ON THE ACTION OF THE VENOMS OF THE COBRA (NAJA TRIPUDIANS) AND OF THE DABOIA (DABOIA RUSSELLII) ON THE RED BLOOD CORPUSCLES AND ON THE BLOOD PLASMA.

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The action of the different varities of snake venoms on the constituents of the blood and on the blood coagulability is a subject which has engaged the attention of various observers in America, Australia and India. A study of the literature of the subject makes it evident that all venoms, which have been worked with, have a marked action on the blood, the effect of which is manifested in the production of changes of a destructive nature on the red corpuscles, as well as in an alteration of the coagulability of the plasma. From such a study, however, it is apparent that no investigator has attempted to carefully note the differences which exist between the various poisons in these respects. So much so is this the case, that Calmette of Lille[2] holds the opinion that all snake venoms have exactly the same physiological actions and only differ from one another in the degree of toxicity which they possess, one being more toxic than another. And, again, Martin of Melbourne[3] has put forward the working hypothesis, founded on his own experiments with the venoms of the Australian snakes, Hoplocephalus and Pseudechis, and on the researches of others with various venoms, that all snake venoms contain two toxic constituents of a proteid nature, A & B: A, incoagulable by heat and acting on the central nervous system; B, coagulable by heat and acting on the blood and heart; and that venoms differ from one another only in the relative proportion of these two constituents.

Along with Dr.Hanna[4] I have shown that Calmette's statement is without foundation, and also that Martin's theory

does not, in all probability, apply to the two venoms with which I propose to deal in this paper. Further, the facts which I have now to record support my previous observations and show without a doubt that in their physiological actions, the effects of which are seen in the changes in the blood constituents and plasma, the venoms of the cobra and of the daboia differ markedly from one another, and that Calmette's statement and Martin's hypothesis can no longer pass unchallenged and must be considerably modified to fit the facts now available. In the present communication, then, I propose to deal, 1), with the action which the venom of the Cobra (Naja Tripudians) and the venom of the Chain-Viper (Daboia Russellii) exert on the red blood corpuscles both 'in vivo' and 'in vitro', the result of which action is manifested by haemolysis: and 2), with the action which these two poisons have, also 'in vivo' and 'in vitro', on the blood plasma, as seen in an alteration of its coagulability.

At the outset it would be well to give a short account of the methods which were employed to collect and to conserve the poisons used in the experiments recorded below so as to ensure that the results obtained were uniform and comparable amongst themselves.

The venom in both instances was collected over a period of some months from a number of healthy living snakes by 'milking', or rather squeezing, the poison glands, the secretion being caught in a watch glass held in a pair of long forceps. The liquid poison was then thoroughly and rapidly dried over calcium chloride. The stock of venom, got in this way, was kept in an air-tight tube away from the light. It has been shown by many investigators that snake venoms, stored dry in this manner, preserve their toxicity unimpaired for an indefinite period. Before being weighed the venom was pounded to a very fine powder and was then kept in a weighing bottle over sulphuric acid or calcium chloride. This precaution is necessary in a climate in which the

atmosphere is saturated with moisture such as is the case in Bombay. As they were required, solutions of definite strengths made with saline solution of varying strength, as indicated in the protocols, were prepared. No solution was kept longer than an hour or two before being used. As the supply of venom was always abundant fresh solutions were prepared every day upon which experiments were made. In this way any diminution of toxicity, which, there is no doubt, takes place when snake venoms are kept in solution for any length of time, was avoided. With this introduction we may now pass on to consider

The action of Cobra Venom on the red blood corpuscles 'in vivo' and 'in vitro'.

It has been stated by some observers that cobra venom has no destructive action on the red blood cells 'in vivo'. This opinion is founded on the fact, that a microscopic examination of the circulating blood of animals injected with large doses of cobra venom shows no structural change of the red cells. The evidence, however, that this poison has a distinct haemolytic power 'in vivo', both in the case of man and of animals, is very strong. Thus, in the case of man after a cobra bite there is in every instance an exudation of a red-stained serous fluid from the punctures. A microscopic examination of this fluid shows the almost total absence of red blood corpuscles. This fact was first pointed out by Wall[5], while in a case of cobra bite, which, along with Dr. Hanna[6], I have put on record, this sanguineous discharge was well marked and lasted for 24 hours. Further, Wall[7] has affirmed, that in every case of death from cobra bite in man there is found on post-mortem examination at the site of the punctures a marked infiltration into the areolar tissues of a purple-blood-like fluid, and that this infiltration contains few red blood corpuscles.

In experimental observations in animals, especially in horses and donkeys, the abscess which sometimes forms, when

a sub-lethal dose of cobra venom is given, contains a thin red-coloured pus. Further, the mucous discharges are sometimes stained red. Thus, I have seen a marked red-stained discharge from the rectum of an ass, which had received a little over the minimum lethal dose of cobra venom. As I have said, however, in animal experiments with cobra poison all observers are agreed that repeated microscopic examinations of the blood of animals, which die from cobra venom intoxication, show an entire absence of any appreciable structural change in the red blood cells. The probable explanation of this fact is, either that, when a large dose has been injected, death takes place so rapidly, that such a marked destruction of the cells has not had time to take place as to be demonstrable by microscopic examination, or that, when a smaller dose has been given and death is delayed, the quantity of venom injected is not large enough to cause any extensive destruction of the red cells.

While this fact is undoubted, Cunningham[8] has shown in several experiments that, if the blood of an animal which has received a large dose of cobra venom, either intravenously or subcutaneously, be withdrawn from the body after death and allowed to clot, the serum which exudes from the clot is of a red colour; also that, if a fowl has been the animal employed, many free nuclei of the red cells are found on examining the blood at death; there has been, in fact, a considerable solution of the bodies of the red cells. Further, this observer has demonstrated that, if the dose of venom injected into a fowl is a very large one, namely, 100 to 300 milligrammes, the blood at death contains a great abundance of free nuclei, and the remaining red cells appear deformed: he has also shown that, if the specimen be allowed to stand, at the end of a few hours complete destruction of the red cells will have taken place. I have been able to confirm these statements of Cunningham. These observations, therefore, conclusively show that cobra venom does have a certain amount of haemolytic action

'in vivo', even when it is injected in moderate doses. When, however, a quantity of venom, which will bring about this effect in a marked degree, is injected into an animal, the animal dies so rapidly, that there has been time for complete destruction of only a comparatively few cells to take place. It would appear, therefore, from 'a priori' reasons that the haemolysis, which results from this action of cobra venom, can have little or no connection with the nervous symptoms which are always observed in cases of intoxication with this poison and cannot be a factor in producing the fatal result. An opposite opinion is, however, strongly held by Cunningham.

The destructive action of cobra venom on the red blood cells 'in vitro' has been investigated by several observers. Thus Cunningham[9] by drawing blood directly into solutions of cobra venom found that the serum which exuded from any clot which formed was dark red or brownish in colour. He demonstrated this phenomenon both with mammalian bloods and with fowl's blood. Kanthack[10], Stephens[11] and Myers[12] have also demonstrated the marked haemolytic action of cobra venom 'in vitro'. These observers, further, noticed that the blood cells of different animals were of different susceptibility to this haemolysing poison, and that this susceptibility of the blood cells bore no relation to the general susceptibility of the animals to subcutaneous injection of the poison.

The most convenient and most reliable method to demonstrate and to estimate this haemolytic action 'in vitro', the method which I have employed in all my experiments, is stated as follows.

The venom is dissolved in a solution of common salt, isotonic[13] for the particular blood under observation. This solution is made of a strength of 1 c.c.=2 milligrammes, namely, 0.2%. From this original solution a succession of two-fold dilutions, made with salt solution of the same strength as the original, is prepared in a series of test

X

102 c.c.

tubes, so that each dilution contains half the amount of poison of the one just above it in the series. A convenient quantity, namely, 0.5 c.c., of each dilution is then measured into a series of small test tubes. To each tube a measured quantity of fresh blood is added by means of a graduated capillary pipette. The qunatity of blood I found most suitable to add to each tube was 0.005 c.c. In these operations care must be taken to avoid, as far as possible, any bacterial contamination. Ordinary precautions, however, of having sterile tubes, sterile salt solution and sterile pipettes are all that are requisite. Control tubes of salt solution and blood alone are prepared in all experiments. The mixtures are allowed to stand at laboratory temperature (about 25°C) for 20 to 24 hours. It is then observed in which tubes complete laking has taken place, in which partial laking and in which no laking at all. When there is any doubt from the naked eye appearances as to whether complete or only partial haemolysis has taken place, a microscopic examination will soon settle the point. Working in this way, I have been able to confirm the general results got by the observers mentioned above, and, further, to estimate fairly accurately the haemolysing power of cobra venom towards the blood of several varieties of animals. But, inasmuch as the results obtained lead to the same conclusions as were arrived at by the observers mentioned above, it is unnecessary to detail these experiments. It is, however, well to point out that slight variations occur from time to time in the minimum amount of venom, which will produce haemolysis of the blood of any particular species of animal. Such variations were also noticed by Kanthack, but they are never so marked as to invalidate the general results. They are, in fact, quite explicable on the ground of experimental error. mentioned on page 1,

In view of Martin's hypothesis, it is important to be able to put on record some experiments which bear on the

effect which heating solutions of cobra venom has on the haemolytic action of this poison. In table I of the protocols, there are tabulated the results of such experiments. From this table it will be seen that, while heating solutions of cobra venom so as to precipitate all the coagulable proteids does not destroy the haemolysing power of the venom, such heating does cause a diminution of this power, and that this diminution is more marked if the solution which is heated be a comparatively weak solution. Thus, two solutions of cobra venom were made of 1% & 0.1% strength respectively. These were heated in the same water bath for half an hour at 73°C. The coagulated proteids were then filtered off. The haemolysing power of the same sample of venom, unheated, and of these two heated solutions was estimated after the method I have described above. A reference to the protocols will show that the greatest dilution of venom, in which complete or almost complete haemolysis was obtained in each instance, was as follows:

1) Unheated venom: Complete haemolysis:-0.5 c.c=0.015 milligramme.

2) 1% heated solution: Nearly complete haemolysis: 0.5 c.c.=0.0312 milligramme.

3) 0.1% heated solution: Complete haemolysis 0.5 c.c.= 0.25 milligramme.

This result is in harmony with, although not so striking as, the fact pointed out by Hanna and myself[14], namely, that, while the power of daboia venom to cause intravascular thrombosis is not completely destroyed, but only diminished, by heating a 1% solution of the poison for half an hour at 75°C, heating a 0.1% solution of this venom for the same length of time at the same temperature completely destroys this clotting power. Further, it lends no support to Martin's theory.

The action of daboia venom on the red blood corpuscles 'in vivo' and 'in vitro'.

There can be no doubt but that daboia venom has a marked destructive action on the red blood corpuscles 'in vivo', and that the results of this action are much more manifest to a clinical observer than is the case in bites from the cobra. Thus, in the recorded cases of daboia bites in man one reads of a large blood-stained extravasation all around the site of the punctures, of an exudation of a reddish-brown fluid from the wounds, of a dark-stained fluid oozing from the various mucous orifices of the body, from the nose, from the bowel, from the mouth, and of marked haematuria or haemoglobinuria. Actual haemorrhages from mucous surfaces are also commonly seen in cases of daboia bite.

