ON SOME EFFECTS UPON THE BLOOD PRODUCED BY THE INJECTION OF THE VENOM OF THE AUSTRALIAN BLACK SNAKE (PSEUDECHIS PORPHYRIACUS).

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In a paper read before the Linnæan Society of New South Wales[1], I showed that the poison of the Australian black snake, one of the largest of our dangerous reptiles, contained one or more albumoses, the injection of which into an animal, caused death with some of the symptoms commonly observed in cases of snake-bite. In a further paper, written in conjunction with Mr McGarvie Smith[2], we showed that the only poisonous constituents present in the venom, were bodies of the nature of the primary albumoses.

In the present paper, I propose to discuss one specific aspect of the physiological effect of the injection of the venom, which has become apparent during the course of an investigation I am now carrying on, into the action of this poison on the animal organism.

A. Effect on the coagulability of dog's blood.

In the general enquiry, I have frequently had occasion to use such methods as are commonly employed for obtaining graphic records of the blood pressure in arteries and veins, and have been struck with the remarkably satisfactory manner in which the blood has behaved as regards clotting. On more than one occasion, a tracing of the venous

[1] "Observations on the poisonous constituents of the venom of the Australian black snake." *Proc. Linn. Soc. N. S. W.* July 1892.

[2] "On the venom of the Australian black snake." *Proc. Roy. Soc. N. S. W.* 1892.

pressure in a dog has continued for some hours, without coagulation occurring in the cannula.

This behaviour suggested some inhibition of the normal clotting power of extravascular blood. During several experiments, I accordingly drew samples of blood. In all of these coagulation was retarded, the specimens clotting only after the lapse of one to twenty hours, or not at all. At this time I was working with the intravenous injection of very small quantities of the poison, viz.—0·00001 to 0·00002 gramme per kilogramme of body weight[1].

I am aware that such an observation on the fluidity of the blood, following the introduction of snake-poison into the system, is by no means new, but in my previous experience with rats, rabbits, guinea-pigs, and dogs, the blood post-mortem, was always found coagulated. Fontana[2], more than one hundred years ago, noticed that the blood remained fluid in animals dead of viper-bite, and Brainard[3] writing forty years back states, that when death occurred *immediately* (the italics are mine) in animals bitten by rattle-snakes, the blood was found at the post-mortem examination to be clotted, but if some time elapsed before the animal succumbed, the blood remained fluid in the vessels. These observations of Brainard were confirmed by Weir Mitchell[4] in 1860, who explained the difference by the hypothesis, that in cases of very rapid death the poison had not had time to affect the blood. A few years later Halford[5] observed the same continued fluidity of the blood, to follow the injection of the venom of some Australian species[6].

More recently Feoktistow[7] has confirmed Fontana's observation on the condition of the blood after the injection of viper-poison (*v. ammodytes* and *v. berus*). The continued fluidity of the blood after death from the bite of the Indian viperine snakes, has been frequently

[1] All the weights mentioned in this paper refer to the venom dried over calcium chloride at the temperature of the laboratory. The freshly ejected venom, as Mr Smith and I have shown, varies greatly in the percentage of solids contained, viz.—from 12 to 65 % according to the time of year, and the conditions of the reptile as regards previous discharge of poison, state of health, and nourishment.

[2] *Fontana on Poisons.* Translated by J. Skinner. London, 1787.

[3] *Smithsonian Reports*, 1854.

[4] *Smithsonian Contributions to Knowledge*, Vol. XII.

[5] *Med. Times and Gazette*, 1873, Vol. II.

[6] Quite recently Dr Skinner, of Beechworth, Victoria, has recorded the same diminished coagulability of the blood in a case of snake-bite which occurred in his practice (*Australian Med. Gaz.* Mar. 15, 1893).

[7] *Mémoires de l'Académie impériale des Sciences de St Pétersbourg*, VIII^e^. Série, T. XXXVI. No. 4, 1888.

noted during past years by numerous observers in that country. This observation contrasts with the negative result in this respect, following the injection of cobra-poison.

On other occasions, when taking carotid pressure tracings during the injection of large doses of venom (0·0002 gramme per kilo. or above this quantity) within a minute of the time when the poison was introduced, the record of the rise in pressure due to the heart's beat disappeared, but the pressure in the manometer remained considerably above zero. On investigation it was found that there were no clots in or near the cannulae, but in each case the artery, at some little distance from the cannula, contained a solid core of clot extending into the aorta and left ventricle. On further dissection the blood in the whole vascular system, excepting only the pulmonary veins and the left auricle, was found to be solid. The clot in the portal venous system was particularly hard.

These experiments I have repeated very many times, in fact one or the other phase of coagulability occurs every time this snake-poison is injected into a vein of a dog. Immediately after the introduction of the venom, the coagulability of the blood increases, and this increase of coagulability, in the case of moderate or large doses (more than 0·0001 gramme per kilo.), culminates in intravascular clotting of greater or less extent. The smallest dose which I have found to produce complete clotting in the systemic circulation was 0·00015 gramme per kilo. Such a dose, however, usually gives rise to thrombosis limited to the portal vein and its branches, and inhibition of coagulability elsewhere.

