon and his colleagues, Morel, Policard, Dubrulle and Sarvonat (15-18), have prepared from the liver and other organs, by auto-claving and by autolysis, material which neutralises the anti-coagulant action of thrombin on shed blood. This material was held by Doyon to be identical with the anti-thrombin supposed to be secreted by the liver. Employing the technique of Doyon, the present writers have prepared active anti-coagulants from yeast and from edestin, and have brought forward evidence indicating that the anti-coagulants obtained from animal sources are *post-mortem* products (Pickering and Hewitt, 19). Doyon has not only assigned the anti-coagulant action of nucleic acid *in vivo* to secretion by the liver of an anti-thrombic nucleoprotein, but he has also maintained that the immunity to anti-coagulant action, which follows the slow intravenous injection of nucleic acid, is due to variation in the secretion of either hepatic cells or those of the vascular endothelium.

The next series of experiments (Nos. 16-22) shows that moderate amounts of

nucleic acid prepared from thymus and from yeast, inhibit the coagulation of the blood of the cat and the rat when rapidly injected into the circulation of animals deprived of hepatic activity. Experiments are also described (Nos. 25-27) showing that slow intravascular injection of like amounts of nucleic acid provokes hyper-coagulability, followed by tolerance culminating in immunity to the anti-coagulant action. This occurs in intact animals and in those with the liver and abdominal viscera out of the circulation.

In the observations illustrated by graphs the points on the upper curve indicate completion of clotting, those on the lower the commencement of that process.

Experiment 16.—Tabby cat, female, 4,060 gm. 0·2 gm. of Doyon's thymus nucleic acid was injected into the circulation at the time indicated on graph No. 1.



GRAPH 1.

The next table illustrates the inhibition of the coagulation of the blood produced by the rapid intravascular injection of nucleic acid into animals after ligature of the thoracic aorta and inferior vena cava.

Table III.

No. of experiment.	Description of animal and weight in gm.	Nucleic acid injected.	Amount of acid injected.	Coagulation times before ligature of vessels.	Coagulation times after ligature of vessels.	Coagulation times after injection of acid.	Coagulation times after partial asphyxiation following injection of nucleic acid.
				min. sec.	min. sec.	min. sec.	min. sec.
17	Tabby cat 2175 ♂	Thymus Levene	0·525	7 50 8 40	7 40 8 40	66 50 140 0	9 40 10 25
18	Black cat 2350 ♀	Yeast Levene	0·375	9 0 10 10	9 0 10 10	15 0 16 0	3 0 6 40
19	Black cat 2400 ♂	Yeast Levene	0·525	8 30 9 50	8 40 9 40	46 30 60 0	7 50 9 10
20	Tabby cat 2175 ♂	Thymus Levene	1·1	8 50 9 40	8 45 9 30	fluid after 360	fluid after 360
21	Brown rat 153 ♂	Thymus Doyon	0·05	4 40 7 5	4 10 7 10	59 0 62 0	59 0 61 0
22	Black rat 150 ♂	Thymus Levene	0·05	5 10 7 10	5 5 7 0	39 0 47 0	36 0 42 0
23	Black rat 160 ♀	Yeast Levene	0·05	5 30 7 25	5 35 7 15	15 10 21 20	15 20 21 30

NOTES :—

1. The upper of each coagulation time shows the commencement of clotting, the lower the completion.

2. The times recorded after the injection of nucleic acid show the longest times of clotting, while the animal was respiring air.

3. Times noted after partial asphyxiation imply that artificial respiration had been discontinued for 1 minute 50 seconds.

