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A STUDY OF THE PROTECTIVE ACTION OF SNAKE VENOM UPON BLOOD CORPUSCLES.

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The present article deals with certain new facts concerning the action of venom upon blood. For the purpose of defining the scope of this study, it will be advisable to review briefly the more important contributions to our knowledge of the action of snake venom upon the blood.

The hitherto well-established effects of venom upon the blood are the hæmolytic and hæmagglutinative action upon the corpuscles, and the inhibitory and accelerating action upon the coagulation of the plasma. The reducing action upon oxyhæmoglobin has also been recognized. It was Mitchell and Reichert,[1] probably, who first definitely described the phenomena which are now known as hæmolysis and agglutination, and these same investigators emphatically called attention to the loss of coagulability of the venomized blood. Martin and Smith,[2] Phisalix,[3] Kanthack,[4] Mitchell and Stewart,[5] and Stephens and Myers [6] pursued the subject further and, while confirming the studies of Mitchell and Reichert, succeeded, at the same time, in extending our knowledge of venom activity. Martin,[7] indeed, found that large doses of the venom of a certain Australian snake produce extensive intravascular clotting, a fact which Lamb [8] also made out for the venom of an Indian viper, *Daboia russelli;* and Flexner and Noguchi [9] pointed out that the blood clots which are found in the right side of the heart and the pulmonary artery after the intravenous infusion of daboia venom contained agglutinated red blood corpuscles. It was Lamb[10] who first demonstrated the power of venom to cause coagulation of citrated blood *in vitro.* Cunningham [11] observed the discolorizing effect of venom upon blood

corpuscles, from which he erroneously concluded that venom exerts its chief toxic action upon blood, and the various phenomena commonly observed in poisoned animals are merely the consequences of the loss of the respiratory function by the altered red corpuscles. Flexner and Noguchi [12] found the mechanism of venom hæmolysis to be analogous to serum hæmolysis, and demonstrated the occurrence in venom of hæmolytic amboceptors in Ehrlich's sense. Calmette [13] discovered the important fact, destined to have very far-reaching consequences, that one of the venom activating principles is thermostable, the other being of the nature of the usual serum complements. This observation of Calmette led immediately to the brilliant discovery of lecithin-venom activation by Kyes,[14] who also succeeded in preparing a crystalline compound of venom hæmolysin and lecithin which possesses all the native hæmolytic strength of the venom. The independence of the hæmatoxic and other toxic principles, the number of which is surprisingly large, has been shown by the later studies of Flexner and Noguchi.[15]

The properties of venom already enumerated depend on principles which, with the possible exception of the hæmolysin in the lecithin compound, have not been separated in a state approaching purity. Mitchell and Stewart [16] described another property of crotalus venom which had apparently escaped the attention of previous investigators. They observed that in a mixture of blood and fresh venom, in equal parts, the corpuscles, instead of undergoing hæmolysis, were actually preserved from disintegration for a period considerably greater than in the control specimens to which no venom had been added. On the other hand, if the amount of venom employed was less than 10 per cent of the mixture, hæmolysis occurred in the usual way. Mitchell and Stewart were led by their experiments to regard the once dried venom as being a less effective preservative than the fresh secretion, and they noted that, of the corpuscles tested, those of the rattlesnake were most perfectly protected by crotalus venom. Stephens and Myers [17] described a similar phenomenon in the case of cobra venom and human blood, although they do not state how long the corpuscles can be preserved in this way. Flexner

and Noguchi [18] in their first studies noted a protection of rabbit corpuscles by means of rattlesnake venom, and, somewhat later, Kyes [19] and Kyes and Sachs [20] encountered the same phenomenon in the course of their studies with cobra venom, and Lamb [29] with daboia venom.

While the earlier investigators merely recorded their results, Kyes and Sachs [21] endeavored to explain the phenomenon. They accounted for it by supposing a complement (lecithin) deviation or "side-tracking" through the action of the large excess of venom hæmolytic amboceptors which were present in the mixture of blood and venom. Their crucial experiments were conducted as follows: Blood corpuscles were treated with a large excess of cobra venom and thoroughly washed to remove the excess of venom. Since, as Flexner and Noguchi [22] had shown, the venom amboceptors became attached to the corpuscles, the latter could now be hæmolyzed by contact with suitable complements (serum, lecithin). In the control experiments the excess of venom was not removed by washing, and in them the tubes containing the larger amounts of venom either failed entirely to show hæmolysis after addition of complement, or the amount of hæmolysis was greatly diminished.

In the course of a recent study of the influence of venom in promoting the hæmolysis caused by physical and chemical agents, this protective action of strong solutions of venom was given special attention, and the present communication deals with the results of this investigation. It may now be regarded as well established that when the optimum of the hæmolytic action of venom is exceeded, the degree of hæmolysis which it is capable of producing diminishes progressively as the dosage of the venom increases. Among the natural biological hæmolysins, venom alone is known to possess this property, but, in the course of a study of bacteriolysis by certain immune sera of high potency, Neisser and Wechsberg [23] observed an inhibition of bactericidal effects when an excess of amboceptors, relative to the complement content, was brought into the bacterial suspension. A similar although probably a distinct phenomenon has been described by Detre and Sellei [24] in their studies of hæmolysis caused by bichloride of

mercury. But excepting the few experiments made by Kyes and Sachs, upon which they base their notion of complement deviation, no really extensive study of this property of venom has been made.

The explanation offered by Kyes and Sachs involves the belief that the principle, which is responsible for the protection, is identical with that which causes, under favorable conditions, dissolution of the corpuscles. If it can, therefore, be shown that it is not the hæmolysin which is protective, but some other principle, the notion that lecithin deviation explains the appearances can not be maintained. It is possible by means of suitably arranged experiments to test fully the assumption of a protective action exerted by venom and due to hæmolysin, and, further, to study other features of this action with a view of acquiring a more complete understanding of it.

The experiments to be related were devised to determine whether the protection is effective only against complements, or whether it operates as well against other hæmolytic agencies from which the biological factors are entirely eliminated. These several aspects of the subject will be dealt with in the following pages.