In the case of experimental injections of daboia venom into animals, while both Wall[15] and Cunningham[16] note that sanious discharges are common sequelae of such injections, these observers do not particularly state that any destruction of the red blood corpuscles has taken place: a perusal of their works, in fact, leaves one with the idea that these discharges are stained with the whole blood and not with haemoglobin only. It is, however, easy to demonstrate that marked haemolysis results when daboia venom is injected into animals intravenously or subcutaneously. Two examples, out of many of which I have records, will be sufficient to put forward here. The first experiment was made along with Professor Martin of Melbourne, to whom I am indebted for much valuable assistance.

1) A horse of 293 kilos in weight received into the jugular vein 3 c.c. of a 1% solution of daboia venom, i.e., 30 milligrammes or about 0.1 milligramme per kilo. Convulsions began 50 seconds after the injection and returned at intervals till the horse died. Death took place 18 minutes

after the venom had run into the vein. Not long before death a bright reddish-stained fluid collected at the nostrils. A microscopic examination of this fluid showed it to contain very few red cells. Samples of blood were drawn from the jugular vein seven minutes before death and at death. Two hours after withdrawal both these samples were still only softly clotted. The red corpuscles had by this time sedimented leaving a dark red serum, which contained only an occasional red cell but plenty of blood platelets.

2) A female donkey, which weighed 89 kilos, received under the skin of the left shoulder 20 milligrammes of daboia venom, i.e. about 0.22 milligramme per kilo. This animal died in about 40 hours after the injection. The post mortem examination, made immediately after death, showed the following condition. Around the site of the inoculation there was a large bloody extravasation. On the front of the thorax at the root of the neck and also in the abdominal wall, namely two dependent parts, there were large oedematous swellings. On cutting into these swellings a dark port-wine coloured fluid escaped. Under the microscope this fluid was seen to contain ~~only~~ very few red cells. After this exudate had been centrifugalised the supernatant fluid remained dark in colour and contained no red corpuscles. The blood collected from a large vein clotted only slightly after standing for an hour. A sample of blood was centrifugalised immediately after it had been withdrawn from the body. The supernatant plasma, which microscopic examination showed to be quite free from red cells, was clear and of a very dark port-wine colour. After the blood had stood for a few hours a firm clot had formed, and the serum which had exuded, was of the same colour as the whole plasma got by centrifugalisation. The urine collected from the bladder was dark in colour like porter. It contained exceedingly few red blood corpuscles, only two or three being visible in a microscopic field.

I have been able to produce similar symptoms and evidence of marked haemolysis by injections of this venom into monkeys, rabbits and pigeons. These observations, therefore, show positively that daboia venom has a very marked and intense destructive action 'in vivo' on the red blood corpuscles of most animals, and that the effects of this action are important factors in the production of the symptoms observed and of the fatal result. To any one who has worked with both cobra and daboia venoms the marked difference which exists between their haemolytic action 'in vivo' must be apparent. The haemolysis produced by cobra venom might easily escape observation, while in the case of daboia poison it is a most prominent feature of the intoxication.

Such being the case one would expect to find that a marked haemolytic action with daboia venom would be easy to demonstrate 'in vitro', similar to the haemolytic action which I have mentioned is easily got 'in vitro' with cobra venom. This surmise, however, does not hold good. Thus Stephens[17], as a result of a few experiments only, has come to the conclusion that, while daboia venom dissolved in saline solution has no haemolysing power 'in vitro' on the red cells of man, this poison has a marked destructive power on the human red corpuscles if it is dissolved in normal horse serum. On the other hand, Cunningham[18] found that, if the blood of a fowl is run into solutions of daboia venom, the serum which exudes from the clot, which he remarks is always soft and tremulous, is dark and red in colour, showing that a considerable destruction of the red cells has taken place. Using a similar technique to that which I have described above in the experiments with cobra venom, I have found that it is possible to demonstrate that daboia venom dissolved in saline solution does produce 'in vitro' laking of the blood of many animals. This action, however, is somewhat variable. Care has to be

taken that the salt solution is not of too great a strength, that, in fact, it is just slightly stronger than the solution, which would of itself produce a certain amount of laking[13]. In table II of the protocols the results of five series of experiments of this description are detailed. The bloods used were those of man, monkey, rabbit, guinea-pig and pigeon respectively.

The strength of the salt solution to be employed for each variety of blood was previously determined by actual experiment. This strength is in each instance indicated in the protocols.

A glance at this table will show that, while the control tube in every instance showed no haemolysis, a marked and even complete laking took place in many of the venom tubes. It will also be seen that the action is somewhat variable, that is to say, the haemolysis obtained in a tube containing a weak solution of venom is sometimes more complete than in tubes containing stronger solutions. Thus, it has often been observed in one series of tubes that while the stronger solutions of venom have only a slight or no destructive action on the red cells, the result of the action of the weaker solutions is an absolutely complete haemolysis. Such a result is well shown in the series of experiments with the blood of a rabbit tabulated in table III of the protocols.

This variability in the haemolytic action of daboia venom 'in vitro' is in marked contrast to the results obtained with cobra venom. This, however, is a point to which I shall return in a moment.

When a weak solution (viz, 0.1%) of daboia venom is heated so as to coagulate the coagulable proteids, the haemolytic property, which the unheated venom has 'in vitro', is completely destroyed.

In table IV of the protocols two parellel series of experiments are detailed in order to demonstrate this fact.

In these observations a 0.1% solution of venom in 0.6% saline solution was divided into two portions. One portion was left unheated, while the other portion was heated in a water bath for half an hour at 75°C. The coagulated proteids were then filtered off. The haemolysing power of each portion, heated and unheated, was then determined in the manner I have already described, the blood of the same rabbit being used in each instance. It will be seen from this table that, while the unheated solution showed a well marked haemolytic action in many of the tubes, no laking was observed in any of the tubes containing the dilutions of the heated venom.

We may pause here for a moment to sift the data contained in the observations and experiments which I have put forward above, with the view of summarising the differences which exist between the haemolysing action of cobra venom and that of daboia venom.

Firstly, as regads the haemolysis which these venoms produce 'in vivo' it is important to note that cobra venom, however introduced into the body, exerts only a slight destructive action on the red cells, so that as a rule no symptoms result. Indeed the results of this particular action of the poison have generally escaped observation. On the other hand, the haemolytic action of daboia venom 'in vivo' is well marked and leads to a train of symptoms, which are very prominent to even a casual observer.

Secondly, when the haemolysing power of these two poisons is estimated side by side 'in vitro', using exactly the same technique in each case, we have seen that, while this action of cobra venom is well marked and regular, varying according to the strength of the venom solution used, no matter the species of animal from which the blood is taken, the destructive effect on the red cells of daboia venom under similar circumstances varies considerably and often, apparently, does not depend on the strength of the

poison solution with which the blood is brought in contact. This action of daboia venom 'in vitro' is, in fact, by no means so definite as the corresponding action of cobra venom.

Thirdly, we have seen that heating a 0.1% solution of cobra venom, while diminishing, does not completely destroy the haemolysing property of the poison, and that heating a solution of daboia venom of the same strength and in exactly the same manner has the effect of completely destroying the haemolytic power, which the unheated solution possessed.

It seems, therefore, justifiable to conclude from these three facts that, as far as the haemolysing substances of these venoms are concerned, we are dealing with substances different but resembling one another, and that in this respect, as in all other respects, cobra venom and daboia venom, although at first sight presenting a superficial resemblance, yet differ materially from one another.

The action of cobra and daboia venoms on the blood coagulability 'in vivo'.

It has been known for some time that many snake venoms have a well marked action on the coagulability of the blood plasma 'in vivo'. This fact was first drawn attention to by Fontana[19], who, more than a hundred years ago, noticed that the blood remained fluid in animals dead of viper-bite. Brainard[19] and Weir Mitchell[19] have also shown that rattle-snake venom has a marked action on the coagulability of the blood. The former observer noticed that in animals, which died immediately after a rattle-snake bite, the blood was clotted; while, if death was delayed for any length of time, the blood remained fluid in the vessels. Weir Mitchell, while confirming these observations, explained these differences by the hypothesis, that in cases of very rapid death the poison had not had time to affect the blood. In Australia, Halford[19] observed the same fluidity of the blood to follow the injection of the venom of some Australian snakes.

Further, the continued fluidity of the blood after death from the bite of the Indian viperine snakes has been frequently noted by numerous observers in this country.

The work, however,[20] of Martin of Melbourne with the venoms of the two Australian snakes (Hoplocephalus and Pseudechis) put this problem on a firmer basis. This careful observer has shown that the venoms of these two species of snakes produce, when injected intravenously in sufficient amount, such a marked increase in the blood coagulability as to lead to an extensive intravascular thrombosis, but that, if the quantity injected is not sufficient to cause death from this thrombosis, then a marked deficiency or negative phase of the blood coagulability follows a short lived phase of increased coagulability. Here, then, was the probable explanation of the results obtained by the American investigators with "crotalus"venom.

Martin, further, put forward the hypothesis, that all snake venoms probably contained, in greater or less quantity, the same proteid substance which brought about this most remarkable effect on the blood coagulability, one venom differing from another in the amount of this constituent. Along with Dr.Hanna[21] I was, however, able to show that this hypothesis was not a sufficient explanation as far as our Indian snakes were concerned. For we proved beyond a doubt that, while the effect of the action of daboia venom on the blood coagulability was apparently the same as Martin had described with regard to the venom of the Australian species, cobra venom did not, at any rate, even when injected intravenously in very large quantity, produce intravascular clotting.

Again, in a paper on "Some effects upon the blood produced by the injection of the venom of Pseudechis",[22] Martin critically sifts the data available in an endeavour to find an explanation of the significance of the phenomena which he described. He arrived at the conclusion that the great changes, which snake venoms bring about on the blood coagulability, are in all probability due to nucleo-proteids set

free from various cells, especially the red blood corpuscles, as a result of their destruction by the venom, and he compares the phenomena he observed with snake venom with the phenomena which Wooldridge described, as resulting from an injection of nucleo-proteids directly into the blood stream of an animal. It is unnecessary to follow this observer in the close reasoning which runs through this paper. It is, however, necessary to point out that at the close of the paper he sums up as follows:-

"Finally, I say that, although I believe that the very striking facts, which I have hitherto observed, do warrant the "hypothesis advanced in this paper, namely, that the phenomena of coagulation following the injection of snake venom "are identical as regards their immediate cause 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."

The observations, which I have now to bring forward, appear to me to reveal facts discordant with this theory, so discordant, indeed, that I am of opinion that Martin's hypothesis can no longer stand, and that the problem of the action of snake venoms on the coagulability of the blood is a much more complicated problem than this observer supposed, and will have to be reconsidered in the light of these new facts. It is also evident that no generalisation can be made, founded on observations with one variety of snake venom, but that every variety of snake poison will have to be considered separately. I propose, then, to take my considerations and observations in more or less chronological order; firstly, stating briefly the action of cobra and daboia venoms on the coagulation of the blood as observed 'in vivo'; and secondly, passing on to consider more in detail their effect on the coagulability of the whole blood and of the blood plasma 'in vitro'.

Wall[13] states in his work on Indian snake venoms, that the blood in the human subject at post-mortem examinations of cases of cobra venom intoxication is as a rule in a fluid condition and clots badly on being withdrawn from the vessels. In animal experiments, however, this observer found that the blood on post-mortem examination was always clotted solidly. On the other hand, Cunningham[14] has shown that, if ~~the~~ an animal received a large quantity of cobra venom and died very shortly after the injection, the blood, drawn just before or immediately after death, remained unclotted for a considerable length of time, and that the clot which did ultimately form was semi-fluid, loose and soft, and no serum separated out. Neither of these observers makes any record of finding <u>immediately</u> after death any intravascular thrombosis. This condition, however, they might easily have overlooked, in the same way as they both failed to observe the intravascular clotting which results from an intravenous injection of daboia venom.