The injection of small doses,—*i.e.* below 0·0001 gramme per kilo.—also gives rise to a condition of increased coagulability of the blood. This increase however, only manifests itself for an extremely short time, and samples must be drawn within two minutes from the time of the introduction of the venom, in order to observe it. This transient positive phase is succeeded by a negative phase, for blood drawn three minutes after the injection, either fails to clot at all, or does so only after the lapse of several hours. The negative phase continues for a considerable period, five or six hours, should the animal live so long; but as every experiment in which I have been able to establish it in a marked degree, has terminated in the death of the dog, I am unable to say what may be its extreme limit.

The blood in all parts of the vascular system does not exhibit the same tendency to clot in the vessels. Coagulation when present always occurs in the portal venous system and the thrombosis is frequently

confined to this area. It also occurs more readily with venous than with arterial blood.

Dogs evince varying degrees of susceptibility in this respect, the same dose per kilo. producing in one animal intravascular clotting, in another inhibition of the coagulation of the shed blood.

B. **Results with animals other than dogs.**

I have examined the condition of the blood after the intravenous injection of snake-poison in rabbits and cats. In these animals both positive and negative phases occur as with dogs. Cats appear more resistant to the action of the venom than dogs and rabbits.

C. **Conditions modifying the result of the injection of the venom.**

(1) *Influence of Respiration.*

I have previously mentioned that I have on no occasion, observed the blood which has just traversed the capillaries of the lungs, to participate in an otherwise general thrombosis. Whatever change may be effected on the plasma during its passage through the pulmonary vessels is very transitory, for in such cases it is only in the pulmonary veins and left auricle where it remains fluid, the blood in the left ventricle and aorta being solid.

When complete clotting of the blood in both systemic arteries and veins follows injection of the poison, respiration continues often for a minute or more, after the circulation is at a standstill. If records of the arterial blood pressure and of the respiratory movements be taken in these experiments, the moment of clotting in the arteries is indicated on the tracing, by the disappearance of heart-beats, and the straight line described by the style, some distance above the abscissa. I have in my possession a number of tracings showing the continuance of respiration for periods up to two minutes after the circulation must have been completely blocked.

These experiments show that as long as the blood will flow through the pulmonary artery and its branches there is nothing to prevent its normal gaseous interchange, as far as the respiratory movements are concerned. That it takes up oxygen as usual is seen by the colour of the blood in the left heart.

To ascertain whether the modification of the coagulability of the

blood during its passage through the lungs, depended on its altered gaseous condition, I followed the method which Wright[1] used to determine the cause of the distribution of thrombosis after injections of Wooldridge's tissue-fibrinogen. This observer rendered animals dyspnoeic by compression of the trachea previous to the injection of doses which, as he had determined would, without this treatment, only give rise to a very limited intravascular clotting. The results of injecting "tissue-fibrinogen" into the circulation when in this venous condition were strikingly altered. My experiments were performed on rabbits of like weight. Several were intravenously injected, under exactly similar circumstances, with gradually decreasing doses of venom, until a dose was found which produced a very limited clotting or none at all. Other rabbits of the same weight were then rendered dyspnoeic by compression of the trachea continued for one minute before the subminimal dose of venom was introduced. These subminimal doses produced in every case complete clotting throughout the whole vascular system.

It would be rash to assert that this difference between the blood in the pulmonary artery and that in the pulmonary vein is simply due to its having lost carbonic acid and gained oxygen, for it is possible that the plasma may be so modified during its sojourn in the pulmonary capillaries in some other way.

In connection with this counteraction of the increased coagulability of the blood, by its passage through the lungs, Pawlow's[2] observation that blood circulating only through the heart and lungs and a Ludwig's automatic stromuhr, lost its power to clot, is interesting.

(2) *Influence of digestion.*

On looking through my notes I find records of eleven experiments in which 0·0002 gramme per kilo. was injected into the jugular vein of a dog. Seven of these animals died within eight minutes, from extensive venous and arterial thrombosis. Three others died within two hours, and one lived for upwards of four hours. The post-mortem examination of this last animal discovered no intravascular clotting and the shed blood remained fluid until it putrefied. In the three which lived from one to two hours after the injection, there was more or less

[1] This *Journal*, Vol. XII. No. 2.

[2] "Einfluss des Vagus auf die Arbeit der linken Herzkammer." *Du Bois Reymond's Arch.* 1887, p. 458.

thrombosis of the portal venous system, but the blood from the rest of the body remained fluid for several hours.

These experiments were not performed with the sole object of observing the effect of venom on the clotting of the blood, and in every one of them, records, including one of arterial blood-pressure, were taken on a kymograph. Consequently I have in each case the exact time and duration of the injection to refer to. In all the experiments the above mentioned amount per kilo. was dissolved in 2 c.c. of a ·7 % solution of NaCl, and the time occupied by the injection, as registered on the tracing, was from five to eight seconds. The importance of the knowledge that the rate of injection was fairly constant, I shall discuss later on.