Preliminary Remarks upon the Action of Venom upon Blood Corpuscles.—In the experiments to be given, the blood of the horse was chiefly used, and it is meant unless another kind of blood is specified. Unwashed horse corpuscles are fairly susceptible to venom hæmolysis, while the corpuscles after washing (three washings in fifteen or twenty times their volume of salt solution at each operation) are insusceptible to venom. The horse has the advantage of yielding small or moderate quantities of blood at not too frequent intervals without causing perceptible changes in the resistance of the corpuscles. By using a single horse and establishing the resistance of its red corpuscles a standard can be obtained that, within small limits, is constant. In carrying out the experiments on resistance this condition was essential.*

* Theobald Smith (*The Journal of Medical Research*, 1904, xii, 385) has described a reduction in resistance to salt solutions of corpuscles of horses sub-

The blood corpuscles of the horse present another advantage in their greater resistance to the mechanical injury involved in defibrination and washing.

The defibrinated blood in 0.9 per cent salt solution is susceptible to cobra, crotalus, and moccasin venoms, the varieties which were employed. A mixture of blood suspension and cobra venom, in which the venom is contained in greater quantity than 5 per cent of the dry poison, will remain undissolved when kept for several hours at 20° C.; but at the end of twelve hours complete hæmolysis will have taken place.

In general it may be stated that rapidity and completeness of hæmolysis depend upon the proportional amounts of venom and blood in the mixtures: the greater the quantity of venom and the smaller the amount of blood, the slower and less perfect the hæmolysis. There is, however, one factor which controls to a large extent the rapidity and degree of hæmolysis, namely, the presence of serum constituents in the mixture. So long as a trace of serum remains, complete dissolution can not be prevented by any practicable concentration of venom. An equal mixture of defibrinated blood and 25 per cent cobra venom* solution will undergo hæmolysis in one or two hours.

It has already been stated that the thoroughly washed horse corpuscles are not hæmolyzed by venom no matter what the degree of concentration. If cobra venom be used in strengths above 4 per cent with a corpuscular suspension of 5 per cent, no change may take place in the mixture in many weeks; while with a quantity of venom as small as 0.1 per cent the corpuscles will disintegrate rather more quickly than in the control tubes which are not entirely sterile.

Corpuscles which had been brought into contact with the stronger solutions of venom were tested for resistance to salt solutions of varying tonicity. These tests disclosed the unexpected fact that corpuscles thus venomized, and in the

jected to repeated bleedings in the production of diphtheria antitoxin. Our practice was to take relatively small quantities of blood at intervals of a number of days from a strong horse that had not previously been used for any experiment.

*The percentage of dried venom is always meant.

presence of an excess of venom, are not hæmolyzable even by water.

At the same time their susceptibility to heat is changed. It has been found that control tubes of blood corpuscles alone are hæmolyzed completely in from one hundred and seventy-five to one hundred and eighty minutes when kept at the constant temperature of 53° C. In the presence of venom of a concentration not exceeding 1 per cent, complete hæmolysis will take place at this temperature in from five to fifteen minutes; with a concentration of venom as low as 0.01 per cent thirty minutes will be required; with a concentration of 10 per cent there is no perceptible change in the corpuscles for the first twenty minutes, after which laking commences. This last laking is not, however, typical. A bright hæmoglobin color does not appear in the fluid, but the cells undergo disintegration, the color of the mixture becomes coffee-like, and in about one hour a turbid precipitate of cell particles and venom granules can be made out under the microscope.

Strong solutions of venom are capable of protecting the corpuscles from destruction by water, but venom solutions below 2 per cent in strength render the corpuscles more sensitive to salt solutions, as measured by the tonicity of the fluid for corpuscles; and as the strength of the venom falls from this limit the susceptibility, as measured by the degree of tonicity, diminishes.

Is the Protective Action Due to Venom Hœmolysin?—The first question to be considered is whether the protective action of strong solutions of venom is in any way dependent on the hæmolytic amboceptors. Since this protection is exhibited against water and heat, it is safe to assume that it bears no necessary relation to complement deviation. However, even these reactions do not prove that the hæmolysin is unconcerned in the process. Some other explanation, one, namely, in which an alteration in solubility of the protoplasmic constituents of the cells is concerned, has more likelihood of fitting the facts observed, but nevertheless it is necessary to exclude by suitable means any possible participation of the hæmolysin.

There is a marked difference between the effects produced by complements present, in an available form, before the venom has acted on the corpuscles and those produced by complements added after the treatment with venom. Should even a trifling quantity of serum be left with the corpuscles, it is not practicable to obtain the protective effect of venom, while if the effect is once produced and the excess of venom allowed to remain, very large amounts of serum or of lecithin fail to bring about hæmolysis.

The first set of experiments relates to the difference in effect produced by two concentrations of venom. In each experiment the washed corpuscles were in 5 per cent suspension, and were allowed to remain in contact with the venom for two hours before any complement was added. The mixtures were all made in 0.9 per cent solution of salt. The two strengths of venom were 10 and 2 per cent. Table I expresses briefly the result.

TABLE I

5 per cent horse corpuscles.	Amount of corpuscular suspension. c.c.	Amount of lecithin and fresh serum. c.c.	Result, at 20° C.
10 % Cobra venom	0.3	+ 0.9 % salt sol. 0.7	= No hæmolysis
	"	+ $\frac{1}{1000}$ N. lecithin 0.7	= No hæmolysis
	"	+ $\frac{1}{100}$ N. " 0.7	= Doubtful result, owing to the formation of granules, but no hæmolysis after 6 hours.
	"	+ horse serum 0.7	= No hæmolysis; strong discoloration after some hours.
2 % Cobra venom	0.3	+ 0.9% salt sol. 0.7	= No hæmolysis.
	"	+ $\frac{1}{1000}$ N. lecithin 0.7	= Complete hæmolysis in 5 minutes.
	"	+ horse serum 0.7	= Complete hæmolysis in 2 hours.
	"	+ horse serum 0.07	= Complete hæmolysis in 4 hours.
Control 0.9 % Salt sol.; no venom	0.3	+ 0.9 % salt sol. 0.7	= No hæmolysis.
	"	+ $\frac{1}{1000}$ N. lecithin 0.7	= Complete hæmolysis in 8 hours.
	"	+ $\frac{1}{100}$ N. " 0.7	= Complete hæmolysis in 1 hour.
	"	+ horse serum 0.7	= No hæmolysis.