In a recent publication along with Dr. Hanna[15] I was able to record definitely the fact, that cobra venom, even when injected in large quantity into a vein, does not produce any intravascular clotting. An amount of cobra poison, as great as 50 times the amount of daboia venom which can cause extensive intravascular thrombosis, was found to have no such effect. It is, therefore, certain that, in whatever other respects these two venoms may differ from another, one they do differ in this particular physiological action, namely, cobra venom does not produce intravascular clotting, while, as we shall see, daboia venom introduced intravenously even in minute doses does possess this remarkable property.

If a non-lethal dose of cobra venom is injected subcutaneously into an animal, one seldom sees any symptoms which might point, as is the case with daboia venom, to any diminution of the blood coagulability. I have, however

observed on several occasions during the process of immunisation of horses and donkeys, which received an injection of this venom over the shoulder, a large oedema of the parts dependent from the site of injection and reaching down the leg for some distance. I have never seen any wide-spread oedema. I have also been able to demonstrate in the case of rabbits, that a slight diminution of blood coagulability does take place as a result of the subcutaneous injection of non-lethal doses of cobra venom. Such a series of observations, made by means of Wright's coagulometer tubes, are detailed in table V of the protocols. A glance at this table will show that in every experiment a slight lengthening of the normal coagulation time of rabbits' blood followed the venom injection, and that this diminution in coagulability was more marked the nearer the quantity of venom injected approached to the minimum lethal dose. These experiments might also explain Wall's failure to observe any post-mortem fluidity of the blood in his animal experiments and Cunningham's success in this respect. For it is apparent that, if a dose of cobra venom which kills in from 6 to 12 hours be given, the deficiency in coagulability may not have had time to develope or may be so slight as to escape observation on post-mortem examination, while a larger quantity, such as Cunningham used, and which killed within a few minutes, may cause a marked deficiency in the blood coagulability of fairly rapid onset by reason of 'mass action'.

To sum up then, it is certain that cobra venom 'in vivo' does not produce any increase but rather a deficiency of the blood coagulability, the amount of this deficiency depending upon the quantity of venom injected, the mode of its injection and the time allowed for its action to take place.

Before passing on to the consideration of the action of cobra venom on the whole blood and on the blood plasma

'in vitro', it would be well, in order to secure a consecutive story, to briefly draw attention to some observations which I have, along with Dr.Hanna, already published[28] on the action of daboia venom on the blood coagulability 'in vivo'.

Both Wall[26] and Cunningham[27] attributed the rapid death, which follows the intravenous or subcutaneous injection of daboia venom, to a direct action of the poison on the central nervous system. They considered that daboia venom in all instances caused a fluidity of the blood, sometimes amounting to complete loss of coagulability. Hanna and I,[28] however, were able to show that this hypothesis was erroneous and that the rapid death which follows an injection of daboia venom is invariably caused by extensive intravascular thrombosis. Further, we showed that, if the amount of venom injected is not sufficient to cause this thrombosis, a negative phase of diminished coagulability results, sometimes amounting to complete inhibition of clotting, and that this diminished coagulability is probably an important factor in the production of some of the symptoms which are observed in these cases. We found that, while 0.1 milligramme of daboia venom per kilo of weight when injected intravenously into a rabbit causes extensive intravascular clotting, 0.4 milligramme has the same effect when injected subcutaneously into a pigeon. This thrombosis may be confined, if the quantity of venom injected has not been excessive, to the portal veins, the right heart and the pulmonary arteries: in these cases the blood collected from the other veins, and from the left heart especially, remains unclotted or clots only after a long interval of time, when the clot is very loose and gelatinous. We also showed that, when the negative phase of diminished coagulability has once set in, it was impossible to produce increased coagulability and thrombosis by the injection of any quantity of venom, which under normal circumstances injected intravenously would have been ample to bring about these phenomena. The action, then, of daboia venom on the blood

coagulability 'in vivo' is very definite and well marked, and corresponds exactly, as far as can be judged from the observation of the symptoms and the results of post-mortem examinations, to the action, as described by Martin, of the venoms of the two Australian colubrine snakes, namely, Hoplocephalus and Pseudechis. Until, however, researches have been made with the specific sera prepared with these poisons, it would be premature even to suggest that daboia venom was of the same nature as Hoplocephalus and Pseudechis venoms even as regards its blood poison.

The action of cobra and daboia venoms on the coagulability of the blood 'in vitro'.

We may now pass on to consider if snake venoms have any definite action on the coagulation of the blood when mixed with this 'in vitro'. In this connection I shall have to put on record some experiments which I have made with cobra and daboia venoms with the whole blood and with the blood plasma freed from red cells.

With the poison of Pseudechis Martin[29] found that, by running fresh blood into a solution (0.1%) of this venom, clotting was considerably delayed, and the clot which did form was soft and on shrinking failed to entangle corpuscles. Working with crotalus poison, Weir Mitchell and Reichert[30] were able to prevent coagulation of the blood altogether 'in vitro'. These observers drew the blood directly into a vessel containing a strong solution of venom surrounded by a freezing mixture. On allowing the temperature to rise gradually they found the blood remained permanently liquid.

Further, both Cunningham[31] and Kanthack[32] have shown that, when fresh blood was run into a strong solution of cobra venom, either it remained permanently unclotted or the clot, which formed after a considerable delay, was soft and gelatinous, and no serum exuded. The former of these observers also states that in three experiments, in which blood was run into solutions of daboia venom, although clotting

took place with normal rapidity, the clots which formed were loose, soft and tremulous.

These observations, therefore, would tend to show that 'in vitro' all snake venoms had a similar action on the blood coagulation, namely, an inhibitory action. In view of the facts recorded above as regards the action of cobra and daboia venoms on the blood coagulability 'in vivo', it appeared to me necessary to further investigate this action 'in vitro' of these two venoms, the only two which were available to me at the time.

After many failures to get any definite results by the method followed by the older investigators, namely, the method of allowing fresh blood to flow directly into a solution of venom, I was led to use blood which was kept liquid by the addition of citrate of soda. At a later date I found that similar results were obtained with citrate and oxalate plasma, which had been completely freed from the red corpuscles by allowing these later to sediment, and then syphoning off the clear supernatant fluid.

The observations, therefore, which I have now to record, naturally fall into two groups, as follows:-

1) Observations on the action of cobra and daboia venoms on the coagulability of citrate whole blood.
2) Observations on the action of cobra and daboia venoms on the coagulability of oxalate and citrate plasma freed from red cells.

<u>The action of cobra and daboia venoms on the coagulability of citrate whole blood.</u>

The blood which was used in these experiments was got from rabbits in the following manner. By means of a canula in the femoral artery a measured quantity of blood was run into a measured amount of a 20% solution of sodium citrate. These quantities were so arranged that the citrate of soda was in the proportion to the blood of 1 to 100 or 1 to 50. In the protocols of the experiments it is invariably recorded in which of these proportions the citrate was present.

The citrate blood was then divided up into a series of small test tubes, 1 c.c. being accurately measured into each test tube. It was then ascertained by direct experiment the minimum amount of a solution of calcium chloride of known strength, which it was necessary to add to each cubic centimetre of citrate blood so as to cause solid clotting in from 1 to 3 minutes, and likewise the maximum amount of calcium chloride solution, which could be added without causing any clot in from 4 to 6 hours or longer.

It was found that these quantities of lime solution varied slightly with each sample of citrate blood used, no matter however carefully the solutions of citrate and of calcium chloride were prepared, and however accurately the blood was measured and divided up. It was, therefore, necessary to make careful control experiments, such as I have indicated, for each series of observations, that is to say, for every different sample of rabbit's blood which was used.

Let us first consider then the action of cobra venom on plasma, as shown by its effect on the coagulability of citrate blood. In view of the fact that cobra venom does not, even when introduced in large quantity directly into the blood stream, produce intravascular clotting, it was not to be expected that this poison would have the property of clotting citrate blood 'in vitro'. Such a surmise was found to be correct. For it will be seen on reference to table VI of the protocols that, when 20, or even 30, milligrammes of cobra venom were added to 1 c.c. of citrate blood, no clot formed after 2 hours, and, further, that on the addition, after this interval of time, of such a $\frac{1}{n}$ amount of soluble lime salt as clotted the control in 3 minutes, the blood remained permanently liquid. As we shall see later, this is a very different result to that got in similar experiments with daboia venom. We can say, then,

that a large quantity of cobra venom does not increase blood coagulability 'in vitro' but completely prevents clotting taking place.

We have also seen above that cobra venom, injected subcutaneously or intravenously even in small sub-lethal doses, has the effect of diminishing the coagulability of the blood. By means of a technique such as I have described above, the optimum quantity of lime solution necessary for rapid clotting of the control being added to each tube two hours after the addition of the cobra venom, a result, comparable to what obtains 'in vivo', can be got 'in vitro' with citrate whole blood.

Thus, I was able to estimate fairly accurately, as judged from a number of experiments, the minimum quantity of cobra venom which can keep 1 c.c. of citrate rabbit's blood permanently liquid 'in vitro', and the minimum quantity which distinctly inhibits, but does not altogether prevent, coagulation.

The result of such an estimation is detailed in table VII of the protocols. From this table it will be seen, that 0.4 milligramme of cobra venom kept 1 c.c. of blood liquid for atleast 20 hours, and that 0.01 milligramme had a distinct inhibitory action on the coagulability of the same amount of blood, lengthening the coagulation time, as compared with the control, by about half an hour. It may be mentioned in passing that the interval of time allowed to elapse between the addition of the venom and the soluble lime salt in some cases affects the results of such experiments, as I have collated above. Especially is this the case when the quantity of poison used is not greatly in excess of the minimum amount requisite to keep the blood permanetly liquid. A reference to table VIII will make the meaning of this statement clear. It requires no further explanation except to state that it was, however, found in many experiments that, even when the cobra venom and the

lime solution were added together, the same inhibition of coagulation resulted, as when a certain interval of time was allowed to elapse. Such a result as I have tabulated in table VIII is exceptional and cannot be depended on.

In view of Martin's hypothesis, that the proteids coagulable by heat are the constituents of snake venoms which affect the blood, it is important to be able to record an observation bearing on the effect which heating a solution of cobra venom, so as to coagulate the coagulable proteids, has on this anti-clotting power of the poison. Such experiments are detailed in table IX of the protocols. Inasmuch as the same technique was used in these experiments, as was employed in the similar series of experiments with unheated venom detailed in table VII, one is able to compare the results recorded in these two tables. From such a comparison it is evident that, while heating a solution of cobra venom at 75°C for half an hour, that is to say, so as to coagulate all proteids coagulable by heat, does not deprive the venom of its anti-clotting action on the blood, such heating, nevertheless, diminishes this particular physiological action of the poison to a considerable extent. For a reference to these two tables will show that, while 0.4 milligramme of the unheated venom suffices to keep 1 c.c. of citrate blood permanently liquid after the addition of the lime solution, 20·4 milligrammes of the heated venom, from which the coagulable proteids had been removed, delayed clotting for a considerable interval of time, but did not prevent it from ultimately taking place. Larger quantities of the heated venom had a still greater inhibitory action on the coagulation of this blood but even two milligrammes did not completely prevent clotting. Such a result would appear to point to the conclusion, that it not the coagulable proteids contained in cobra venom which affect the blood coagulability, but rather some non-coagulable proteid which,

however, is considerably diminished in activity by heating. This result, therefore, lends no support to Martin's theory, a conclusion the same as was arrived at with respect to the haemolysing constituent of cobra venom. These two similar results, in fact, are strong proof against the correctness of this hypothesis. Such, then, is the effect of the action of cobra venom on the coagulability of citrate whole blood. We may now pass on to the considerations of some similar experiments which were made with daboia venom.