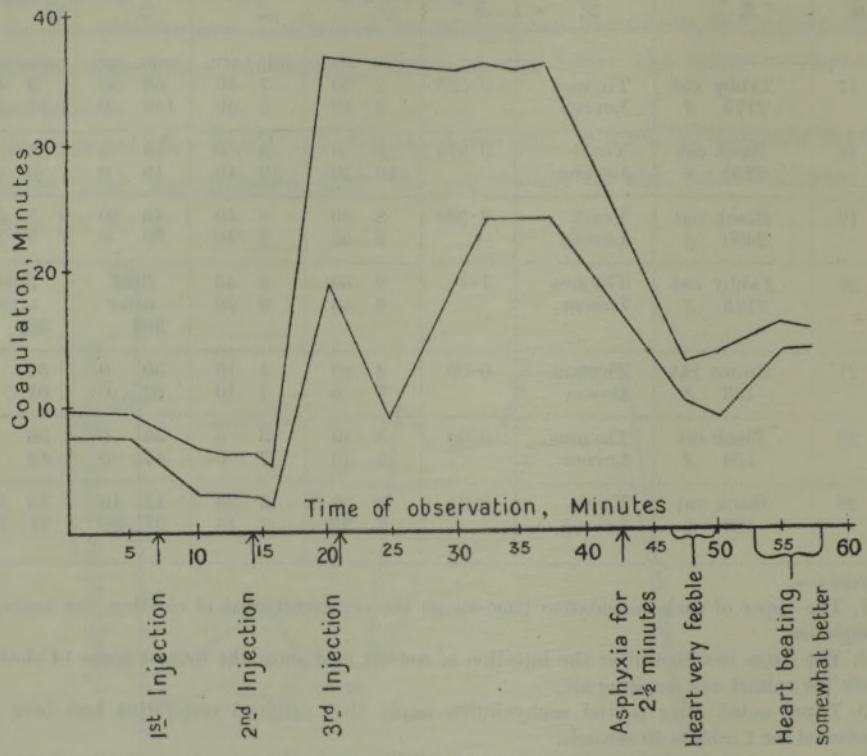
4. In one of the repeat experiments of No. 20 a minute clot was observed after 3 minutes in a sample of blood shed 4 minutes after injection of nucleic acid. The remainder of this blood kept fluid for 6 hours. Five other samples of blood from this animal behaved like that in No. 20.

5. The protein-free nucleic acids prepared by Dr. Levene are shown to be less active than the material supplied by Prof. Doyon.

Pithed cats, which had remained on the operating table for prolonged periods and which had suffered considerable hæmorrhage, were found to be more susceptible to the toxic action of nucleic acid than were less exhausted animals. The intravascular injection of 0·525 gm. of either preparation of thymus nucleic acid under these conditions usually caused cessation of heart beats. After the injection of a like amount into unexhausted animals, which had suffered but little hæmorrhage, the hearts continued beating strongly for

from one to two hours. The effect of a smaller dose on the speed and type of clotting of the blood of an exhausted animal is shown in the next experiment.

Experiment 24.—Cat, male, weight 3,500 gm. After pithing under A.C.E., but prior to ligaturing the vessels, the animal had been on the operating table for one and a half hours, and 25 c.c. blood had been withdrawn. The clotting times before the hæmorrhage were :—Commencement, 8 minutes 5 seconds ; completion, 9 minutes 25 seconds. After bleeding, these times



Graph 2.

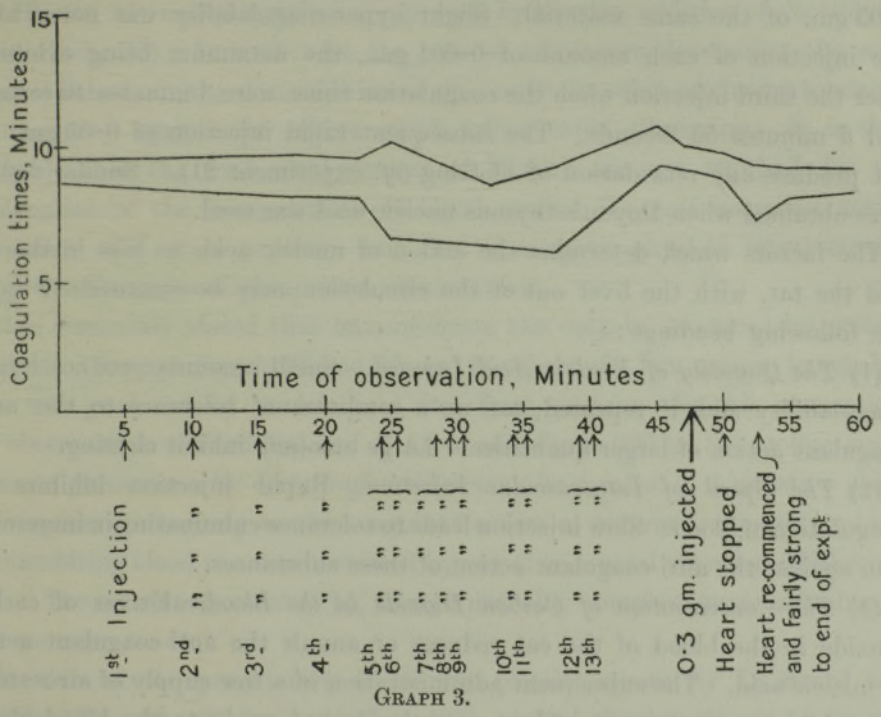
Note.—A comparison with the record of experiment No. 16 shows the differences in coagulability resulting respectively from slow and rapid injection.

were 5 minutes 30 seconds and 7 minutes 40 seconds respectively. These rates remained constant for 15 minutes. 0·25 gm. of Doyon's thymus nucleic acid was then injected rapidly into the heart, which stopped for two minutes but then resumed feeble beating. Blood shed four minutes after the injection showed a membranous clot when 26 minutes had elapsed. Five minutes later an almost complete gel was formed enclosing the membranous clot, and after the lapse of a further five minutes the gel showed signs of liquefaction. In a further 15 minutes the lysis of the gel was apparently complete, but the

membranous clot had contracted. Blood withdrawn nine minutes after the administration of the nucleic acid revealed a minute clot after four minutes but a complete gel was not formed till 24 minutes had elapsed. This gel contracted normally.