Examination of the table shows that the corpuscles which are acted on by 10 per cent venom are not hæmolyzable by quantities of fresh horse serum and lecithin, which far surpass the amounts theoretically required to bring about solution; while with the venom in 2 per cent solution the corpuscles were perfectly dissolved by lecithin and serum. The quantity of complements used in the experiments given in the table was in great excess, and the excess in the tests with 10 per cent venom was ten times greater than in the tests with 2 per cent venom, and one hundred times greater than actually required under optimal conditions.

Before discussing the participation of the venom-hæmolysin in the protective action, it will be necessary to describe the influence of heat upon the reaction. As a preliminary step, the influence of high temperatures upon the hæmolytic action of cobra venom was made. It was found that the filtrate from the coagulum of venom heated for five minutes at 100° C. was still hæmolytic, and, indeed, quantitatively in the same degree as the unheated venom.

Tubes containing a 25 per cent solution of cobra venom in 0.9 per cent salt solution were heated to 95°, 100°, and 135° C., respectively. The temperatures of the first two tubes were maintained for five minutes, while the last tube was kept at the high temperature in the autoclave for one hour. When venom in solution is heated to 95° C. it becomes milky in appearance, while at 100° C. coarse particles of coagulated matter separate. At the temperature of 135° C. the coagulum shrinks to a solid, spongy mass, leaving the fluid portion clear. The heated venom was now tested and it was found that the specimen heated to 95° C. was practically unaltered in its hæmolytic and protective action; while at 100° C. it had lost a portion of its effect only, and at 135° C. it had lost entirely both of these properties.

The next series of tests was made with the separated portions, coagulum and filtrate. For this purpose the specimen heated to 95° C. was employed, and to obtain a perfectly clear fluid, filtration through a Berkefeld bougie was resorted to. The results of this test were conclusive; the filtrate had no protective action,

while the coagulum when suspended again was protective. The filtrate from the specimen heated to 100° C. was also non-protective. The filtrates from both of these fractions contain the hæmolytic principle in undiminished amounts.

These experiments prove definitely that the protective principle is distinct from the hæmolytic amboceptors, and it is interesting to note that the coagulum from the specimen heated to 95° C. is still capable of exerting protective influence upon blood corpuscles. It seems safe to state that the coagulated proteid is not the protecting body; and hence it is a fair assumption that in process of coagulation the protective principle is mechanically carried down under conditions of union which still permit its entering into combination with blood cells.*

Although the filtrate from a solution of venom heated to 95° or 100° C. has no protective action, such a filtrate exerts an injurious action on the corpuscles, whereby they are rendered more easily hæmolyzed by salt solution. The washed corpuscles of the horse are laked by 0.44 per cent salt solution, but after treatment with the filtrates mentioned they are instantly laked by solutions of 0.5 per cent. The temperature of 135° C. destroys not only the protective but also the injurious body; and at that temperature the filtrate is without hæmolytic action even in the presence of suitable complements.

Our experiments lead us, therefore, to regard corpuscular protection by strong solutions of venom as a peculiar effect which is opposed to hæmolytic action, and one which has thus far received neither adequate attention nor probable explanation. Moreover, it would seem that the hæmolyzing principle in venom is directly injurious to corpuscles even in cases in which suitable complements are absent. Until a special injurious agent which predisposes corpuscles to laking by physical agents is discovered in venom, we are inclined to believe that the injurious action of the filtrates is due to venom hæmolysin.

* In view of the fact that in his experiments which led to the view of complement deviation by venom amboceptors Kyes employed rabbit corpuscles, I have made a series of tests with these cells. My results show that rabbit corpuscles are somewhat less perfectly protected than some other cells, but

A passing consideration should be given to venom agglutinins, since it is at least conceivable that the agglutinated masses of corpuscles arising from venom treatment are less readily affected by laking agents than the separated corpuscles. But since the

they exhibit unmistakable evidences of protection to heat, saponin, and water. Moreover, they also show the paradoxical reaction to salt solution, since the super-venomized corpuscles show at one point a greatly lessened resistance to solutions of sodium chloride. A table is appended in which these results may be observed.

Amount of 5 % cobra venom solution c. c.	1 c. c. of 5 % suspension of rabbit blood (washed)		
	Blood No. I.		Blood No. II.
	Temperature of 53° C.	1 % saponin sol.	Reaction to water and NaCl solution.
1	No hæmolysis, precipitate formed, cells disintegrated in two hours.	Slight hæmolysis	Slight hæmolysis in water.
0.5	No hæmolysis, precipitate formed, cells disintegrated in two hours.	" "	Partial hæmolysis in water.
0.25	Complete hæmolysis in 9 minutes	Partial "	Complete hæmolysis in 0.5 % salt sol.
0.1	" " 5 "	Complete "	" " 0.53 % " "
0.075	" " 3½ "	" "	" " 0.68 % " "
0.05	" " 2 "	" "	" " 0.566 % " "
0.025	" " 2½ "	" "	" " 0.55 % " "
0.01	" " 9 "	" "	" " 0.535 % " "
0.005	" " 17½ "	" "	" " 0.52 % " "
0.001	" " 35 "	" "	" " 0.48 % " "
0.0005	" " 42 "	" "	" " 0.458 % " "
Control (no venom)	" " 130 "	" "	" " 0.43 % " "

In repeating Kyes's experiments I found that the points of protection coincided with the points of so-called deviation of complement as is shown in the next table.