We have seen above that daboia venom, injected into animals in sufficient quantity, so increases the blood coagulability as to lead to extensive and rapid intravascular clotting, and further that, when the quantity injected is not sufficient to cause thrombosis, a negative phase of marked diminution in the blood coagulability sets in. As regards the 'in vitro' experiments with citrate rabbit's blood, now to be put on record, it is unnecessary to again detail the technique used, which was similar to that employed in the experiments with cobra venom. But in as much as the first series of experiments was made with the object of ascertaining if daboia venom had any effect on increasing the coagulability of citrate blood, it is apparent that, in order to demonstrate such an action to the best advantage, it was not feasible to add the lime solution to each tube in such amount as would clot the control in from 2 to 3 minutes. It was found that a good result was obtained by the addition of a very small amount of calcium chloride, a quantity, in fact, which did not clot the control even after six hours.

A reference to table X will show the results which were obtained by working in this way. The control tube with a small quantity of lime added remained unclotted after six hours: the tubes to which from 1 to 0.0078 milligrammes of daboia venom were added clotted solidly in from 5 to 10 minutes, and that to which 0.0002 milligramme was added clotted solidly after one hour. We have here, then, a

perfect demonstration 'in vitro' of the positive phase of increased coagulability caused by daboia venom. A further series of experiments was then made with the object of ascertaining whether or not the negative phase of diminished coagulability could be demonstrated with citrate blood 'in vitro'. A technique similar to that used in the series of experiments noted above was employed. But much smaller quantities of venom were used, and the poison was added to the blood one hour before the addition of the lime solution. Further, the quantity of lime solution added to each tube was slightly increased, so that a clot formed in the control tube in about four hours. It is evident, then, that these conditions of experimentation were in every way favorable to show any diminution in the coagulability of the blood which the venom might cause. No such diminution, however, took place. In fact, quite the opposite result was obtained. For a glance at table XI of the protocols will show that very minute quantities of daboia venom had a distinct effect in increasing the coagulability of citrate blood, even 0.0000009 milligramme added to 1 c.c. of citrate blood causing a slight shortening of the coagulation time as compared with the control. A similar series of experiments to that tabulated in table XI was then made, the only difference being, that 0.3 c.c. of lime solution (0.5%) was added to each tube instead of 0.1 c.c. This amount of lime clotted the control tube in about two minutes. As it was found that the contents of all the tubes, to which the venom had been added, clotted in about the same time as the control, some of them, in fact, even more quickly, this series of experiments need not be further considered. It confirms the results already plainly demonstrated in table XI, namely, that daboia venom, even in minute quantities, does not cause any diminution in the coagulability of citrate blood 'in vitro', similar to the effect which is produced by cobra venom. This result, therefore, throws no light on how

the negative phase of coagulability caused by daboia venom 'in vivo' is brought about. It does show, however, that this action of daboia venom is something quite different from the action of cobra venom in this respect, and that, in all probability, it is not due to ~~the~~ a direct action of the poison on the blood cells or on the plasma.

In the above experiments with daboia venom a small quantity of soluble lime salt was added to the citrate blood. I have now to put forward the results of some experiments in which no lime salt was added, daboia venom alone being run into each tube of citrate blood. Two series of experiments of this description are tabulated in the protocols (tables XII & XIII).

Firstly, in the series of observations detailed in table XII the citrate was present in the proportion of 1 to 100 to the blood, that is to say, the minimum amount which can be depended on to keep rabbit's blood permanently liquid.[33] A reference to this table will show that 0.5 milligramme of venom clotted 1 c.c. of the citrate blood in a little over half an hour, and 0.0312 milligramme in about two hours. Smaller quantities than this caused no clot in two hours.

Secondly, in the series of experiments detailed in table XIII of the protocols the citrate was present in the proportion of 1 to 50 to the blood, namely, a quantity double the amount which is necessary to keep rabbit's blood unclotted 'in vitro'. Under this condition it was found that 2 milligrammes of venom were required to cause clotting of 1 c.c. of blood in half an hour, that 0.6 milligramme caused clotting only after $1\frac{3}{4}$ hours and that 0.2 milligramme caused only a slight clot after two hours. Thus it is seen that the presence of an excess of citrate, while not preventing the clotting action of the venom, certainly lessens this power to some extent. The results, then, of these two series of experiments, taken with the results of the two series detailed in tables X & XI, show that daboia

venom can of itself cause clotting of well citrated blood 'in vitro', but that the clotting is more rapid and the clot is firmer, the nearer the quantity of lime salts in solution in the blood approaches to that contained in normal blood. I do not for a moment urge that daboia venom of itself is able to cause clotting of blood, which contains no lime salt in solution, as the citrate of lime is not an absolutely insoluble salt. However, the fact, that daboia venom does of itself clot citrate blood 'in vitro', is important and interesting, as I know of no other substance which has this action without the addition of a small quantity of soluble lime salt.

In a recent paper by Dr.Hanna and myself[34] we pointed out the fact that heating a weak solution of daboia venom completely destroys the property which this poison has of causing any increase of the blood coagulability 'in vivo'. We showed that after heating a 0.1% solution for half an hour at 75°C a large quantity of the filtrate can be introduced into the blood stream of animals without causing any symptoms.

I have found that a comparable result is obtained with citrate blood 'in vitro'; that is to say, heating a weak solution of daboia venom at 75°C for half an hour completely destroys the clotting action the unheated venom has on citrate blood. Such an experiment is detailed in table XIV of the protocols. From this table it will be seen that even 2 milligrammes of venom (0.2% solution), heated for half an hour at 75°C, does not clot 1 c.c. of citrate blood after three hours. When we consider that 0.03 milligrammes of the unheated venom clots this amount of blood, citrated to the same extent in 2 hours, we must conclude that heating in this way completely destroys this constituent of the poison.

I have shown above that it is not possible, even with minute quantities of venom, to demonstrate 'in vitro' the

negative phase of diminution of blood coagulability, which negative phase is such a marked phenomenon in chronic cases of daboia intoxication. I have also shown in the case of cobra venom that a marked diminution in the blood coagulability produced by this poison is capable of demonstration both 'in vivo' and 'in vitro'. It was thought that the failure of daboia venom to cause diminished coagulability of the blood 'in vitro' might possibly be due to the fact, that the constituent of this poison which causes clotting is a different substance to that which causes the diminution of coagulability 'in vivo', and that this latter is not able to exert its specific action 'in vitro' in the presence of the former. Now we have seen that heating a weak solution of cobra venom diminishes, but does not destroy, the power which this poison has to prevent coagulation of citrate blood 'in vitro', while heating a solution of daboia venom completely destroys its power to cause an increase of coagulability. If the above hypothesis ~~was~~ correct, then, it would be found, that by heating a weak solution of daboia venom, thereby completely destroying the constituent which causes coagulation of citrate blood, the heated poison would now have the effect of completely inhibiting or diminishing the coagulability of citrate blood. This theory, however, was not supported by experimental data. For a reference to table XV of the protocols will show that 2 milligrammes of daboia venom, heated in weak solution, after being in contact with 1 c.c. of citrate blood for 3 hours had no effect in lengthening the coagulation time of the blood, when a quantity of lime solution sufficient to clot the control in about two minutes was added. This result, then, strongly supports the conclusion I have already put forward on page 26, namely, that the substance in daboia venom, which causes the diminution of coagulability 'in vivo', differs, both in nature and in method of action, from the substance in cobra venom, which has such a marked inhibitory effect on the blood coagulability both 'in vivo' and 'in vitro'.

Further, it is evident that the observations on the

action of daboia venom on the coagulability of the blood 'in vitro' neither support nor disprove Martin's hypothesis, namely, that the constituent of all snake venoms which acts as a blood poison is a proteid coagulable by heat. Finally, when we consider the results of all the observations with cobra and daboia venom detailed above, there can be no possible doubt but that in their action on the ~~same~~ cell constituents of the blood and on the blood plasma the differences between these two venoms are fundamental differences, and not only differences in degree of toxicity. These observations, in short, support my contention, that Calmette's statement, that all snake venoms are alike in their physiological action, is quite untrue and not evidently founded on experimental data carefully worked out.

The action of cobra and daboia venoms on the coagulability of citrate and oxalate plasma.

We may now pass on to consider whether or not results, similar to those, which have been collated above in the case of citrate whole blood, can also be obtained with citrate or oxalate plasma, which has been freed from red cells by a process of sedimentation. The importance of such an investigation is evident in view of the theory put forward by Martin (page 15), namely, that the action of snake venoms on the blood coagulability 'in vivo' probably depends on the fact that, as a result of a great destruction of cells, especially of the red cells of the blood, by the poison, a large quantity of nucleo-proteids is set free, and that it is these nucleo-proteids which affect the blood coagulability.

Some of the experiments detailed below were made along with Professor Martin, to whom I am indebted for much valuable suggestion and encouragement.

In these experiments the same samples of citrate and oxalate plasma were used throughout, so that it will suffice, once for all, to give a short description of the manner in which these were obtained and kept. They were both got

from the horse. In the case of the citrate plasma one litre of blood was drawn into 50 c.c. of a 20% solution of citrate of soda (0.75% saline solution), that is to say, there was 1% citrate in the whole blood. This was kept in an ice chest (temperature about 14°C.) for 48 hours. Then the supernatant plasma was syphoned off into small bottles, which were stored in an ice chest till required.

In the case of the oxalate plasma one litre of blood was drawn into 40 c.c. of a 5% solution of oxalate of potash (0.75% saline solution), that is to say, there was 0.2% oxalate in the whole blood. This was then treated in exactly the same way as the citrate blood. The plasma was likewise stored in the ice chest till required. Strict aseptic precautions were observed in the collection and storing of these samples of plasma.

Just before use in both cases the bottles were well shaken up and heated in an oven at 37°C. for a short time.

The next step was to determine the amount of soluble lime salt which it was necessary to add to a given quantity of each plasma to procure rapid and firm clotting. To save repetition it may be stated here that in all these experiments 2 c.c. of plasma was the amount run into each tube, and that a 1% solution of calcium chloride was employed as the lime solution.

The results of this estimation are detailed in table XVI of the protocols. From this table it will be seen that 0.5 c.c. of the lime solution clotted 2 c.c. of citrate plasma in less than 8 minutes, and that 0.4 c.c. of lime solution clotted 2 c.c. of oxalate plasma in 10 minutes. These, then, were the quantities of lime solution used in the experiments with cobra venom.

Having, by preliminary observation, ascertained that cobra venom did prevent clotting of both citrate and oxalate plasma, I next proceeded to estimate the amount of venom which was able to effect this, when added to the plasma two

hours before the addition of the quantity of lime solution sufficient to cause clotting of the control in a few minutes.