In this and other experiments of a like nature the death of the animal precluded further observation. There was no intravascular clotting.

Experiment 25.—Black cat, male, 2,075 gm. Three intravascular injections each of 0·0625 gm. of Doyon's thymus nucleic acid were made at the



GRAPH 3.

NOTE.—Comparison with experiment No. 17 illustrates the difference in coagulability when a similar quantity of this nucleic acid is injected in one amount.

times indicated on graph No. 2. The total amount of acid administered was 0·1875 gm. Ligature of the abdominal blood supply precluded renal excretion.

Experiment 26.—Tabby cat, male, 2,200 gm. Coagulation times before ligaturing the aorta and inferior vena cava were :—Commencement, 8 minutes 20 seconds ; completion, 9 minutes 30 seconds. Fifteen minutes after ligature they were 8 minutes 30 seconds and 9 minutes 30 seconds respectively. Thirteen intravascular injections, each of 0·0175 gm. of Levene's thymus nucleic acid, were made at the times indicated by the small arrows on

graph No. 3, followed by an injection of 0·3 gm. of the same substance at the time indicated by the large arrow.

Experiment 27.—Brown rat, male, 145 gm. The coagulation times before ligaturing the blood vessels were :—Commencement, 4 minutes 50 seconds ; completion, 8 minutes. After ligature these times were 4 minutes 45 seconds and 8 minutes 5 seconds respectively. Five injections each of 0·001 gm. of Levene's thymus nucleic acid were made at approximately equal intervals of time over a period of 30 minutes, followed by the rapid administration of 0·05 gm. of the same material. Slight hyper-coagulability was noted after the injection of each amount of 0·001 gm., the maximum being exhibited after the third injection when the coagulation times were 3 minutes 40 seconds and 6 minutes 55 seconds. The subsequent rapid injection of 0·05 gm. did not produce any retardation of clotting (*cf.* experiment 21). Similar results were obtained when Doyon's thymus nucleic acid was used.

The factors which determine the action of nucleic acids *in vivo* in the cat and the rat, with the liver out of the circulation, may be summarised under the following headings :—

(1) *The Quantity of Nucleic Acid Injected.*—Small amounts produce hyper-coagulability and, if repeated, set up a condition of tolerance to the anti-coagulant action of larger quantities. Large amounts inhibit clotting.

(2) *The Speed of Intravascular Injection.*—Rapid injection inhibits the coagulation of blood. Slow injection leads to tolerance culminating in immunisation against the anti-coagulant action of these substances.

(3) *The Concentration of Carbon Dioxide in the Blood.*—Excess of carbon dioxide in the blood of the cat reduces or annuls the anti-coagulant action of nucleic acid. The subsequent administration of a free supply of air restores the inhibition of clotting. These remarks do not apply to the blood of the rat.

(4) *The Degree of Exhaustion of the Animal.*—Exhausted animals are more susceptible to the toxic action of nucleic acid on the heart than are those in a more normal condition. The type of clotting may be abnormal and similar to that which has been observed after acute poisoning by peptone.

Evidence is thus forthcoming that hepatic secretion plays no part in the production of either tolerance or of immunity to the anti-coagulant action of nucleic acid on blood. The phase of hyper-coagulability, or tendency to aggregation of the colloids of the plasma is intelligible on the view that the changes producing clotting are similar to those leading up to precipitation and may be due to inequality and irregular distribution of electric charge.

The immunisation produced appears similar to the immunity, described by the present authors, against the anti-coagulant action of peptone in cats and rats deprived of hepatic activity, to the negative phase of clotting following the slow intravenous injection of tissue extract (Wooldridge, 20), or their slow addition to bird's blood (Pickering and Hewitt, 21), to the *in vitro* reactions of Dansyz (22) with the toxins and antitoxins of diphtheria and of ricin, to the fractional neutralisation of the toxic action of arsenious acid by ferric hydroxide, to the inhibition of precipitation of gelatin (Pickering and Hewitt, 21), and of certain inorganic sols by the slow addition of electrolytes (Freundlich, 23). In each case the rate of admixture determines the subsequent behaviour, the slow addition of the one to the other producing tolerance or immunity against the effects which follow rapid admixture. It is thus probable that the conceptual devices of Ehrlich are not required for the explanation of the immunisation of blood against the anti-coagulant action of nucleic acid or of peptone, and that a common physical interpretation applies to the series of phenomena under review.