Amount of 5 % cobra venom solution c. c.	1 c. c. of 5 % rabbit blood (defibrinated)		
	Blood No. I.	Blood No. II.	Blood No. III.
1	No hæmolysis	No hæmolysis	No hæmolysis
0.5	" "	Trace "	" "
0.25	Trace "	Marked "	Slight "
0.1	Moderate hæmolysis	" "	Moderate hæmolysis
0.075	Almost complete hæmolysis	Complete hæmolysis	Complete "
0.05	Complete hæmolysis	" "	" "
0.025	" "	" "	Almost complete hæmolysis
0.01	" "	Almost complete hæmolysis	" " "
0.005	" "	" " "	Marked hæmolysis
0.001	Almost complete hæmolysis	Much hæmolysis	" "
0.0005	Trace hæmolysis	No "	No "
Control (no venom)	No "	" "	" "

venom agglutinins are destroyed at relatively low temperatures their participations can be quickly eliminated. I have also tested abrin and ricin, which are strongly hæmagglutinative, without finding a corresponding protection. Indeed, corpuscles treated with abrin are slightly reduced in resistance, while corpuscles treated with ricin show about the normal reaction to salt solution.

The serum of the rattlesnake is highly agglutinative and hæmolytic for corpuscles of the horse, and yet it does not protect them in any degree. The corpuscles, after a contact of twelve hours with the inactivated rattlesnake serum in excess, were hæmolyzed by salt solution of 0.45 per cent strength.

That other coagulated proteids and mucin do not contain any such protecting principle is shown by the negative results obtained with coagulated serum of the horse and of the rattlesnake, and with salivary mucin.

The Effect of Washing the Corpuscles after Venomization.—Attention has been drawn to the fact that the property of venom which is being described is operative only in the presence of an excess of venom. Corpuscles once protected can be diluted with water indefinitely without any laking taking place, so long as the excess of venom is not removed by washing, and other evidences of protection, such as increased resistance of the corpuscles to heat and to various chemical laking agents, can be demonstrated. If, however, the venomized corpuscles are subjected to repeated and thorough washing with 0.9 per cent salt solution, it is found that the protection disappears and, moreover, the corpuscles are now more easily laked than normal blood cells.

Four mixtures of washed corpuscles and venom were prepared. The corpuscles were used in 3 per cent suspension in salt solution, and the venom strengths were 10, 1, 0.1, and 0.01 per cent. The period of contact was six hours. A portion of each mixture was removed and washed in 0.9 per cent saline four times, twenty times the volume being used at each washing. Two sets of tubes, after dilution four times with saline solution, were exposed to the temperature of 53° C. and the results noted. They are reproduced in Table II.

TABLE II

Strength of venom solution.	Washed venomized corpuscles.	Unwashed venomized corpuscles.
10 %	Complete hæmolysis in 20 minutes	No typical laking, but general disintegration of cells in 60 minutes; the fluid presented a light brownish hue.
1 %	Complete hæmolysis in 25 minutes	Complete hæmolysis in 6 minutes.
0.1 %	Complete hæmolysis in 25 minutes	Complete hæmolysis in 12 minutes.
0.01 %	Complete hæmolysis in 30 minutes	Complete hæmolysis in 30 minutes.
Control (no venom)	Complete hæmolysis in 170 minutes	Complete hæmolysis in 175 minutes.

Table II shows that horse corpuscles suspended in the venom solutions of strengths above 0.1 per cent absorb about the same quantity of venom irrespective of the actual concentration of the solutions. The quantity of venom absorbed sufficed to bring about laking in twenty-five minutes, the mixtures having been kept during this period at 53° C.; and this amount of venom is apparently the maximum quantity which the corpuscles are able to retain after repeated washings with the saline solution.

There is no method of ascertaining with absolute accuracy whether this quantity was originally absorbed, or whether it is the part of the original quantity fixed to cells and retained with great avidity. An approximate estimation could, of course, be made of the amount of venom remaining in the fluid part of the mixture after simple contact of the blood-cells and venom; but since, in carrying out this estimation, no account can be taken of the venom which is loosely and mechanically held by the corpuscles, figures approaching accuracy are impossible.

That venom directly injures blood corpuscles has been mentioned, but Table II is valuable in showing that this injury can be expressed in quite definite figures. I know of no series of experiments in which laking by means of heat has been used to

determine the degree of decreased resistance of blood cells. The method seems to promise a fair degree of accuracy, and it also would seem to promise the discovery of substances of primary injurious nature, which until now have been assumed to be inactive except in the presence of suitable complements.

In order that this primary injurious action may be expressed, the concentration of the venom solution must be kept between 0.1 and 1 per cent. Higher concentrations either begin to be protective or do not increase the injury, because they are not so entirely absorbed. In any case an excess of venom would appear not to be present in the unheated mixtures, for hastening of heat hæmolysis is not observed in the venomized corpuscles which have previously been washed. Hence the question arises whether at the high temperature more venom is not absorbed than at 20° C.; from this increased fixation and injury perhaps the greater susceptibility to laking proceeds. No answer to this question is attempted at present; but I shall now proceed to the next series of experiments which are concerned with the effect of repeated washings in saline solution upon the tonicity of the venomized corpuscles, as measured by their resistance to salt solutions of different strengths.

TABLE III

Strength of venom solution.	Venom removed.	Venom not removed.
10 %	0.51 %	Not hæmolyzable in water.
1 %	0.49 %	0.48 %
0.1 %	0.48 %	0.465 %
0.01 %	0.465 %	0.44 %
Control (no venom)	0.44 %	0.44 %

The striking difference in the manner of reaction to hypotonic solutions between the corpuscles acted upon by strong venom solutions and by solutions of weaker concentration is well illustrated in this table. After the action of 10 per cent venom, the corpuscles can be diluted indefinitely with water without showing any tendency to dissolve, while the same corpuscles, after

repeated washing with 0.9 per cent salt solution, show a higher hypotonic index than normal—0.51 per cent as compared with 0.44 per cent. The significance of this behavior is quite plain. Strong venom solutions react in some manner with the corpuscles so that they are rendered insoluble in water; the insoluble compound is, however, capable of being broken up by certain chemicals—in this case sodium chloride. But whether it is the sodium chloride as such, or the free ions in the mixture, the experiment does not show. It may be remarked here that the protective property is not quickly removed by the saline solutions, but several washings are required to remove it. It would seem, therefore, that an excess of sodium chloride alone does not suffice, but some other factor is concerned.