At the time of these experiments I attributed the variations in results to idiosyncracies possessed by the dogs. On reconsidering the matter however, I am inclined to attribute them to the effect of digestion. The seven first mentioned experiments were performed in sequence and the four last also in sequence. During the former series I was working in the afternoon, whilst the experiments forming the latter series were performed in the forenoon, and I find that the dogs have always been fed at 11 a.m. Thus, for the first series dogs which had had a meal three hours previously were used, whereas the last four experiments were made upon animals which had fasted for nearly 24 hours.

(3) *Influence of rapidity of injection.*

The variations in effect caused by alterations in the rapidity of injection are very marked. So much so, that it is impossible to compare the results from different experiments, unless the rate with which the poison is introduced is a constant factor. Intravascular clotting is produced most readily if the dose be rapidly thrown into a vein near the heart (*e.g.* the jugular). On increasing the time of delivery of the poison into the circulation, either by employing diluted solutions, or by pressing down the piston of the syringe more slowly, the positive phase (increased coagulability of the blood) is less and less pronounced. If the duration of the injection be still further prolonged, the positive phase, if present, is so rapidly succeeded by the negative variation that this latter appears to be the only result. The negative phase becomes more and more pronounced as larger quantities of the venom are allowed to slowly enter the circulation. For slow injections I have connected a burette containing a very dilute solution of venom

in ·7 % NaCl with the cannula in the vein, by a piece of rubber tube, the calibre of which was controlled by a screw clamp. With this arrangement I have been able to introduce large doses (0·005 gramme per kilo.) and yet only to produce inhibition of the coagulability of the blood.

The discovery that the effects on the blood, after slow injections, are vastly different from, in fact exactly opposite to, those following rapid introduction of the venom, explains why, in my earliest experiments, I did not obtain the same fluid condition of the blood as Halford[1], who used either subcutaneous injection of the venom, or else allowed a snake to bite the dog. By these methods delivery into the circulation would necessarily be slow, and comparable with results obtained by intravenous injection, only in cases in which such injection was very gradual.

(4) *Influence of previous injection of the venom into an animal on the result of a subsequent injection.*

If a small dose of venom (less than 0·0001 gramme per kilo.) be introduced into an animal, the blood, as previously mentioned, exhibits for about two or three minutes after the completion of the injection, an increased coagulability. That is to say samples drawn during this period clot sooner than control samples taken previous to the injection. At the same time the arterial blood-pressure falls to one half or one third of its former height. After twenty or thirty minutes the pressure begins slowly to rise and one hour to one hour and a half from the time of the injection may have reached to nearly its original height. During the whole of this period with the exception of the first two or three minutes, samples of blood taken from an artery, clot only after some hours, or not at all.

If now, one hour or more after the first injection a second injection containing a much larger quantity (ten or twenty times the first dose) be made, the introduction of this second dose does not increase the coagulability of the blood in the least. Samples of blood drawn after the second injection do not coagulate any sooner than those drawn before. I have always introduced at the second injection a quantity of the venom which would inevitably produce complete arterial and venous thrombosis in an animal which had not been previously sub-

[1] *loc. cit.*

jected to an injection of a dose sufficient to establish a negative variation.

On one occasion I gave a dog yet a third injection containing comparatively speaking, a very large dose, viz.:—·002 gramme per kilo. This amount, dissolved in 3 c.c. of ·7 % NaCl solution, was injected as rapidly as possible into the femoral vein. It also failed to produce any increased coagulability of the blood, for the samples drawn immediately after the injection did not clot at all.

It would thus appear that the establishment of a negative variation confers an immunity, as far as intravascular clotting is concerned, against further injections of the venom. This immunity is very speedily produced; how long it may last I am at present not in a position to say.

These facts are capable of explaining how it is one can introduce large quantities of the venom into the circulation, provided this be done slowly, without producing intravascular clotting. The first portion of the injection causes a transitory positive phase, *i.e.* increased coagulability, but not sufficient to cause actual thrombosis. This is immediately superseded by a negative phase, and the establishment of this inhibitory phase confers immunity against the remainder of the injection.

Before discussing the bearings of these facts, I shall indicate the results of an examination of shed blood which has been subjected to the action of the venom, and of some of the reactions of the non-coagulable blood itself.

D. Action of a solution of venom on the coagulation of shed blood.

To determine whether a solution of venom were capable of exerting any influence upon shed blood, I dissected out three inches of the femoral artery of a dog, and bled from this, directly into a solution of the poison. The cut end of the artery was immersed in a ·7 % solution of NaCl, containing 0·1 % of venom. By releasing the compression of the fingers a small amount of blood—equal to the volume of the salt solution—was allowed to flow into the vessel. The two fluids were at the same time freely mixed, by agitating the end of the artery. The blood began to thicken in 56 seconds, but clotting did not proceed in a normal manner. At the end of an hour a small soft clot formed, which on shrinking failed to entangle all the corpuscles. The vessel containing the mixture of blood and venom solution, could at no time be

inverted without upsetting the contents, as could be done to a vessel containing a control with salt solution alone.

On no occasion have I been able to prevent coagulation altogether, as was done with the venom of the rattle-snake, by Weir Mitchell and Reichert[1]. These authors drew the blood directly into a vessel containing a solution of venom (a stronger solution than mine) surrounded by a freezing mixture. On allowing the temperature of the blood to rise gradually, they found it remained permanently fluid.