The results of these estimations are detailed in tables XVII and XVIII of the protocols. In the case of citrate plasma (table XVII) it was found that 1 milligramme of cobra venom was sufficient to keep 2 c.c. of plasma permanently liquid, and that amounts less than this, down to 0.2 milligramme, had a very marked inhibitory action on coagulation, the clot which had formed after 20 hours being always soft and loose. The control, as with the same amount of lime as the venom tubes, clotted solidly in a few minutes. The similarity between this result and that detailed in table XII, the series of experiments in which citrate whole blood was used, allowance being made for the plasma being double the amount of the whole blood, is striking and worthy of note. In the case of oxalate plasma (table XVIII) it was found that even 0.2 milligramme of cobra venom was able to keep 2 c.c. of plasma unclotted for a period of at least 20 hours. The control with the same quantity of lime added clotted solidly in ten minutes. These results, then, confirm and further extend the observations with citrate whole blood, which I have already recorded. They show that the haemolytic action of cobra venom is quite independent of its action in inhibiting coagulation, that, in fact, the action which this poison has on the blood coagulability does not depend on the destructive effect which it has on the red blood corpuscles and other cells. It would, therefore, appear certain that the action of one venom at any rate, namely, cobra venom, on the blood coagulation 'in vivo' cannot be explained by Martin's hypothesis, that is to say, the setting free of nucleo-proteids as a result of the great destruction of the cells of the body in general, and of the red cells in particular, which is caused by this poison. It cannot, however, be said from these experiments that nucleo-proteids play no part in this phenomenon, as

plasma prepared in the manner I have described is rich in these substances, derived, as they are, from disintegration of the white cells of the blood after its withdrawal from the vessels.

With the view of further investigating this action of cobra venom two series of experiments were made with a sample of hydrocele fluid. This sample did not clot on the addition of a soluble lime salt, but was clotted solidly by the addition, either with or without a small amount of lime, of a small quanity of citrate donkey plasma (1%). It was found that 0.1 c.c. of this plasma clotted 2 c.c. of hydrocele fluid in about 20 minutes. A reference to tables XIX and XX of the protocols will show that even 0.1 milligramme of cobra venom was sufficient to completely inhibit the clotting of this sample of hydrocele fluid, as brought about by the addition of a small amount of citrate plasma, when the venom was allowed to act on the plasma for ten minutes before the addition of the hydrocele fluid. The addition of a small quantity of soluble lime salt did not affect this result in any way. Taking these observations along with the others already recorded into consideration, I can only conclude that cobra venom owes its anti-clotting power either to some obscure chemical combination which it effects with the nucleo-proteids, rendering these substances inert as far as coagulation is concerned, or to an action of a destructive nature on the hypothetical fibrin ferment contained in plasma. I have been unable, so far, to procure nucleo-proteids, except as contained in plasma, which were able to cause clotting of any sample of hydrocele fluid which I could procure. It is impossible, therefore, to exclude the action which the venom may have on any ferment which may be contained in plasma. Further experiments are, however, in progress and will form the subject of another communication.

I may now pass on to put on records some experiments, which bear on the action which daboia venom has on the

coagulability of citrate and oxalate plasma 'in vitro'. As the same samples of plasma, which were employed for the experiments with cobra venom, were used for these experiments, it is unnecessary to again detail the method of their preparation.

We have already seen (tables X to XV) that daboia venom has a marked action on the coagulability of citrate whole blood. It has been demonstrated that it can, even without the addition of any soluble lime salt, produce solid clotting of citrate whole blood and, further, that it contains no substance strictly analogous to the substance contained in cobra venom, a substance which has such a remarkable power of completely inhibiting the coagulation of citrate blood as brought about by the addition of a soluble lime salt.

Three series of experiments were made with daboia venom and citrate and oxalate plasma.

In the first series varying quantities of the venom alone were added to a series of tubes each containing 2 c.c. of citrate plasma. A reference to the protocols (table XXI) will show that, as in the case of citrate whole blood, daboia venom causes clotting of citrate plasma without the addition of any lime. Thus any quantity of venom from 3 to 5 milligrammes clotted 2 c.c. of plasma in less than 3 hours: with an amount between 0.4 and 1 milligramme clotting occurred, but was delayed for about 20 hours: while 0.2 milligramme caused only a slight clot after 20 hours.

The second series of experiments (table XXII) was similar to the above series, only oxalate plasma was substituted for citrate plasma. The results obtained show that daboia venom causes very slight clotting of plasma of this nature, for even 5 milligrammes of venom produce only a feeble clot in 2 c.c. of plasma after 20 hours; smaller amounts down to 0.6 milligramme had the same effect, while 0.4 milligramme produced no clotting at all.

The third series of experiments was made with the object of ascertaining the reason of this difference between

citrate and oxalate plasma. In this series (table XXIII) to each tube of oxalate plasma, at the same time as the daboia venom was added, there was run in a small amount of soluble lime salt, an amount, however, not sufficient to clot the control in 20 hours. As a result of this addition of lime the contents of each tube clotted solidly in about 10 minutes. It is, therefore, apparent that the failure of daboia venom to produce solid clotting of oxalate plasma, while it does clot citrate plasma, is due to the fact that the oxalate of lime is a more insoluble salt than the citrate. The coagulation of plasma by daboia venom does not take place independent of a certain amount of lime salt in solution. The venom acts, evidently, by increasing the coagulability of the plasma, but solid formation of clot cannot take place unless a small quantity of lime in solution, in some form or other, is present as well.

With a view of further searching for a working hypothesis to explain this action of daboia venom on blood plasma, experiments were made to ascertain whether or not this poison caused clotting of hydrocele fluid, either with or without the addition of a soluble salt of lime. The sample of hydrocele fluid used was clotted solidly on the addition of a small amount of oxalate plasma; it was, in fact, the same sample which was employed in the experiments with cobra venom detailed in tables XIX and XX of the protocols.

The results of both these series of experiments were, however, entirely negative (table XXIV of protocols). Even 5 milligrammes of venom produced no clotting in 2 c.c. of hydrocele fluid either alone or when lime was added. It is evident, then, that in whatever manner daboia venom does act in increasing coagulability, it cannot replace the substances in plasma, be they nucleo-proteid, ferment or both, which are so intimately connected with the phenomenon of coagulation.

Further, Martin's hypothesis, namely, that the

remarkable intravascular clotting, which some snake venoms produce 'in vivo', is due to the setting free of a large quantity of nucleo-proteids, the result of the great destruction by the venom of cells, and of red blood corpuscles in particular, receives no support from the observations with daboia poison which I have collated above. When we consider that this venom is able to markedly increase the coagulability of citrate or oxalate plasma, which no doubt contains nucleo-proteids but is free from cells, it is at once apparent that this 'destruction' theory of Martin does not furnish a sufficiently definite mental picture of the phenomena, when looked at in the light of these new facts which I have put forward above. It would appear, rather, that the ultimate cause of this increase of coagulability produced by daboia venom is some obscure interaction between the poison and the nucleo-proteids, or between the poison and the clotting ferment. It is certainly not the result of the setting free of normal nucleo-proteids through the destruction of the cells by the poison.

We have seen that cobra venom can prevent the coagulation of citrate plasma, which takes place on the addition of a soluble salt of lime, and that, on the other hand, daboia venom has a marked action in increasing the coagulability of this plasma. It is interesting, now, to be able to record a series of experiments, which were made with the object of determining, what would be the result of the addition of cobra and daboia venoms together to citrate plasma. In this series of experiments 5 milligrammes of cobra venom were added to each tube of a series containing 2 c.c. of plasma. This, as we have seen, is a quantity of venom five times greater than the amount, which can completely inhibit the coagulation of citrate plasma that takes place on the addition of a soluble salt of lime. Half an hour afterwards a different amount of daboia venom was run into each tube. The amount varied from 5 to 0.2 milligrammes. When we compare the results obtained in this

series of experiments (table XXV) with the results obtained in the series of experiments already detailed (table XXI), in which daboia venom alone was added to the plasma, it will be seen that the previous addition of this large quantity of cobra venom had no effect in even delaying the time in which the various amounts of venom brought about coagulation of the plasma. In other words, the presence of a large quantity of cobra venom does not inhibit in the slightest degree the clotting action of daboia venom.

Although it is hardly justifiable to make deductions, from the results of experiments 'in vitro' as to what obtains 'in vivo', it appears to me interesting and important to draw attention to the following considerations.

I have mentioned in an early part of this communication that, when a quantity of daboia venom not sufficient to cause intravascular clotting is introduced directly into the blood stream of an animal, the blood coagulability becomes markedly diminished. Further, along with Dr.Hanna, I have shown that, when this negative phase of blood coagulability has once developed, the injection of even large quantities of daboia venom does not increase the blood coagulability, and that in consequence it is now impossible to cause death from intravascular thrombosis. Again, I have shown in this paper, that this negative phase of blood coagulability is not obtained in experiments with daboia venom and citrate blood 'in vitro'. When we consider these facts in the light of the experiments both 'in vivo' and 'in vitro' with cobra venom, the results of which have been collated above, namely, that this poison has both 'in vivo' and 'in vitro' a marked power of preventing coagulation or diminishing the coagulability of blood, and in the light of the experiments 'in vitro' with cobra and daboia venoms together, great support is given to the conclusion, which I have already stated, namely, that the substances contained in cobra venom which affect blood coagulability are quite different, both in nature and in action, from the

substances contained in daboia venom which likewise affect the coagulability of the blood. While, therefore, superficial and cursory observations might lead one to conclude that the action of cobra and daboia venoms on blood plasma only differed in degree and not in nature, I hold that these observations are sufficient to show that this is not the case, and that in this, as in other respects, these two snake venoms differ widely from one another.

Finally, I may now sum up the conclusions, which the observations detailed in this paper, would appear to justify :

1) Cobra venom and daboia venom have a marked haemolytic action both 'in vivo' and 'in vitro'. While the haemolytic action of cobra venom is better observed 'in vitro' than 'in vivo', the reverse holds good in the case of daboia venom. The haemolysing constituent of cobra venom is not a proteid coagulable by heat.

2) Cobra venom never produces intravascular clotting. Injected either intravenously or subcutaneously it brings about a certain, but not well marked, diminution in the blood coagulability. No symptoms as a rule result from this diminution of blood coagulability.

On the other hand the result of the injection of daboia venom in sufficient quantity is rapid death, due to an extensive intravascular clotting. Should, however, the quantity of poison injected not be sufficient to cause this clotting, a negative phase of marked diminution of blood coagulability supervenes. Symptoms of an urgent nature may result from this diminution of blood coagulability.

3) 'In vitro' cobra venom completely prevents the clotting of citrate whole blood, of citrate plasma and of oxalate plasma, which results normally from the addition of a small quantity of a soluble salt of lime.

'In vitro' daboia venom has a marked action in increasing the coagulability of citrate whole blood, citrate

plasma and oxalate plasma. Even in small amounts it does not produce 'in vitro' any negative phase of blood coagulability which is such a prominent action 'in vivo'.

The constituent of cobra venom which affects the blood coagulability is not a proteid coagulable by heat.

4) Cobra venom and daboia venom differ widely as regards the nature of their action on the red blood cells and on the coagulability of the blood plasma. Calmette's statement, therefore, that all snake venoms are alike in their physiological action is, by this fact alone, shown to be untrue and must be modified.