Since it has now been shown that the entire protective effect of the venom, as regards water hæmolysis, can be removed by washing the corpuscles with the saline solution, the question arises whether this process restores the susceptibility of the corpuscles to lecithin and serum hæmolysis. It has already been stated that the presence of serum in the mixture will prevent the strongest solutions of venom from causing protection; and now it may be said that the protection once removed by salt solution the corpuscles become susceptible to serum and lecithin hæmolysis.

The next table gives the results obtained with lecithin and horse serum. The conditions of the experiment were similar to those of the preceding experiments. The period of contact of venom and washed corpuscles was two hours; and the washing was always done with 0.9 per cent salt solution. The volume of suspended corpuscles was 3 cubic centimeters, to which 0.5 cubic centimeter of $\frac{1}{1000}$ N. lecithin or 1 cubic centimeter of horse serum was added. The observations in the case of the horse serum were made at the end of four hours.

It appears that washing with salt solution exposes the protected corpuscles to laking with serum and lecithin. This susceptibility to laking is due to the fixation of venom hæmolysin by the corpuscles. When the activity of very strong solutions, after removal of all unattached venom, is compared with the

activity of weak solutions, in which subsequent washing is omitted, a marked difference in the rapidity with which hæmolysis occurs is apparent. The difference may depend, possibly on one of two causes, first, that the corpuscles in the absence of complement actually fix an insufficient quantity of hæmolysin to produce rapid laking; or, next, the washing removes a part of the hæmolytic amboceptors already attached.

The Action of Certain Substances upon the Venomized Blood Corpuscles.—The effects of water, salt solution of different concentrations, and heat upon the venomized corpuscles have been

TABLE IV

Strength of venom solution.	LECITHIN.		HORSE SERUM.	
	Venom removed.	Venom not removed.	Venom removed.	Venom not removed.
10 %	Complete hæmolysis in 28 minutes	No hæmolysis	Complete hæmolysis	No hæmolysis.
1 %	Complete hæmolysis in 28 minutes.	Complete hæmolysis in 7 minutes	" "	Complete hæmolysis.
0.1 %	Complete hæmolysis in 29 minutes.	Complete hæmolysis in 9 minutes.	Partial hæmolysis	" "
0.01 %	Complete hæmolysis in 30 minutes.	Complete hæmolysis in 28 minutes.	" "	" "
Control (no venom)	Complete hæmolysis in 8 hours.		No hæmolysis	

recorded. The next series of experiments deals with the action of still other definite agents, such as acids, alkalies, etc., upon the protected cells. The method of procedure was as follows: Four tubes, containing 4 cubic centimeters each of 5 per cent cobra venom in 0.9 per cent salt solution and 6 per cent of washed horse corpuscles, were allowed to stand for two hours at 20° C. Tube No. 1 received 4 cubic centimeters of water, and tube No. 2 received 20 cubic centimeters of water; the corpuscles were permitted to subside when the clear fluid was decanted and the volume of the residue brought up to 8 cubic centimeters. Tube No. 3 received an additional 4 cubic centimeters of salt solution, while the residue in tube No. 4 was washed once in 20 cubic centimeters of salt solution and suspended in 8 cubic centimeters of saline solution. The actual tests were made by measuring 2

cubic centimeters of each mixture into a test-tube, to which the solutions of chemicals were added gradually until an amount was introduced which produced instantaneous hæmolysis. The chemicals were dissolved either in water or in saline solution, according as they were to be added to blood cells diluted in one or the other of these fluids. The results are given in the next table.

TABLE V

Venomized corpuscles.	$\frac{1}{50}$ N. HCl c. c.	$\frac{1}{50}$ N.NaOH c. c.	$\frac{1}{50}$ N. NH_3 c. c.	0.1 % saponin. c. c.
1. Dilution with water	0.125	0.75	1.75	No hæmolysis.
2. Sedimented and re-suspended in water	0.12	0.7	1.	" "
3. Dilution with saline solution	0.33	1.5	2.	" "
4. Washed with and re-suspended in saline solution	0.25	0.8	0.3	Complete hæmolysis, 0.35
5. Control, no venom	0.5	1.75	0.4	" " 0.45

An analysis of this table shows an essential agreement between the manner of action of hydrochloric acid and sodium hydroxide. These substances cause hæmolysis in smaller quantities when added to the watery suspensions than with suspensions of corpuscles in saline solution, the difference being approximately as 1 : 2 or 1 : 2.5. On the other hand, after removal of the excess of venom by washing in the saline solution, the corpuscles show a greatly reduced resistance, since they are about twice as readily laked by these chemicals as the control without venom. On the other hand, in the watery medium the super-venomized corpuscles are about four times as susceptible to hydrochloric acid and twice as susceptible to sodium hydrate as the control specimens. This apparent paradox is probably explained by the nature of the medium, for, the insoluble compound of venom and proteid once dissolved, the previously protected corpuscles

immediately become the victims of water-laking. The reduction of resistance exhibited by the venomized cells suspended in salt solution is an expression of the injury inflicted by the venom directly upon the corpuscles in the manner already described.

On the other hand, ammonia permits unmistakable evidences of protection to be exhibited. In the first place, in the presence of excess of venom, ammonia proved to be relatively a weak solvent for the protecting compound, and, in the next place, in the case of the washed venomized cells the susceptibility of the corpuscles to the solvent action of the ammonia is increased beyond the control, although this difference is again less marked than with hydrochloric acid and sodium hydrate.