I have never used solutions stronger than 0·1 % as my supply of poison is too limited, and owing to the incidence of the winter season, it cannot for the present be reinforced.

E. Examination of samples of blood exhibiting the negative variation.

Negative phase blood always shows marked retardation in clotting. The delay is so great in some cases that putrefaction sets in before coagulation has occurred. The loss of coagulability is the more pronounced the greater the amount of venom which has been introduced. There is not the slightest difficulty in producing this negative phase: it is only necessary to introduce the poison sufficiently slowly at first, in order that the initial positive variation may not reach the pitch, when intravascular clotting occurs. The duration of time before coagulation sets in, is in most cases considerably shortened by keeping the samples at 37° C.

The blood of the negative phase takes up oxygen and gives it up in the usual manner and with the exception of its loss of spontaneous coagulability and the fact that some of the haemoglobin is dissolved in the plasma, does not appear to depart from the normal. I have however not yet examined the condition of the blood in respect to the amount of gaseous constituents.

Samples of blood in which the negative phase is not too pronounced may be made to clot by the addition of the following—

(1) Alexander Schmidt's fibrin-ferment,
(2) Calcium chloride (sometimes),
(3) Wooldridge's tissue-fibrinogen,

and by

(4) dilution,
(5) passage of CO_2 through the liquid.

[1] *loc. cit.*

Frequently samples of blood will not clot on the addition of any or all of the above list, even when kept at 38° C. Of all the various agents mentioned, tissue-fibrinogen exerts the most powerful influence in producing a clot. Specimens which still remain fluid after treatment with all the other four methods, may often be induced to clot by the addition of a few drops of an alkaline solution of "tissue-fibrinogen." The addition of "tissue-fibrinogen" to the serum from such blood as coagulates feebly on standing, produces a second clotting.

F. Significance of these phenomena.

These phenomena of intravascular coagulation exhibiting positive and negative phases, together with the conditions which I have found to modify the result, show the closest parallelism with the effects observed by Wooldridge[1], Wright[2], and Halliburton[3], to follow the introduction of "tissue-fibrinogen" into the circulation. This parallelism is still further evident when one compares the behaviour of the fluid blood, drawn from an animal poisoned with venom, in which the negative phase is pronounced, with the reactions which have been found to characterise the blood drawn during the corresponding phase following the intravenous injection of "tissue-fibrinogen."

Wooldridge in one of his papers[4] thus summarises the results obtained by injecting tissue-fibrinogen:—

"If a solution of tissue-fibrinogen be injected into a dog in varying quantity, the effects observed are: with very small quantities no discoverable intravascular clotting occurs, but the blood, drawn off after the injection clots very slowly, 1—2 hours intervening; with larger quantities, intravascular clotting takes place, being as a general rule, chiefly confined to the portal venous system—the extent of clot being

[1] "On intravasc. coag." *Proc. Roy. Soc.* 1886. "Ueber intravasc. Gerinnung." *Du Bois Reymond's Arch.* 1886. "Uebersicht einer Theorie der Blutgerinnung." *Ludwig's Festschrift*, 1887. "On haemorrhagic Infarction of the liver." *Proc. Path. Soc.* 1887. *The Nature of Coagulation.* Hamsworth and Co. London, 1888. "Ueber Schutzimpfung auf chemischem Wege." *Du Bois Reymond's Arch.* 1888.

[2] "On the conditions which determine the distribution of the coagulation following the intravascular injection of Wooldridge's tissue-fibrinogen." This *Journal*, XII. No. 2. "A study of intravascular coagulation produced by the injection of Wooldridge's tissue-fibrinogen." *Proc. Roy. Irish Acad.* 3rd Series, Vol. II. No. 2. "On tissue or cell fibrinogen in its relation to the pathology of blood." *Lancet*, Feb. 27 and Mar. 5, 1892.

[3] "The proteids of kidney and liver cells." This *Journal*, XIII. Supplementary No. "The chem. physiol. of the animal cell." Goulstonian Lectures, lecture 3. *B. M. J.* Mar. 25, 1893.

[4] *Nature of Coagulation*, p. 31.

greater as more tissue-fibrinogen is injected. The shed blood [from other areas] will not clot. The more tissue-fibrinogen there has been injected the more complete is this prevention of the [extravascular] clotting—the interval between the drawing off and the clotting varying from 2 to 30 hours. In most cases the blood can be readily made to clot firmly by additions, such for instance, as the ordinary fibrin-ferment, and in the great majority of cases it clots firmly on standing."

A few pages further on in the same paper he says:—

"Further, the injection of a very large quantity of tissue-fibrinogen always leads to the production of a shed blood entirely non-coagulable, either spontaneously, or on addition of leucocytes, or tissue-fibrinogen. The only difficulty is this, that frequently the circulation is arrested before the required quantity is got in, and then only marked slowing of the shed blood is produced."

A summary of my experiments showing the effect on the coagulability of the blood from the intravenous injection of different amounts of venom, could well enough be given in the same words as those used by Wooldridge, substituting only "venom" for "tissue-fibrinogen."