5) Martin's hypothesis, namely, that all snake venoms contain at least two toxic proteids, A & B, A, being a neuro-tropic poison incoagulable by heat; B, a haemo-tropic poison coagulable by heat, and that the venom of one variety of snakes differs from that of another variety only in the proportionate amounts of the constituents A & B contained therein, is shown to be a theory which does not fit in with the facts now available. This conclusion was foreshadowed in ~~the~~ a paper on daboia venom which I published along with Dr. Hanna.[35]

6) Martin's other hypothesis, namely, that the action of snake venoms on the blood coagulability 'in vivo' is due to the setting free of a large quantity of nucleo-proteids, the result of the destruction of cells, and especially of red blood cells, by the venom, is not a theory which forms a complete mental picture of the phenomena in the light of the new facts I have put forward. It is evident that it will have to be considerably modified to suit these facts.

Table I

Experiments to show the effect of heating solutions of cobra venom of different strengths on the haemolysing power of this poison.

A sample of cobra venom was dissolved in 0.6% salt solution. The strength of this solution was 1%. From this original solution a tenfold dilution was prepared. Portions of these two solutions were heated for half an hour at 75° C and then filtered. An estimation of the haemolytic power of the original unheated venom and of these two heated samples was made. Each tube in the series contained 0.5 c.c. of venom solution, which was half the strength of the solution in the tube next above it in the series. The dilutions were made with 0.6% saline solution. Fresh blood of the same animal, namely, a rabbit, was used. The amount of blood added to each tube was 0.005 c.c. The observations were recorded 20 hours after the preparations were made.

Strength of venom solutions	Unheated	Heated 1% solution	Heated 0.1% solution.
0.5 c.c.=0.5 mgr	C.H.	C.H.	C.H.
0.5 c.c.=0.25 mgr	C.H.	C.H.	C.H.
0.5 c.c.=0.125 mgr	C.H.	C.H.	Trace H.
0.5 c.c.=0.0625 mgr	C.H.	Nearly C.H.	Nil
0.5 c.c.=0.0312 mgr	C.H.	Nearly C.H.	Nil
0.5 c.c.=0.0156 mgr	C.H.	Trace H.	Nil
0.5 c.c.=0.0078 mgr	Trace H.	Nil	Nil
0.5 c.c.=0.0039 mgr	Nil	Nil	Nil
0.5 c.c.=6% Salt Solution (control)	Nil	Nil	Nil

P.T.O.

Note. In this and the following tables the following degrees of haemolysis were recorded.-

Complete Haemolysis (C.H.)

Haemolysis (H)

Slight Haemolysis (Slight H)

Trace Haemolysis. Trace H.

No Haemolysis. Nil.

Table II.

Experiments to demonstrate the result of the haemolytic action of daboia venom 'in vitro' on the blood of man, monkey, rabbit, guinea-pig and pigeon.

The salt solution used to dissolve the venom in each series was of such a strength as did not produce of itself any laking. It was about 0.1% stronger than the solution which did produce slight laking. The dilutions were made with the corresponding salt solution in the same way as in the previous series. The amount of fresh blood added to each tube was 0.005 c.c. The observations were recorded 20 hours after the preparations were made.

Strength of venom solutions	Human Blood (.6% saline)	Monkey's Blood (.6% saline)	Rabbit's Blood (.6% saline)	Guinea-pig's Blood (.7% saline)	Pigeon's Blood (.7% saline)
0.5 c.c. = 2 mgr.	—	—	—	—	H.
" = 1 mgr.	Nearly C. H.	Slight H.	—	C. H.	C. H.
" = 0.5 mgr.	Slight H.	C. H.	Nearly C.H.	C. H.	C. H.
" = 0.25 Mgr.	C.H.	C. H.	C. H.	C. H.	Trace H.
" = 0.125 mgr.	Trace H.	C. H.	C. H.	C. H.	Trace H.
" = 0.0625 mgr.	Trace H.	H.	C. H.	C. H.	Trace H.
" = 0.03125 mgr.	Trace H.	Trace H.	H.	C. H.	H.
" = 0.0156 mgr.	Nil	Trace H.	Slight H.	Slight H.	Nil
" = 0.0078 mgr.	Nil	Nearly C.H.	Nil	Trace H.	Trace H.
" = 0.0039 mgr.	Nil	Nil	Nil	Nil	Nil
" Salt solution (control)	Nil	Nil	Nil	Nil	Nil

Table III.

Experiments to demonstrate the variability ~~of~~ in the haemolytic action of daboia venom 'in vitro'.

The same technique as the previous series of experiments was used : 0.005 c.c. of fresh rabbit's blood was added to each tube. Observations were recorded 20 hours after the preparations were put up.

Strength of venom solution	Result.
0.5 c.c. = 1 mgr.	Trace H.
" = 0.5 mgr.	Nil
" = 0.25 mgr.	Trace H.
" = 0.125 mgr.	C. H.
" = 0.0625 mgr.	C. H.
" = 0.0312 mgr.	C. H.
" = 0.0156 mgr.	C. H.
" = 0.0078 mgr.	Trace H.
" = .6 % saline (control).	Nil

Table IV.

Experiments to show the effect of heating a weak solution of daboia venom on the haemolysing power of this poison.

A 0.1 % solution of daboia venom in 0.6 % saline solution was divided into two portions. One portion remained unheated and the other portion was heated for half an hour at 75° C. and then filtered. The dilutions were made in the same way as the series of experiments detailed in table I. To each tube was added 0.005 c.c. of the same rabbit's blood. The observations were recorded 20 hours after the preparations had been made up.

Strength of venom solutions	Unheated solution	Heated solution.
0.5 c.c. = 0.5 mgr	Slight H.	Nil
" = 0.25 mgr	H.	Nil
" = 0.125 mgr	H.	Nil
" = 0.0625 Mgr	C. H.	Nil
" = 0.0312 mgr	H.	Nil
" = 0.0156 mgr	Nil	Nil
" = 0.0078 mgr	Nil	Nil
" = 0.0039 mgr	Nil	Nil
(Control) 0.5 c.c. = 0.6 % saline	Nil	Nil

Table V.

Experiments to demonstrate the result of the action of non-lethal doses of cobra venom injected subcutaneously on the blood coagulability of rabbits, as estimated by Wright's coagulometer tubes.

A 0.1 % solution of cobra venom was used. The observations were made at intervals of 24 hours. The lethal dose of cobra venom for a rabbit varies with the sample of poison from 0.3 to 0.5 milligrammes per kilo of body weight. The normal coagulation time of rabbit's blood, as estimated by Wright's tubes, is from 2 to 3 minutes.

Animal	Weight in grammes	Amount of venom per kilo in milligrammes	Coagulation time in minutes			Remarks.
			24 hours after injection	48 hours after injection	72 hours after injection	
Rabbit 1	1640	0.35	8	10½	4½	Was very ill and lost weight
Rabbit 2	1680	0.3	5¾	6½	4½	Was ill and lost weight
Rabbit 3	1370	0.3	6	5	—	Was very ill and lost much weight
Rabbit 4	1820	0.25	6	4	4½	Was ill and lost weight
Rabbit 5	1620	0.25	4½	5¼	4½	Lost slightly in weight
Rabbit 6	1620	0.2	4	—	—	No symptoms

Table VI.

Experiments to demonstrate the result of the action of a large quantity of citrate blood 'in vitro' *cobra venom on the coagulability of*.

Citrate rabbit's blood (1-50) was used: each tube contained 1 c.c. of this blood. The strength of the cobra venom solution was 1 c.c.=25 milligrammes. The soluble lime salt, namely, a 2% solution of calcium chloride, was added two hours after the venom.

Citrate blood (1 in 50)	Cobra venom (2.5 % solution) in milligrammes.	Calcium chloride solution (2 %)	Result.
1 c.c.	30	0. 3 c.c.	Quite liquid
1 c.c.	20	0. 3 c.c.	24 hours afterwards
1 c.c.(control)	Nil	0. 3 c.c.	Clotted solid in 3 minutes.

Table VII.

Estimation of the amount of cobra venom which can inhibit the coagulation of citrate blood 'in vitro'.

The solutions of cobra venom used were of three strengths viz., 0.2 %, 0.1 %, and 0.01 % respectively. These were prepared with saline solution (0.6 %). A quantity of a solution of calcium chloride, sufficient to clot the control in 3 minutes, was added to each tube of 1 c.c. of blood 2 hours after the cobra venom has been run in. This quantity was 0.3 c.c. of a 1 % solution. Observations were made at periodical intervals.

Citrate blood(1-50)	Cobra venom in milligrammes	Calcium chloride solution(1 %)	Result.
1 c.c.	2	0.3 c.c.	Liquid after 20 hours
1 c.c.	1.2	0.3 c.c.	do
1 c.c.	0.8	0.3 c.c.	do
1 c.c.	0.6	0.3 c.c.	do
1 c.c.	0.4	0.3 c.c.	do
1 c.c.	0.2	0.3 c.c.	Liquid after 4 hours. Slight clot after 20 hours.
1 c.c.	0.1	0.3 c.c.	Liquid after 4 hours. Clotted after 20 hours.
1 c.c.	0.08	0.3 c.c.	Clotted, but not solid, after 2 hours.
1 c.c.	0.06	0.3 c.c.	Clotted, but not solid, after 2 hours.
1 c.c.	0.04	0.3 c.c.	Clotted solid after 2 hours.
1 c.c.	0.02	0.3 c.c.	Clotted solid after 2 hours.
1 c.c.	0.01	0.3 c.c.	Clotted solid after half an hour.
1 c.c. (Control)	Nil	0.3 c.c.	Clotted solid in 3 minutes.

Table VIII.

Experiments to show the effect of varying the interval of time between the addition of cobra venom and the addition of the soluble lime salt to citrate blood in the coagulation-inhibitory action of the venom.

Citrate rabbit's blood (1-50) was used. A fixed amount of venom, namely, 1 milligramme, dissolved in 0.5 c.c. saline solution, was added to each tube, containing 1 c.c. of citrate blood. A quantity of calcium chloride solution, namely, 0.5 c.c. of 1 % solution, was added to each tube at varying intervals after the venom. This amount clotted the control in 2 minutes.

Citrate blood (1-50)	Cobra venom, 1 milligramme, added at	Calcium chloride solution (1 %) 0.5 c.c. added at	Result.
1 c.c.	2.23 p.m.	2.23 p.m.	Solid clot in 2 hours.
1 c.c.	2.43 p.m.	2.58 p.m.	Slight clot in 2 hours.
1 c.c.	2.17 p.m.	2.47 p.m.	Liquid after $2\frac{1}{2}$ hours.
1 c.c.	2.17 p.m.	3.17 p.m.	Liquid after $2\frac{1}{2}$ hours.
1 c.c. (Control)	Nil	3 p.m.	Solid clot in 2 minutes

Table IX.

Experiments to demonstrate the result of the action of the filtrate, which results after heating and filtering a solution of cobra venom, so as to remove all proteids coagulable by heat, on the coagulability of citrate whole blood.

A 0.2% solution of cobra venom was heated at 75°C. for half an hour. The coagulated proteids were then removed by filtration.

A different amount of the filtrate, as indicated below, was added to each of a series of tubes containg 1 c.c. of citrate rabbit's blood. A quantity of calcium chloride solution, namely, 0.3 c.c. of 1% solution, sufficient to clot the control in $2\frac{1}{2}$ minutes, was added to each tube two hours afterwards. The weights refer to the original unheated venom.