Another remarkable fact is the inability of saponin to cause laking of the protected corpuscles. According to Kobert,[25] saponin is hæmolytic probably because of its property of dissolving lecithin, which it withdraws from the corpuscles with the effect of destroying the integrity of the cells. It would seem, therefore, that the protection of the corpuscles against saponin hæmolysis might be associated with a change in the availability of the intracellular lecithin. It is improbable that venom extracts the lecithin, for if it did it is likely that the corpuscles would be directly disintegrated by the venom, but such disintegration does not occur. It is far more probable that the venom action is to bring about some physical or chemical alteration in the cells which prevents the usual effect of saponin upon the corpuscles. That, indeed, the effect of venom is not permanent, as would be the case were it to abstract the lecithin, is shown by the results of washing the protected corpuscles in the saline solution and subsequent exposure to saponin. Such washed cells are hæmolyzed by saponin even more readily than the controls. Hence the evidence is in favor of the view that venom fixation of the corpuscles is due to some change in the character of the cellular constituents, and that the venom acts also directly upon the corpuscles, producing an injury even though this injury is not at once evident. We shall return to the first aspect of this subject. The strongest solutions of venom which it is practicable

to use, like the weaker solutions, act injuriously upon blood corpuscles. The stronger solutions, however, exert a second action —a kind of fixation of the cells—which obscures the injurious action. Table VI gives the results obtained with saponin and corpuscles in the presence of an excess of venom and after its removal. The volume of the venomized blood suspension in each instance was 4 cubic centimeters. Saponin was employed in 0.1 per cent solution, which was added in increasing quantities until instantaneous hæmolysis was produced.

TABLE VI

Strength of venom solution.	Washed venomized corpuscles + 0.1 % saponin.	Unwashed venomized corpuscles + 0.1 % saponin.
10 %	0.3 c. c.	No hæmolysis *
1 %	0.35 "	0.35 c. c.
0.1 %	0.4 "	0.4 "
0.01 %	0.45 "	0.45 "
Control (no venom)	0.45 "	0.45 "

Strong venom solutions afford no protection against formic acid hæmolysis, and the protection against oleic acid is not absolute, but the degree of resistance against this acid was not wholly worked out. Ether in large quantities fails to lake the venom-protected corpuscles.

The Rapidity and the Limits of the Protective Action.—In order to determine the rapidity of the fixation of the corpuscles by venom a series of tests was made. A 10 per cent solution of cobra venom was prepared in 0.9 per cent salt solution, and to this washed horse corpuscles were added in quantity of 3 per cent. The mixture was allowed to stand at 20° C. for the period of the experiment, and at the intervals mentioned in the table portions were withdrawn and tested against salt solutions of different degrees of hypotonicity. A parallel series of tests was carried on to determine what concentration of venom in 0.9 per cent saline solution is necessary to protect the corpuscles against suspension in water. In the latter series a contact of five hours

* 2 % saponin in the mixture caused no hæmolysis after 10 days.

was employed. The following table presents the results of these experiments:

TABLE VII

DETERMINATION OF RAPIDITY OF ACTION.		DETERMINATION OF MINIMAL LIMIT OF ACTION.	
Period of contact of venom and corpuscles.	Reaction to sodium chloride solution.	Concentration of venom solution.	Reaction to sodium chloride solution.
5 minutes	Partial hæmolysis in 0.3 % sol.	2 %	Complete hæmolysis in 0.7 % sol.
10 "	Trace hæmolysis in 0.3 % sol.	3 %	Trace hæmolysis in 0.1 % sol.
20 "	No hæmolysis in 0.3 % sol.; trace in water.	4 %	No hæmolysis in water.
30 "	No hæmolysis in water.	5 %	No "
60 "	" " "	6 %	No "
		7 %	No "
		8 %	No "
		Control (no venom)	Complete hæmolysis in 0.44 % sol.

Estimation of the Protective Action of Moccasin and Crotalus Venom.—The tests under this heading were made in the manner previously given. Different strengths of the venoms were prepared in 0.9 per cent saline, and to them washed horse corpuscles were added in 3 per cent suspension. The mixtures were allowed to stand for six hours at 20° C., and were then tested against salt solutions of varying tonicity. The removal of the venom in some of the experiments was done by means of 0.9 per cent saline.

TABLE VIII

Concentrations of venom.	CROTALUS VENOM.		WATER-MOCCASIN VENOM.	
	Venom removed. Tonicity of NaCl sol.	Venom not removed. Tonicity of NaCl sol.	Venom removed Tonicity of NaCl sol.	Venom not removed. Tonicity of NaCl sol.
20 %	0.54 %	0.18 %	0.57 %	0.2 %
10 %	0.528 %	0.2 %	0.48 %	0.25 %
5 %	0.5 %	0.3 %	0.47 %	0.35 %
1 %	0.49 %	0.465 %	0.47 %	0.465 %
0.1 %	0.47 %	0.454 %	0.465 %	0.45 %
0.01 %	0.454 %	0.444 %	0.45 %	0.445 %
Control (no venom)	0.44 %		0.44 %	

The above table brings out the interesting fact that, while moccasin and rattlesnake venoms possess the property of

protecting blood corpuscles, they are in this respect much inferior to cobra venom. With neither moccasin nor rattlesnake venom was there produced absolute protection against water hæmolysis. It may be worth while to point out in this place that in ultimate hæmolytic power with defibrinated horse and dog corpuscles these venoms were about the equal of cobra venom, although crotalus venom acts more slowly and is quantitatively a little weaker than the others.* The fact of the similarity in hæmolytic power and dissimilarity in protective action is another proof of the distinction between the several principles causing the two phenomena.

TABLE IX

Period of contact before heating.	Time required for complete hæmolysis at 53° C.
Immediate	10 minutes.
10 minutes	11 "
30 "	20 "
60 "	38 "
2 hours	30 "
6 "	25 "

The Influence of Small Amounts of Venom upon Heat Hæmolysis.—In the course of the experiments a phenomenon came under my observation which will now be described. Mixtures of washed corpuscles and venom of low concentration were exposed, after varying intervals of contact, to the temperature of 53° C. At first the effect, as measured by the degree of heat hæmolysis, is small, but after a time it becomes marked. However, after a still longer interval of contact the acquired resistance undergoes diminution. A series of tests is given in Table IX. The mixture exposed to heat consisted of 20 per cent washed horse corpuscles

* For complete hæmolysis under the conditions mentioned, crotalus venom requires at 20° C. twenty-four hours and, in addition, occasional agitation of the tubes. If the comparison of hæmolysis be made after six hours and at the upper limit of dosage, the difference between cobra and moccasin venom, on the one hand, and rattlesnake venom, on the other, may be as 1 : 20, but if the reading be taken after twenty-four hours it will be as low as 2 : 3 or, at most, 2 : 4.

in 30 cubic centimeters of 0.9 per cent salt solution, and 0.2 per cent cobra venom in 1.5 cubic centimeters of the same strength salt solution. Four cubic centimeters were removed at a time at different intervals and tested.