Wooldridge also found, that variations in the condition of an animal, were followed by alterations in the amount and distribution of the thrombosis, and Wright[1] in following up the researches of this observer, has very considerably extended our knowledge in this respect, as regards both dogs and other animals.

One of these modifying conditions observed by Wooldridge was that in fasting dogs, unless large quantities of the "tissue-fibrinogen" were injected, the intravascular clotting was limited to the portal area; whereas the same dose given to an animal in full digestion produced clotting extending to the general venous system, right heart and even into the arteries. I have shown reason for believing that the blood of animals in full digestion exhibits the same increased sensitiveness towards the action of the venom.

I have previously had occasion to mention an experiment of Wright's in which he rendered an animal dyspnoeic by compression of the trachea, before injecting tissue-fibrinogen. Wright found that the injection of an amount of "tissue-fibrinogen" which would, in an animal not so treated, give rise to clotting only in the portal venous system, was under these circumstances followed by intravascular clotting throughout the whole vascular system.

[1] *loc. cit.*

Similar experiments with venom produced results corresponding in every case to those detailed by Wright. I may add that our able assistant Mr Robert Grant who had had the opportunity of helping my predecessor Dr A. E. Wright in his experiments, could not indicate the slightest difference in the two sets of results.

Another experimental condition which influences in an exactly corresponding manner the results from injections of both "tissue-fibrinogen" and venom, is the rapidity with which they are introduced into the circulation. Wright[1] has already drawn attention to this factor as influencing the results from the injection of "tissue-fibrinogen." I have experimented with "tissue-fibrinogen" in order to determine this point and find that, as I have indicated to be the case with venom, one can produce at will the positive or negative phase by the injection of the same amount of "tissue-fibrinogen," simply by varying the rate at which it is allowed to enter the circulation. Both "tissue-fibrinogen" and venom may be introduced in great quantity, without increasing the coagulability of the blood, once the initial positive phase is passed and a negative phase established, if the solution be allowed to flow very slowly into a vein.

In 1888 Wooldridge[2] published a remarkable result he had obtained with the injection of his "fibrinogens," viz.—that the injection of one dose conferred upon the animal immunity from further injections. I have repeated this experiment with "tissue-fibrinogen" with the same result; and as I have shown, exactly the same occurs with the venom. The first injection produces an immunity, as far as the clotting of the blood in the vessels is concerned, from the effects of future doses, even when these are very large.

There is however one result of the injection of "tissue-fibrinogen" recorded by Pekelharing[3], viz.—the presence of "peptone" in the plasma during the negative phase—which I have been unable to discover in animals after the injection of venom.

From the very conspicuous analogy between the behaviour of the non-coagulable blood obtained from an animal after "fibrinogen" injection, Wright[4] has formulated the hypothesis, that the want of spontaneous coagulability is due to the same cause in both cases. This

[1] *Proc. Roy. Irish Acad.* 3rd Series, Vol. II. No. 2.

[2] "Schutzimpfung auf chemischem Wege." *Du Bois Reymond's Arch.* 1888.

[3] *Verhand. d. Konink. Akad. v. Wetenschappen te Amsterdam.* Tweede Sectie, Deel 1, No. 3.

[4] *Proc. Roy. Irish Acad.*

theory of Wright's is further supported by the fact that conditions which influence the result of peptone injections also modify the negative phase of blood after fibrinogen injections; the above-mentioned statement of Pekelharing, and Wright's own observation that under the same circumstances albumoses appear in the urine, both point in the same direction.

My inability to confirm Pekelharing's statement as to the presence of peptone, appeared to show, that although the two series of phenomena had up to this point exhibited such a striking similarity, in this important respect they differed.

I accordingly thought it advisable to make experiments with tissue-fibrinogen, with the object of confirming Pekelharing's statement or otherwise. The determination of this question I found more difficult than I had anticipated, firstly because I am unfortunately unable in Sydney to consult Pekelharing's original paper (I am indebted to my friend Prof. Halliburton for the reference to his work) and so am ignorant of what method he employed to separate any "peptone" from the plasma. Secondly, I conceive it very probable, that he may have used "peptone" to include albumoses, and I knew of no method free from sources of error by means of which small amounts of albumoses could be separated from the other proteids in plasma[1].

I have however found a method, which is entirely satisfactory, for separating not only peptone, but all the albumoses, from plasma. I will here merely state that I have been unable to discover the slightest trace of albumose, or peptone in the non-coagulable blood produced by the injection of either venom or "tissue-fibrinogen," and reserve full details of the experiments and the methods employed for a further communication.

The parallelism, as far as the coagulation of the blood is concerned, between the results of the injection of "tissue-fibrinogen" and of venom, is maintained when one examines the conditions under which the two kinds of fluid blood may be induced to clot.