It is commonly stated that immunisation can only be produced by protein material. In experiments 26 and 27, and in others of a like nature, immunisation was produced by nucleic acid free from protein. In this connection, the observations of Ford (24) and of Acree and Syme (25) on the production of immune phenomena by certain glucosides may be recalled. A negative phase or temporary immunity to the coagulant action of certain synthetic products on circulating blood was demonstrated by one of us several years ago (Pickering, 26). The substances referred to, though probably complex, were undoubtedly not protein.

In view of the preparation of an anti-platelet serum by Le Sourd and Pagniez (27) and also by Ledingham (28), and of the hypothesis of Cramer and Pringle (29) that the coagulation of the blood is primarily induced by the lysis of platelets, it became desirable to enquire whether the anti-coagulant action of nucleic acid is dependent on the presence of platelets. The series of observations described in experiment 28 answers the question in the negative.

Experiment 28.—Cat, male, 2,300 gm. The coagulation times before ligaturing the aorta and inferior vena cava were :—Commencement, 5 minutes 30 seconds ; completion, 6 minutes 20 seconds. After ligature these times were unchanged. Blood films were prepared and examined for platelets. + signifies platelets present, — signifies platelets absent, while ? indicates aggregates present which may be platelets, detritus of platelets or the pseudo-platelets described by Sacerdotti (31).

Table V.

Number of observation.	Time after the injection of 0·375 gm. of Levene's thymus nucleic acid.	Coagulation times in minutes and seconds.	Presence of platelets.	Air supply of animal.
	min. sec.	min. sec.		
1.	0 0	5 20	+	Constant
		6 30	+	,,
2.	3 0	58 30	+	,,
		65 0	+	,,
3.	7 0	74 30	+	,,
		95 0	+	,,
4.	12 0	71 0	?	,,
		85 0	?	,,
5.	16 0	70 0	—	,,
		85 0	—	,,
6.	20 0	7 0	—	At 17 minutes asphyxia for 1 minute 30 seconds.
		8 0	—	

Note.—The upper of the coagulation times shows the commencement of clotting, the lower the completion of that process.

These observations are concordant with those of Achard and Aynaud (30) and of Sacerdotti (31) who found that platelets could be absent or present in peptone blood without altering its coagulability. By centrifuging blood peptonised *in vitro* the present authors have shown that the anti-coagulant action of peptone on blood is independent of leucocytes and erythrocytes, and the same remark applies to plasma *in vitro* kept fluid by addition of nucleic acid. The conclusion is thus reached that the anti-coagulant action of each of those substances is on the plasma and not on the formed elements of the blood.

VI. The Action of Nucleic Acid on Blood in vitro.

The blood was shed through paraffined cannulæ into clean glass vessels from pithed animals respiring air; the liver was out of the circulation.

In the following tables the upper of the coagulation times is that of commencement, the lower of completion of clotting.

Table VI.

No. of Experiment	29	30	31	32	33	34
Percentage of Doyon's thymus nucleic acid in blood	0·01	0·05	0·10	0·15	0·175	0·2
Coagulation times of control blood	min. sec. 8 5 9 25	min. sec. 8 5 9 25	min. sec. 7 20 9 5	min. sec. 7 20 9 5	min. sec. 7 20 9 5	min. sec. 8 5 9 25
Coagulation times after addition of nucleic acid	min. sec. 6 0 8 10	min. sec. 7 45 9 0	min. sec. 33 40 39 0	min. sec. 31 0 45 0	min. sec. 42 25 60 0	min. sec. 130 0 ?

NOTE.—In experiment No. 34 the blood was found clotted on the following morning. No deposit was found on the surfaces of the containing vessel.

Similar results were obtained when Levene's thymus nucleic acid was substituted for that of Doyon. Similar results were also obtained when a plasma prepared by centrifuging blood in paraffined tubes at 4° C. was substituted for whole blood.

Table VII.