We can see in these results the opposed action of the two properties of venom when acting together. The first effect to declare itself is apparently partial fixation of the corpuscles, but when the venom solution is too weak to accomplish this entirely, the directly injurious action of the poison makes itself felt. Hence it would seem that with such weak solutions of cobra venom as were employed in this experiment, the injurious action upon the corpuscles, in the absence of available complement, was more marked than the protective action.

The Action of Venom upon the Blood Serum, the Stroma, and the Soluble Contents of the Blood Corpuscles.—With a view of ascertaining whether venom forms with the blood constituents any difficultly soluble compound, it was tested separately upon the fluid and solid portions. Horse blood being used, the serum, the stroma, and the corpuscular contents soluble in water were tested against various venom solutions.

The stroma was prepared by laking the twice-washed, condensed corpuscles. The final product was a gelatinous mass of pale rose color, which could be suspended in water or in saline solution. The contents of the corpuscles were readily obtained by laking with water and centrifuging. They could also be diluted to any degree with water or salt solution.

The results given in the table show clearly that venom is a precipitant for certain of the constituents of the blood. The chief precipitate is obtained with the water-soluble contents of the corpuscles, although a partial reaction is also obtained with diluted serum. The stroma reacts in a minor degree with the venom, but whether or not this reaction depends on the venom agglutinin or is due to a precipitant in the real sense was not determined.

The precipitate formed with the contents of the corpuscles in pure water is dense, viscid, and finely divided, while in saline solution it is coarser and less in amount. The original color of

TABLE X

Cobra venom sol.	10 % SERUM.		10 % STROMA.		10 % CORPUSCLE-CONTENTS *		CONTROL VENOM SOLUTIONS.	
	in water.	in 0.9 % salt sol.	in water.	in 0.9 % salt sol.	in water.	in 0.9 % salt sol.	in water.	in 0.9 % salt sol.
5 %	Partial precipitation.	Cloudy, but evenly so.	Cloudy, but not more than the control.	Cloudy, but not more than the control.	Dense, viscid, and soft precipitate formed quickly. The precipitate separates out, leaving clear, slightly tinted fluid near the surface.	A coarser precipitate is formed after 18 hours. By diluting with a large volume of water a more abundant and finer precipitate appears.	Cloudy, but evenly so.	Cloudy, but not quite opaque.
1 %	A definite precipitation.	Slightly cloudy.	Slightly cloudy; the stroma settled down to the bottom.	Slightly cloudy; the stroma settled down to the bottom.	Similar to the above; but precipitate much less opaque.	A definite precipitation, but less than with water. Dilution with water caused increase of precipitate.	Slightly cloudy.	Slightly cloudy.
0.1 %	A slight cloudiness.	Almost no cloudiness.	Almost clear; the stroma sunken to the bottom.	Almost no cloudiness; the stroma is on the bottom.	More cloudy than the control, but no definite precipitate.	About same as the control.	Almost no cloudiness.	Almost no cloudiness.
No venom (control)	Almost no cloudiness.	Almost no cloudiness.	Perfectly clear; the stroma is all sedimented.	Perfectly clear; the stroma is all sedimented.	Perfectly clear.	Perfectly clear.		

* Contained hæmoglobin also.

the solution is bright red from the presence of dissolved oxyhæmoglobin, but venom quickly changes it to dark brown. The pigment is carried down with the precipitate in the watery mixture, but in the saline mixture it remains for some time in the supernatant fluid. Salt, in certain concentrations would seem, therefore, to prevent, more or less, the formation of the precipitate. Indeed, if the saline solution described is further diluted with water a more abundant precipitation takes place.

Since the protected corpuscles are quickly dissolved by acids and alkalis, the question naturally arises whether the precipitate is also easily soluble in these chemicals. It was found that acids and alkalis dissolve the precipitate in approximately the same degree as they are capable of bringing about hæmolysis of the venom-treated corpuscles.

The Action of Venom upon the Separated Constituents of Serum and Corpuscles.—Venom, as has been stated, causes a dense precipitate with an aqueous extract of washed corpuscles. When, however, sodium chloride is present in the solution in a strength equalling or exceeding 0.9 per cent, the precipitation does not take place, or occurs only imperfectly. Now, it is my belief that venom owes the protective action upon corpuscles described to its ability to form a compound insoluble in water with certain corpuscular constituents. It is, therefore, desirable to ascertain whether any particular constituent of the corpuscles is concerned in the formation of the insoluble substance, or whether all the proteids of the cells are fixed by the venom. Moreover, since salt in weak concentration is able to prevent the development of the precipitate in the corpuscular extract, it is also possible that it prevents, in serum, a similar reaction. The next experiments were made with serums in which the saline percentages had been reduced by dilution with water. The results of these tests are given in Table XI.

That simple dilution with water affects the precipitability of a compound formed by venom and serum when brought together, is shown by the results given in the table. Another way of approaching this problem is to remove the salts of the serum by dialysis. This was done for the serum of the horse and dog, and

dialysis was continued until a considerable amount of globulin had separated from the serums. It was found that only a trace of chlorides remained in the fluid. The two parts—fluid and precipitated globulin—were tested separately. The globulin which had separated belonged chiefly to the euglobulin fraction, but there still remained in the fluid parts of the serums considerable globulin precipitable by one-third saturation with ammonium sulphate. During the dialysis the volume of the serums had increased two and one-half times.