Both these kinds of blood remain fluid, or clot only after the lapse of some hours, the time intervening before the onset of coagulation

[1] In the report of Halliburton's Goulstonian Lectures in the *B. M. J.* for Mar. 25, 1893, speaking of trichloracetic acid Prof. Halliburton says: "This reagent precipitates all the proteids except proteoses and peptone." This I find is not absolutely correct, as this reagent precipitates the larger proportion of the albumoses, especially of hetero-albumose, which Pollitzer (this *Journal*, Vol. VII.) showed to be the most active in producing non-coagulable (peptone) plasma.

depending in both cases on the amount of the agent injected. In each case clotting may be accelerated, if the vessels containing the blood be placed in an incubator at 37° C. Both kinds may in most cases be made to clot by the addition of the following—"tissue-fibrinogen," fibrin-ferment, calcium chloride, and by dilution and the passage of CO_2 through the liquid.

So close is the parallelism between the two series of phenomena, that one is inevitably driven to enquire, whether, after all, there may not underlie them both, an actual identity of process. But in thus enquiring, one is confronted by the very striking fact that, in the venom experiments, only an exceedingly small quantity of the active agent is required to produce an effect negative or positive on the coagulability of the blood.

Wooldridge stated that it required 1·5 to 2 grammes of his "tissue-fibrinogen," to produce intravascular clotting, in a medium-sized dog. This I should imagine, from my own experiments with "tissue-fibrinogen," is a rather high estimate. However this may be, it is certain that in the one case we are dealing with the injection of grammes and in the other with thousandths or ten-thousandths of a gramme. He also found that his "fibrinogen" disappeared in the process of coagulation and presumably took part in the formation of the clot[1].

On account of the minute quantity of venom required to produce the intravascular clotting, it is hard to conceive that it can operate by any such direct action. An important difference between the two series of phenomena which I have always noticed is, that whereas with injections of "tissue-fibrinogen" the clotting occurs practically instantaneously; so much so, that it frequently causes blocking in the vein before one has had opportunity to finish the injection; with venom-injections there is an interval of, between (90—100 secs.) before clotting occurs in the arteries, but notwithstanding this delay in onset, the clotting in cases of venom-injections is much firmer and more extensive than I have ever been able to obtain with "tissue-fibrinogen."

Pekelharing[2], Wright[3], and Halliburton[4] have recently shown that the bodies Wooldridge designated "tissue-fibrinogens" consist

[1] *Nature of Coagulation* and *Ueber intravasc. Gerinnung.*

[2] *Virchow's Festschrift*, Bd. I.

[3] *Proc. Roy. Irish Acad.* 3rd Series, Vol. II. No. 2.

[4] This *Journal*, Vol. XIII. Supplementary No.

largely of nucleo-albumens, and that these nucleo-albumens are the active agents in producing intravascular clotting.

The venom of the Australian black snake does not contain any body of the nature of a nucleo-albumen. If a clear solution of venom be subjected to artificial gastric digestion, it is quite unaltered in appearance (no precipitate of nuclein). Moreover by such treatment, it is not deprived of its toxic properties.

The question has now to be answered whether, notwithstanding the negative result of the examination of the venom for nucleo-albumens, there is yet any source, from which through the direct or indirect action of the venom (*e.g.* the production of this toxic agent within the organism itself, under the influence of the specific toxic activity of the venom) a sufficient supply of nucleo-albumen could be derived.

With the idea of determining whether venom was capable of disintegrating cells, and in this way setting free nucleo-albumens sufficient to account for the symptoms, I turned my attention first to the organised elements of the blood. Wooldridge[1] produced intravascular coagulation by injecting the stromata of mammalian blood corpuscles, and the similar results of Köhler[2] obtained by injection of the haemaglobin-stained fluid expressed from warm blood-clot, may no doubt be explained as due to broken up corpuscles. Krüger has confirmed Wooldridge's result as regards the stromata of bird's blood.

That the disintegration of red blood cells gives rise to nucleo-albumens, was shown as early as 1881 by Wooldridge[3] himself. It is true he did not give a name to this body which he derived from stromata, but he refers to it as, "Ein Eiweisskörper, der mit einem anderen an Phosphor reichen Molecül verbunden ist." Wooldridge came to this conclusion from the fact that by digesting this body with gastric juice, peptone and a phosphorous-body, which he compares with Miescher's nuclein, were formed.

Groth[4] and Krüger[5] have found intravascular coagulation to follow the introduction of a saline extract of lymph-glands, containing leucocytes, into the circulation. Wooldridge criticised these results of Groth and Krüger on the ground that *well-washed* leucocytes have no such effect, and he ascribed the consequences of their injection to the

[1] *Practitioner*, 1886.

[2] "Ueber Thrombose etc." *Inaug. Diss.* Dorpat, 1887.

[3] "Zur Chemie der Blutkörperchen." *Du Bois Reymond's Arch.* 1886.

[4] "Ueber die Schicksale der farblosen Elemente im kreisenden Blute." Dorpat, 1884.

[5] *Zeitschr. f. Biologie.* Vol. XXIV.

fluid and not to the cells. As nucleo-albumens are set free by the disintegration of cells, Wooldridge would by his washings separate the products of disintegration, whereas Groth and Krüger would inject them.

There is then abundant evidence that the disintegration of both kinds of blood cells outside the body, sets free nucleo-albumens, which if introduced into the circulation may give rise to intravascular clotting, and I do not think it is an unwarrantable assumption that if any agent could be found to produce this cell destruction in a wholesale manner in the circulation, clotting would result.