No. of Experiment	35	36	37	38	39	40
Percentage of Levene's yeast nucleic acid in blood	0·01	0·1	0·15	0·18	0·225	0·3
Coagulation times of control blood	min. sec. 9 0 10 30	min. sec. 9 0 10 30	min. sec. 9 0 10 10	min. sec. 9 0 10 30	min. sec. 9 10 10 30	min. sec. 9 0 10 10
Coagulation times after addition of nucleic acid	min. sec. 7 0 8 15	min. sec. 13 10 15 0	min. sec. 14 0 15 45	min. sec. 17 50 19 10	min. sec. 24 10 26 5	min. sec. 36 0 42 0

NOTE.—No deposit was found on the surfaces of the containing vessels.

The experiments recorded in Table VI show that moderate concentrations of nucleic acid prepared from the thymus inhibit the clotting of blood *in vitro* provided the blood has not been in contact with damaged tissue. A like but less marked anti-coagulant action is exhibited by yeast nucleic acid (*vide* Table VII).

Doyon (3) has maintained that the anti-coagulant action of nucleic acid *in vivo* differs essentially from that exhibited *in vitro*, the former being assigned to secretion by the liver of an anti-thrombic nucleoprotein, the latter to specific

action of the nucleic acid on the blood. In support of this view Doyon has pointed out that anti-coagulant action is more marked *in vivo* than *in vitro*. This difference in degree is, however, intelligible on the view that nucleic acid combines more readily or more completely with circulating blood than with blood which has suffered change by contact with surfaces which are wetted by blood.

On this view the action of nucleic acid, both *in vivo* and *in vitro*, would be specific in nature and similar to that of peptone, which restrains clotting *in vitro* when surface changes in the plasma are minimised, but fails to inhibit clotting when these changes have arisen from contact with disturbing materials.

Summary.

The intravascular injection of Witte's peptone into tortoises deprived of hepatic activity inhibits the coagulation of blood subsequently shed. The inhibition of clotting is reduced or annulled by an increase in the concentration of carbon dioxide in the blood. In this respect, the blood of the tortoise behaves like that of the cat.

The addition of moderate concentrations of peptone to the blood of the tortoise *in vitro* causes prolonged inhibition of clotting, provided the blood has not been in contact with damaged tissue.

Tortoise blood kept fluid by admixture *in vitro* with peptone clots on dilution with distilled water, gives positive results when examined for fibrinogen, and ultimately coagulates if left exposed to the air.

Tortoise blood mixed with peptone after contact with lacerated tissue clots more slowly than does pure blood under like conditions, but coagulates more rapidly than blood shed on to glass.

Intravascular injection of peptone into rats with pigmented fur inhibits coagulation of the blood. The retardation of clotting is as great in animals deprived of hepatic circulation as in those with the liver fully active. An increase of concentration of carbon dioxide in the blood of this animal does not reduce the anti-coagulant action of peptone.

Partly pigmented rats are more resistant to the anti-coagulant action of peptone and to its toxic effect on the heart than are animals with completely pigmented fur. Very large amounts of peptone fail to inhibit the coagulation of the blood of the albino rat. The hearts of albino rats are very resistant to the toxic action of peptone.

Attention is directed to a relationship in certain rodents between the

pigmentation of the animals and their power of resistance to the action of peptone and of nucleic acid.

Peptone has little or no action *in vitro* on the blood of pigmented rats.

It is suggested that variations in the surface conditions of the blood of one animal from those of another may account for the toxic effect following the intravenous injection of a heterologous blood.

Dogs exhibit wide variations in their susceptibility to the anti-coagulant action of peptone on circulating blood. Such anti-coagulant action may be demonstrated in animals deprived of hepatic activity.

Attention is directed to the failure of the thrombin theories of coagulation to account for the cases of normal coagulability of the blood after massive intravenous injection of peptone.

The rapid intravascular injection, or addition *in vitro*, of thymus or yeast nucleic acids inhibits the coagulation of the blood shed from cats and rats which have been deprived of hepatic activity.

An increase of carbon dioxide in circulating blood of the cat decreases or annuls the anti-coagulant action of nucleic acid. A like effect was not observed with the blood of the rat.

Exhausted cats are more susceptible to the toxic action of thymus nucleic acid than are more normal animals.

Serial intravascular injections of thymus nucleic acid into cats or rats deprived of hepatic activity produce hyper-coagulability, followed by tolerance, culminating in immunity to the anti-coagulant action of nucleic acid.

Immunisation against the anti-coagulant action of thymus nucleic acid can be produced with material free from protein.

The rapid intravascular injection of thymus nucleic acid may cause a temporary disappearance of platelets. The anti-coagulant action of nucleic acid is exhibited during the presence and absence of platelets.

Reasons are given for rejecting the current views on the inhibition of clotting by nucleic acid. It is suggested that nucleic acid acts by union with plasma components, forming a more stable complex than that existent in normal circulating blood.

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