Citrate Blood (1 in 50)	Heated cobra venom in milligrammes	Calcium chloride solution (1%)	Result.
1 c.c.	2	0.3 c.c.	No clot after 2 hours. Solid clot after 20 hours.
1 c.c.	1	0.3 c.c.	No clot after 2 hours. Solid clot after 20 hours.
1 c.c.	0.6	0.3 c.c.	Slight clot after 1 hour. Solid clot after 20 hours.
1 c.c.	0.4	0.3 c.c.	Clotted nearly solid after 2 hours.
1 c.c.	0.2	0.3 c.c.	Clotted nearly solid after 2 hours.
1 c.c. (Control)	Nil	0.3 c.c.	Solid clot after $2\frac{1}{2}$ minutes.

Table X.

Experiments to demonstrate the result of the action of daboia venom on the coagulability of citrate whole blood 'in vitro'. A small quantity of soluble lime salt was added to each tube as well as the venom.

Each tube contained 1 c.c. of citrate rabbit's blood (1 in 50). A solution of venom (0.6% saline) of strength 1 c.c.=2 milligrammes was prepared. From this solution successive two-fold dilutions were made. To each tube of citrate blood there was added 0.5 c.c. of each dilution of the poison solution; at the same time there was added 0.05 c.c. of a 0.5% solution of calcium chloride, an amount not sufficient to clot the control in 6 hours.

Citrate Blood (1 in 50)	Daboia venom in milli-grammes	Calcium chloride solution (0.5%)	Result.
1 c.c.	1	0.05 c.c.	Clotted solid in 6 minutes
1 c.c.	0.5	0.05 c.c.	Clotted solid in 10 minutes
1 c.c.	0.25	0.05 c.c.	Clotted solid in 8 minutes
1 c.c.	0.125	0.05 c.c.	Clotted solid in 7 minutes
1 c.c.	0.0625	0.05 c.c.	Clotted solid in 5 minutes
1 c.c.	0.03125	0.05 c.c.	Clotted solid in 5 minutes
1 c.c.	0.01562	0.05 c.c.	Clotted solid in 8 minutes
1 c.c.	0.00781	0.05 c.c.	Clotted solid in 9 minutes
1 c.c.	0.0039	0.05 c.c.	Clotted solid in 15 minutes
1 c.c.	0.0019	0.05 c.c.	Clotted solid in 21 minutes
1 c.c.	0.0009	0.05 c.c.	Clotted solid in 33 minutes
1 c.c.	0.0004	0.05 c.c.	Clotted solid in 36 minutes
1 c.c.	0.0002	0.05 c.c.	Clotted solid in 60 minutes
1 c.c. (Control)	Nil	0.05 c.c.	Quite liquid after 6 hours

Table XI.

Experiments to ascertain if any diminution in the coagulability of citrate whole blood, results from the addition of small quantities of daboia venom.

Citrate rabbit's blood (1 in 100) was used. Each tube contained 1 c.c. of this blood. From an original solution of daboia venom of 0.2% strength a succession of two-fold dilutions was prepared as in the previous experiment. The high dilutions only were used, and 0.5 c.c. of each of these was added to each tube of citrate blood, One hour after the addition of the venom the same quantity of calcium chloride solution was run into each tube. This quantity clotted the control tube in about 4 to 5 hours. The time of clotting noted refers to the interval of time between the addition of the lime and the occurrence of solid clotting.

Citrate Blood (1 in 100)	Daboia venom in milligrammes	Calcium chloride solution (0.5%)	Result.
1 c.c.	0.000976	0.1 c.c.	Solid clot in 20 minutes
1 c.c.	0.000488	0.1 c.c.	Solid clot in 21 minutes
1 c.c.	0.000244	0.1 c.c.	Solid clot in 21 minutes
1 c.c.	0.000122	0.1 c.c.	Solid clot in 34 minutes
1 c.c.	0.000061	0.1 c.c.	Solid clot in 1¾ hours
1 c.c.	0.0000305	0.1 c.c.	Solid clot in 1 hour
1 c.c.	0.0000152	0.1 c.c.	Solid clot in ¾ hour
1 c.c.	0.0000076	0.1 c.c.	Solid clot in 1¼ hours
1 c.c.	0.0000038	0.1 c.c.	No clot after 2 hours
1 c.c.	0.0000019	0.1 c.c.	Nearly solid clot in 1 hour: then lost
1 c.c.	0.0000009	0.1 c.c.	Solid clot in 2½ hours
1 c.c. (Control)	Nil	0.1 c.c.	Nearly solid clot after 4 hours

Table XII.

Experiments to demonstrate the result of the action of daboia venom on the coagulability of citrate whole blood when the citrate is present in an amount just sufficient to keep the blood permanently liquid. No soluble lime salt was added.

Rabbit's blood was received into a solution of citrate of soda so that the latter was in the proportion of 1 to 100 of the blood, namely, 15 c.c. of fresh blood was mixed with 0.75 c.c. of a 20% solution of citrate. Each tube contained 1 c.c. of this citrate blood. From an original solution of daboia venom of a strength of 1 c.c.=1 milligramme a series of two-fold dilutions was prepared: 0.5 c.c of each of these dilutions was added to each tube of blood. No soluble lime salt was added.

Citrate blood (1 in 100)	Daboia venom in Milligrames	Result
1 c.c.	0.5	Solid clot in 35 minutes
1 c.c.	0.25	Solid clot in 50 minutes
1 c.c.	0.125	Solid clot in 1 hour
1 c.c.	0.0625	Solid clot in 1½ hours
1 c.c.	0.03125	Solid clot in 2 hours
1 c.c.	0.0156	No clot after 2 hours
1 c.c.	0.0078	No clot after 2 hours
1 c.c.	0.0039	No clot after 2 hours

Table XIII.

Experiments to demonstrate the result of the action of daboia venom on the coagulability of citrate whole blood when the citrate is present in excess, namely, double the minimum amount necessary to keep the blood liquid. No soluble lime salt was added.

Rabbit's blood was received into a solution of citrate of soda, 15 c.c. being run into 1.5 c.c. of a 20% solution of this salt. The proportion of citrate to blood was, therefore, as 1 to 50. The blood was then divided up into a series of test tubes, 1 c.c. being run into each test tube.

Varying amounts of a 0.2% solution of daboia venom were added to each tube. No lime solution was added.

Citrate blood (1 in 50)	Daboia venom in Milligrammes	Result
1 c.c.	2	Solid clot in 37 minutes
1 c.c.	1.6	Solid clot in 39 minutes
1 c.c.	1.4	Solid clot in 41 minutes
1 c.c.	1.2	Solid clot in 43 minutes
1 c.c.	1	Solid clot in 1 hour and 12 minutes
1 c.c.	0.8	Clotted but not solid in 1 hour
1 c.c.	0.6	Solid clot in 1¾ hours
1 c.c.	0.3	Clotted but not solid in 2 hours
1 c.c.	0.2	Clotted but not solid in 2 hours

Table XIV.

Experiments to show the effect of heating a 0.2% solution of daboia venom on its power of clotting citrate blood 'in vitro'.

Citrate rabbit's blood (1 in 100) was used. The venom solution(0.2% in 0.6% saline) was heated for half an hour at 75° C.: it was then filtered. Varying quantities of the filtrate were added to 1 c.c. of the citrate blood. The weights of venom refer to the original unheated dried poison.

Citrate blood (1 in 100)	Heated daboia venom in milligrammes	Result
1 c.c.	2	No sign of clotting after three hours
1 c.c.	1	
1 c.c.	0.6	

Table XV.

Experiments to ascertain if daboia venom heated for half an hour at 75° C has the effect of diminishing the coagulability of citrate blood.

Citrate rabbit's blood (1-50) was used: 1 c.c. of this blood was measured into each tube.

A solution of daboia venom (0.2% in 0.6% saline solution) was heated for half an hour at 75° C and then filtered. Varying quantities of the filtrate were added to each tube. The weights refer to the original unheated venom. Three hours after the addition of the venom 0.15 c.c. of a 2% calcium chloride solution was added to each tube, a quantity sufficient to clot the control in less than 2 minutes.

Citrate blood (1 in 50)	Heated daboia venom in milligrammes	Calcium chloride solution 2%.	Result
1 c.c.	2	0.15 c.c.	
1 c.c.	1	0.15 c.c.	Solid clot in less than 2 minutes after the addition of the li...
1 c.c.	0.6	0.15 c.c.	
1 c.c. (Control)	Nil	0.15 c.c.	Solid clot in less than 2 minutes.

Table XVI.

a) Estimation of the amount of soluble lime salt required to determine clotting of citrate horse plasma (1%).

To each tube containing 2 c.c. citrate plasma (1 in 100) there was added a different amount of a solution of calcium chloride (1%).

Citrate plasma 1%	Calcium chloride solution. 1%	Result
2 c.c.	0.1 c.c.	Found clotted in 4 hours
2 c.c.	0.2 c.c.	Found clotted in 3 hours
2 c.c.	0.3 c.c.	Found clotted in 3 hours
2 c.c.	0.4 c.c.	Found clotted in 1 hour
2 c.c.	0.5 c.c.	Found clotted in 8 minutes

b) Estimation of the amount of soluble lime salt required to determine clotting of oxalate horse plasma (0.2%)

To each tube containing 2 c.c. oxalate plasma (0.2%) there was added a different amount of a solution of calcium chloride (1%).

Oxalate plasma (0,2%)	Calcium chloride solution 1%	Result
2 c.c.	0.1 c.c.	No clot after 20 hours
2 c.c.	0.2 c.c.	No clot after 20 hours
2 c.c.	0.3 c.c.	Clotted in 20 minutes
2 c.c.	0. 4 c.c.	Clotted in 10 minutes
2 c.c.	0.5 c.c.	Clotted in 20 minutes

Table XVII.

Estimation of the amount of cobra venom which can inhibit the coagulation of citrate horse plasma.

Each tube contained 2 c.c. of citrate horse plasma (1%). To each tube was added a different amount of a solution of cobra venom, which was made up in two strengths, namely, 1 % and 0.2 % respectively. Two hours after the addition of the cobra venom 0.5 c.c. of 1 % calcium chloride solution was run into each tube. This quantity of lime clotted the control in 3 minutes.

The following was the result obtained:-

Citrate plasma 1%	Cobra venom in milligrammes	Calcium Chloride solution 1 %	Result
2 c.c.	5	0.5 c.c.	No clot after 20 hours
2 c.c.	4	0.5 c.c.	No clot after 20 hours
2 c.c.	3	0.5 c.c.	No clot after 20 hours
2 c.c.	2	0.5 c.c.	No clot after 20 hours
2 c.c.	1.5	0.5 c.c.	No clot after 20 hours
2 c.c.	1	0.5 c.c.	Mere trace of clot after 20 hours
2 c.c.	0.8	0.5 c.c.	Slight clot after 20 hours
2 c.c.	0.6	0.5 c.c.	Clotted but not firm after 20 hours
2 c.c.	0.4	0.5 c.c.	Clotted but not firm after 20 hours
2 c.c.	0.2	0.5 c.c.	Clotted but not firm after 20 hours
2 c.c.(control)	Nil	0.5 c.c.	Clotted in 3 minutes

Table XVIII.

Estimation of the amount of cobra venom which can inhibit the coagulation of oxalate horse plasma.

A similar technique was used in this series of experiments, as was employed in the series detailed in table XVII. Oxalate plasma (0.2 %), however, was substituted for the citrate plasma, and the amount of lime solution added to each tube was 0.4 c.c. of a 1% solution, an amount which clotted the control in ten minutes.