The first animal the blood of which I submitted to the action of the venom, was the handy frog. A drop of frog's blood was mixed with an equal bulk of a ·7 % solution of NaCl containing ·1 % of venom. The mixture was made on a slide, and speedily covered with a cover glass and the edges smeared with oil to prevent concentration of the salt solution by evaporation. Within a few moments a disintegration of the red cells occurred. They lost their shape, their nuclei became apparent and the haemoglobin dissolved out; afterwards they appeared larger and circular, and became more and more indistinct, until finally nothing could be seen of them, but shrivelled granular nuclei. These shrivelled nuclei soon began to swell, the granules became less distinguishable and eventually disappeared. The disappearance of the red cells was so complete that at the end of fifteen minutes there was nothing except the slight colouration of the field, to distinguish the preparation from one of lymph.

The action on the white cells was much slower. For the first fifteen minutes I could discover no change in them, but they exhibited no amoeboid movements. At the end of this time the nuclei in some of them were very distinct, as if fixed by acetic acid. They then became intensely granular, and soon began to swell and their outlines to grow less distinct, until they disappeared, leaving a small heap of granules to mark their grave.

During this time, control specimens situated under similar circumstances showed no change and the leucocytes were exhibiting active amoeboid movements.

The action of the poison on the corpuscles of pigeon's blood is similar to the above, but the dissolution of the red cells, with poison of this concentration, only occurs after the lapse of about an hour and proceeds slowly.

Did the same extensive destruction of corpuscles take place by the

action of dilute solutions of venom, with the blood of dogs and rabbits, one would I think be justified in considering this destruction as a sufficient source of nucleo-albumens to explain the phenomena of intravascular coagulation.

Such is however not the case. If the human blood or that of a dog, or rabbit, be treated in exactly the same way as was the frog's, and in addition kept at the temperature of the body, by means of a warm stage, microscopic examination reveals no change in the red corpuscles. The only difference between the leucocytes of the venom specimens, and those of the controls is that whereas the latter exhibit amoeboid movement the former are still.

Fontana[1] obtained similar negative results by mixing blood (from what animal?) with solutions of viper-poison. Feoktistow[2], using 2% solutions of viper-poison obtained dissolution of the red corpuscles after 18—24 hours. I have not thought it necessary to examine the result of such strong solutions, nor to continue the observations longer than a few hours, because the phenomena with which this paper is concerned, take place with minute doses and within a few minutes.

On examining the sanguineous exudation, which collects after the lapse of some hours, at the point of inoculation, in animals subcutaneously injected with the poison, I have repeatedly found haemoglobin in solution. It would thus appear that the venom does in time, directly or indirectly, exert a destructive action on the red corpuscles.

I have mentioned that venom, when mixed with blood on a slide, prevents the display of amoeboid activity on the part of the white cells. That the same interference with the vital activity of the leucocytes occurs in the body is shown by the following experiment, which is one of a series with similar result.

Two small pieces of sterilised sponge about 1 m.m. cube were aseptically introduced into the abdominal wall of a guinea-pig. One of these little sponges had been soaked in a ·7% solution of NaCl containing 1% of venom, the other in the saline without the venom. Both sponges were pushed about a centimetre away from the incision, which was afterwards drawn together by a horse-hair suture and covered with collodion. After two hours a swelling arose around the venom-containing sponge, whereas the other remained of the original size. At the expiration of 5 hours the animal was killed and both sponges very carefully withdrawn and plunged into absolute alcohol.

[1] *loc. cit.* [2] *loc. cit.*

Sections of the two sponges treated in the same way presented very different appearances. The control was infiltrated with leucocytes which stained well with ordinary nuclear stains; the other contained leucocytes only near the margins and many of these were broken down and took the stain badly or not at all.

From these sponge experiments I conclude, that whereas, into the control sponge the leucocytes could by their amoeboid movements penetrate unharmed, in the other, their activity was paralysed, they succumbed and were eventually disintegrated by the solution of the venom.

I have also microscopically examined specimens of blood which had been taken from dogs subsequent to the intravenous injection of venom. The only departure from the normal that I could discover was that many of the leucocytes were gathered together into groups. I cannot say definitely under such circumstances whether the number of leucocytes be diminished or not, as owing to the massing together of the white cells, their enumeration with a haemocytometer is very difficult. One may examine numerous squares and meet with very few leucocytes and in another square encounter a mass of them. On this account a conclusion drawn as to their increase or decrease would be worthless.

These results with the blood of dogs do not indicate an immediate destruction of corpuscles. There is one fact however which points to their destruction to a small extent, viz. plasma obtained from negative phase blood is always stained with haemoglobin. This has also been the case in my experiments with plasma after the injection of "tissue-fibrinogen" and Halliburton[1] has noted the same fact in his experiments, so that one cannot accept it as evidence of the direct action of venom.