The following was the result obtained:-

Oxalate plasma(0.2%)	Cobra venom in Milligrammes	Calcium chloride solution 1%	Result
2 c.c.	5	0.4 c.c.	No clot after 20 hours
2 c.c.	4	0.4 c.c.	do
2 c.c.	3	0.4 c.c.	do
2 c.c.	2	0.4 c.c.	do
2 c.c.	1	0.4 c.c.	do
2 c.c.	1	0.4 c.c.	do
2 c.c.	0.8	0.4 c.c.	do
2 c.c.	0.6	0.4 c.c.	do
2 c.c.	0.4	0.4 c.c.	do
2 c.c.	0.2	0.4 c.c.	do
2 c.c.(control)	Nil	0.4 c.c.	Clotted in 10 minutes

Table XIX.

Experiments to ascertain if cobra venom can prevent the clotting of hydrocele fluid which results from the addition of a small quantity of citrate plasma.

It was by experiment found that 0.1 c.c. of citrate donkey's plasma (1 %) clotted 2 c.c. of a sample of hydrocele fluid in about 20 minutes.

A series of tubes was prepared each containing 0.1 c.c. of this plasma and varying amounts of cobra venom. The strengths of venom solution used were 1% and 0.1%. Ten minutes afterwards 2 c.c. of hydrocele fluid was added to each tube.

Citrate donkey plasma 1%	Cobra venom in milligrammes	Hydrocele fluid	Result
0.1 c.c.	5	2 c.c.	No clot after 20 hours
0.1 c.c.	4	2 c.c.	do
0.1 c.c.	3	2 c.c.	do
0.1 c.c.	2	2 c.c.	do
0.1 c.c.	1	2.c.c.	do
0.1 c.c.	0.5	2 c.c.	do
0.1 c.c.	0.4	2 c.c.	do
0.1 c.c.	0.3	2 c.c.	do
0.1 c.c.	0.2	2 c.c.	do
0.1 c.c.	0.1	2 c.c.	do
0.1 c.c.(control)	Nil	2 c.c.	Clotted solid in 19 minutes

Table XX.

Experiments to ascertain if cobra venom can prevent the clotting of hydrocele fluid which results from the addition of a small quantity of citrate plasma and soluble lime salt.

A similar technique was used as was employed in the series of experiments detailed in table XIX, the only difference being that a small quantity of lime solution, namely, 0.4 c.c. of a 1% solution of calcium chloride was added to the mixture of citrate plasma and venom solution at the same time as the hydrocele fluid was run in..

Citrate donkey plasma 1%	Cobra venom in milligrams	Hydrocele fluid	Calcium chloride solution 1%	Result
0.1 c.c.	5	2 c.c.	0.4 c.c.	No clot after 20 hours
0.1 c.c.	4	2 c.c.	0.4 c.c.	do
0.1 c.c.	3	2 c.c.	0.4 c.c.	do
0.1 c.c.	2	2 c.c.	0.4 c.c.	do
0.1 c.c.	1	2 c.c.	0.4 c.c.	do
0.1 c.c.	0.5	2 c.c.	0.4 c.c.	do
0.1 c.c.	0.4	2 c.c.	0.4 c.c.	do
0.1 c.c.	0.3	2.c.c.c.	0.4 c.c.	do
0.1 c.c.	0.2	2 c.c.	0.4 c.c.	do
0.1 c.c.	0.1	2 c.c.	0.4 c.c.	do
0.1 c.c. (control)	Nil	2 c.c.	0.4 c.c.	Solid clot after 20 minutes

Table XXI.

Experiments to demonstrate the result of the action of daboia venom on the coagulability of citrate horse plasma. No soluble salt of lime was added.

Two cubic centimetres of citrate horse plasma (1%) were measured into each tube. To each tube of plasma there was added a different amount of daboia venom. The strengths of the venom solution used were 1% and 0.2%. It was dissolved in normal saline solution.

The following was the result obtained.-

Citrate Horse Plasma. 1%.	Daboia venom in milligrammes	Result.
2 c.c.	5	Slight clot after 2 hours Solid clot after 3 hours
2 c.c.	4	ditto
2 c.c.	3	Trace of clot after 2 hours Solid clot after 3 hours
2 c.c.	2	Slight clot after 3 hours Solid clot after 20 hours
2 c.c.	1	Trace of clot after 5 hours Solid clot after 20 hours
2 c.c.	1	ditto
2 c.c.	0.8	No clot after 5 hours Solid clot after 20 hours
2 c.c.	0.6	ditto
2 c.c.	0.4	ditto
2 c.c.	0.2	Slight clot after 20 hours.

Table XXII.

Experiments to demonstrate the result of the action of daboia venom on the coagulability of oxalate horse plasma. No soluble salt of lime was added.

Two cubic centimetres of oxalate horse plasma (0.2%) were measured into each tube. To each tube of plasma there was added a different amount of daboia venom. The strengths of the venom solutionswere 1% and 0.2%. It was dissolved in normal saline solution. The following results were obtained -.

Oxalate horse plasma 0.2 %	Daboia venom in milli-grammes	Result
2 c.c.	5	Slight clot only after 20 hours
2 c.c.	4	do
2 c.c.	3	do
2 c.c.	2	do
2 c.c.	1	do
2 c.c.	1	do
2 c.c.	0.8	do
2 c.c.	0.6	do
2 c.c.	0.4	No clot after 20 hours
2 c.c.	0.2	do

Table XXIII.

Experiments to demonstrate the result of the action of daboia venom on the coagulability of oxalate horse plasma when a small amount of soluble salt of lime, namely, an amount not sufficient to clot the control, is added at the same time.

Two cubic centimetres of oxalate horse plasma (0.2%) were measured into each tube. To each tube there were added a different amount of daboia venom and 0.2 c.c. of a 1% solution of calcium chloride. The strength of the daboia venom solution used was 0.2%.

The following was the result obtained:-

Oxalate horse plasma(0.2%)	Daboia venom in milligrammes	Calcium chloride solution 1%	Result
2 c.c.	1	0.2 c.c.	Clotted in 10 minutes
2 c.c.	0.8	0.2 c.c.	do
2 c.c.	0.6	0.2 c.c.	do
2 c.c.	0.4	0.2 c.c.	do
2 c.c.	0.2	0.2 c.c.	do
2 c.c.(control)	Nil	0.2 c.c.	No clot after 20 hours

Table XXIV.

Experiments to ascertain if the addition of daboia venom to hydrocele fluid clots this fluid, either without or with the addition of a soluble salt of lime.

(a) Without the addition of a soluble salt of lime.

To 2 c.c. of hydrocele fluid, measured into each test tube, different amounts of daboia venom were added. The strength of the poison solution was 1%.

Hydrocele fluid	Daboia venom in milligrammes	Result
2 c.c.	5	No clot after 20 hours
2 c.c.	4	do
2 c.c.	3	do
2 c.c.	2	do
2 c.c.	1	do

(b) With the addition of a soluble salt of lime.

The technique used was the same as in the above series of experiments, only 0.5 c.c. of a 1% solution of calcium chloride was added to the hydrocele fluid as the same time as the venom.

Hydrocele fluid	Daboia venom in milligrammes	Calcium chloride solution 1%	Result
2 c.c.	5	0.5 c.c.	No clot after 20 hours
2 c.c.	4	0.5 c.c.	do
2 c.c.	3	0.5 c.c.	do
2 c.c.	2	0.5 c.c.	do
2 c.c.	1	0.5 c.c.	do

Table XXV.

Experiments to determine the effect of the addition of cobra venom and daboia venom together to citrate plasma, no lime solution being added.

To each tube containing 2 c.c. of citrate plasma, 5 milligrammes of cobra venom were added. This, as we have seen, is five times the quantity which can prevent the coagulation of citrate plasma, which is caused by the addition of a soluble salt of lime (vide table XVII). Half an hour after the addition of the cobra venom, a different amount of daboia venom was added to each tube. The strengths of the daboia venom solutions used were 1% and 0.2%.

The following was the result obtained.

Citrate horse plasma 1%	Cobra venom in Milligrammes	Daboia venom in Milligrammes	Result
2 c.c.	5	5	Slight clot after 2 hours Solid clot after 3 hours
2 c.c.	5	4	do
2 c.c.	5	3	do
2 c.c.	5	2	No clot after 2 hours Solid clot after 3 hours
2 c.c.	5	1	Solid clot after 4 hours
2 c.c.	5	1	do
2 c.c.	5	0.8	Slight clot after 4 hours Solid clot after 20 hours
2 c.c.	5	0.6	No clot after 5 hours Clotted after 20 hours
2 c.c.	5	0.4	do
2 c.c.	5	0.2	Only slight clot after 20 hrs.

Notes and References.

1) A complete bibliography of the subject is to be found at the end of Professor Martin's article on 'Snake Poison and Snake Bite' in Allbutt's "System of Medicine", Volume II, p 809.

2) 'Notice sur le sérum antivenimeux et sur le traitement des morsures de Serpents: Lille: 1901.

3) 'Snakes, Snake Poisons and Snake Bites'. Heimes Medical Supplement: Dec.4th, 1895.

4) 'Journal of Pathology and Bacteriology'. Edinburgh & London, 1902, Volume VIII. page 1.

5) Wall: "Indian Snake-poisons, their nature and effect": W.H.Allen & Co; London, 1883.

6) 'Lancet', January 5th, 1901: p 25.

7) Loc.Cit.

8) 'Scientific Memoirs by Medical Officers of the Army in India': Part IX, 1895, page 1, and Part XI, 1898, page 1.

9) Loc.Cit.

10) 'Scientific Memoirs by Medical Officers of the Army in India'. Part XI, 1898, page 31.

11) 'Journal of Pathology and Bacteriology': Edinburgh & London; 1900, Volume VI: page 273.

12) 'Journal of Pathology and Bacteriology': Edinburgh & London 1900, Volume VI: page 415.

13) The term 'isotonic' is not used in the strictly physiological sense. I have employed it to mean the weakest strength of salt solution in which no 'laking' of the blood was observed: in other words, 'laking' and not swelling of the corpuscles was taken as a standard.

14) Loc.Cit.

15) Loc.Cit.

16) Loc.Cit.

17) 'Journal of Pathology and Bacteriology': Edinburgh & London: 1900, Volume VI. p 273.

18) Loc.Cit.

19) Quoted from a paper by Professor Martin: 'On some effects upon the blood produced by the injection of the venom of the Australian Black Snake (Pseudechis Porphyriacus)' "Journal of Physiology", Volume XV., No.4, 1893.

20) 'Proceedings of Royal Society of N.S.Wales': July 3rd, 1895. Clifford Allbutt's 'System of Medicine', VolumeII, page 809.

21) Loc.Cit.

22) 'Journal of Physiology', Volume XV, No.4, 1893.

23) Loc.Cit.

24) Loc.Cit.

25) Loc.Cit.

26) Loc.Cit.

27) Loc.Cit.

28) 'Journal of Pathology and Bacteriology': Edinburgh & London, 1902, Volume VIII. page 1.

29) 'Journal of Physiology' Volume XV, No.4, 1893.

30) Quoted by Martin in above paper.

31) Loc.Cit.

32) Loc.Cit.

33) Many experiments were made to ascertain the minimum amount of citrate of soda which could be relied on to keep rabbit's blood permanently liquid. It was found that when the citrate was present in the proportion of 1 to 150 clotting sometimes occurred and sometimes didn't, and when in the proportion of 1 to 100 the blood always remained fluid for an indefinite time.

34) Loc.Cit.

35) 'Journal of Pathology and Bacteriology': Edinburgh & London, 1902, Volume VIII: page 1.