It is possible that venom may have some action on plasma itself, by which nucleo-albumen may be set free. Pekelharing[2] was able to separate a small amount of these bodies from plasma and Wooldridge[3] obtained from serum a body possessing the same properties, which he termed "serum-fibrinogen." Owing to the fact that nucleo-albumens disappear so rapidly from the blood, I cannot at present conceive of an experimental method, by means of which one could discover whether or not injections of venom do produce such a body from plasma.

Wooldridge obtained his "tissue-fibrinogen" from very various

[1] Goulstonian Lectures, 1893. [2] *loc. cit.*

[3] "On a new Constituent of Blood Serum." *Proc. Roy. Soc.* 1887.

kinds of cells and Pekelharing and Halliburton have increased the range. One must therefore conclude that the destruction of all manner of cells gives rise to these bodies in greater or less amount. Wright[1] has shown that by the extensive disintegration of cells which occurs in a resolving pneumonia, nucleo-albumens are set free. He was able to separate these bodies from the lungs of patients who had died in this stage of the disease, and has by injecting them into the veins of a dog produced intravascular clotting. In the case of pneumonia the nucleo-albumens would only reach the blood in small quantities at a time, and would not produce a marked effect.

From experiments with "tissue-fibrinogen" which I have quoted in this paper, it will I think be obvious, that if by the action of this agent, the coagulability of the blood is to be so increased as to cause intravascular clotting, they must first be present in the blood in some quantity. Even when large areas of cells are disintegrated, as in pneumonia, the rate of absorption is so slow, that this condition is not fulfilled, though Wright has suggested that it may explain the increased coagulability of the blood which has been observed in this disease.

It would be quite otherwise, were the nucleo-albumens the product of disintegration of cells in proximity to the blood stream, as are the endothelial cells lining the whole of the vascular system. In such a case the conditions would be practically the same as if these toxic agents were injected from without.

That a solution of rattle-snake venom *is* capable of playing havoc with these cells lining the blood vessels has been shown by Weir Mitchell and Reichert[2]. These authors moistened the mesentery of a cat, with a solution of rattle-snake poison, and observed under the microscope the rapid formation of extensive capillary haemorrhages.

In a few minutes the whole mesentery became, by the coalescence of the numerous haemorrhagic foci, absolutely infiltrated with blood. I have repeated these experiments, using black-snake venom, and although the action of this poison is less rapid than was the case in Mitchell's experiments, the results were identical. The mesenteries of cats or dogs were arranged under a microscope, so as to obtain a good view of the circulation in the capillaries. A minute fragment of the poison was then placed near that portion of the mesentery, in the field of the microscope. In a few moments, without any previous

[1] *Lancet*, 1892. [2] *loc. cit.*

clouding of the field, or stasis, small haemorrhages appeared. These invariably occurred first in those positions where the hydrostatic pressure was greatest, viz. from the wall of the capillary near to its origin from an arteriole, and where these capillaries branched or joined one another at right angles. These tiny haemorrhages increased in number and extent until the whole field was one mass of corpuscles. These appearances are very characteristic and altogether distinct from the escape of corpuscles in diapedesis and I think there is no doubt that the venom first damages the capillary wall, and that the pressure within causes an actual solution of continuity, the blood escaping through this rupture.

One of the most prominent symptoms in dogs, after the injection of a very small dose of venom (*i.e.* so small that the animal may live a sufficiently long time, for the full development of the symptoms) is the occurrence of extensive haemorrhages. I have often seen the whole of the mucous membrane of the alimentary canal, pancreas and spleen, one mass of haemorrhage; and sections of these organs appeared on microscopic examination absolutely infiltrated with blood corpuscles. Frequently too, more than half of the substance of the lungs is solid from haemorrhage into the alveoli and interalveolar tissue, and the intima of the aorta and endocardium more or less haemorrhagic. Under these circumstances the bowels are full of grumous bloody fluid and the pleural and pericardial fluid bloodstained.

In a case such as I have depicted, the damage to the endothelial wall is demonstrated by the passage of the red corpuscles through it, but I conceive that the amount of nucleo-albumen at once set free, is insufficient to so far increase the coagulability of the blood, as to cause intravascular clotting. With larger doses, though one can hardly suppose such damage to be less, the demonstration by the leakage of red corpuscles out of the capillaries, is often diminished, owing to the simultaneous great fall in blood-pressure. With still larger doses no demonstration occurs, for the blood coagulates almost at once.

Finally I may say that although I believe that the very striking facts which I have hitherto observed do warrant the hypothesis advanced in this paper, viz. that the phenomena of coagulation following the injection of snake-venom are *identical,* as regards their immediate causation, with those described by my friend the late Dr Wooldridge as consequent upon the injection of "tissue-fibrinogens," I am yet quite prepared to entertain a different explanation should further observation reveal facts discordant with this theory. But what

does appear to me of real and assured import is, that the observations which have been here recorded reveal highly important modifications in the constitution and character of the blood, under the influence of venom, and that in relation to these facts the snake-venom question assumes a somewhat novel aspect.

In conclusion I wish to express my gratitude to my friend Professor Wilson, for much valuable criticism, to Mr Grant for his enthusiastic help with the experiments and to the New South Wales branch of the British Medical Association for its generosity in granting me a sum of money, which has gone far towards defraying expenses.