TABLE XI

	MIXTURE A.	MIXTURE B.	MIXTURE C.	MIXTURE D.
	Undiluted serum 1.0 c. c. 20 % cobra venom 0 2 c. c.	Mixture A 0.2 c. c. Water 1.0 c. c.	Serum diluted with 4 vols. of water 1.0 c.c 20% cobra venom 0.2 c. c.	Serum diluted with 4 vols. of 0.9 % salt solution 1.0 c. c. 20 % cobra venom 0.2 c. c.
Horse serum	Clear	Moderate precipitate	Much precipitate	Clear
Rabbit "	"	Moderate "	Moderate "	"
Rat "	"	Much "	Much "	"
Guinea-pig "	"	" "	" "	"
Dog "	"	" "	" "	"
Pigeon "	"	" "	" "	"
Crotalus plasma	"	Much precipitate, resembling fibrin.	" "	"

REMARKS: Controls without venom remained perfectly clear for more than 24 hours, while the controls with venom alone showed only a slight amount of precipitate in water and in 0.9 % salt solution.

The dialyzed horse serum was tested as follows: First tube—1 cubic centimeter of the fluid portion was mixed with 0.2 cubic centimeters of 20 per cent cobra venom solution; moderate precipitation. Second tube—The same mixture plus sodium chloride to 0.9 per cent strength; no precipitation. Next, the precipitate of globulin was washed in water by centrifuging and dissolved or suspended in salt solutions of the following concentrations: 0.8, 0.6, and 0.4 per cent. In the first there was perfect, in the second almost perfect, in the third imperfect solution. To the clear filtrates the 20 per cent venom solution was added, and rapid and nearly complete precipitation of the dissolved globulins occurred. When the globulin is merely suspended, as in water or weak saline, the venom quickly brings down the sus-

pended particles which, left to themselves, subside very slowly, if at all. A comparison of the amounts of sediment obtained from the fluid part of the dialyzed serum and the redissolved globulin shows that the globulin is more completely precipitable than the other constituents.

It was now decided to fraction the serum, and for the purpose the method of Freund and Joachim [26] was employed. The fractions—euglobulin, pseudoglobulin, serum albumin—were freed from ammonium sulphate by dialysis and tested with cobra venom. The most marked reaction was given by the first, the pseudoglobulin fraction giving a less reaction, and the serum-albumin fraction no precipitate. The results of the two series of tests, therefore, agree in showing the globulin to be the constituent acted upon by the venom.

The water-soluble contents of the corpuscles were also fractioned. The first fraction, yielded by one-third saturation, was proportionately large and contained a part of the coloring matter of the corpuscles. The second fraction, obtained by one-half saturation, was less in amount than the first, but contained the greater part of the oxyhæmoglobin. The third fraction, obtained by saturation, was small in quantity. The salts were removed by dialysis.

The first two fractions were deep red in color. One cubic centimeter of the solution of each was mixed with 0.2 cubic centimeters of 20 per cent cobra venom; the result was precipitation. The second fraction gave the more abundant precipitate, leaving the fluid clear of coloring matter. In order to obtain similar clearing of the first fraction additional dilution with water was required. The third fraction yielded no precipitate with the venom solution.

Since in this series of experiments the globulins and hæmoglobin were associated and it was desirable to know whether the reaction takes place between hæmoglobin and venom, as well as between the globulins and venom, separation of the hæmoglobin in a pure state was necessary.

Oxyhæmoglobin from horse corpuscles was prepared by Zinoffsky's [27] method, while that from dog's corpuscles was prepared by

laking the washed corpuscles with ether and removing the stroma in the separatory funnel. The clear solution of hæmoglobin was shaken with a large volume of ether and allowed to stand for twenty-four hours at 0° C. A large quantity of crystalline oxyhæmoglobin, which was purified by being twice recrystallized, was thus obtained.

For testing against venom the hæmoglobin was used in 0.2 to 0.1 per cent solutions in water or 0.9 per cent sodium chloride. To 1.0 cubic centimeter of the solutions of oxyhæmoglobin 0.1 cubic centimeter 20 per cent venom was added. The results were as follows: in the presence of sodium chloride no reaction occurs; in aqueous solution there is complete precipitation of the hæmoglobin of the horse, but no reaction with that of the dog. That pure hæmoglobin may react with venom is shown by these experiments; that the reaction is similar to that with the globulins in respect to solubility in salt is also shown; and that a difference in composition exists between dog's and horse's oxyhæmoglobin the experiments also indicate.

In view of these results it seemed desirable to test venom directly upon globin, which was prepared from the oxyhæmoglobin of the horse and dog by Schulz's [28] method. The solutions of globin were neutral to litmus and gave the reactions described by Schulz. To 1.0 cubic centimeter of globin solution 0.1 cubic centimeter of 20 per cent venom was added. Dog's globin gave no precipitate; horse's globin gave a voluminous precipitate which was easily soluble in acids and alkalis, but not in salt solution. Indeed, a small quantity of salt hastens the appearance of the precipitate with horse's globin.

This series of experiments would tend to show that the precipitability of oxyhæmoglobin by venom depends on the globin constituent, and that the globins of horse and dog corpuscles are distinct substances as regards, at least, their reaction with venom.

The question has already been mooted as to what the nature of the protective process described in this paper really is. That the phenomenon is independent of the hæmolytic reaction has been conclusively proven. Moreover, the immediately preceding experiments show that venom is a precipitant for several proteids

of the blood. It is doubtless upon the reaction which takes place between the venom and these precipitable proteids that the phenomenon of protection depends. One of the striking appearances of the reaction is the carrying down of the hæmoglobin by the venom; and it seems not improbable that this property might be utilized as a measure of the protection of the corpuscles, for when it is absent it is found that venom does not shield the corpuscles from water laking. The following table shows that this relation actually exists:

TABLE XII.

Origin of corpuscles.	20 % cobra-venom solution 2.0 c. c. Washed blood corpuscles 0.3 c. c.		1 % intracorpuscular contents 1.0 c. c. 20 % cobra-venom solution 0.1 c. c.	
	Behavior of the corpuscles left in the above mixture.	Behavior of the corpuscles when brought into water.	Precipitation of the hæmoglobin.	Solubility of the precipitate* in 0.9 % sod. chloride solution.
Horse	No hæmolysis	No hæmolysis	Complete	Dissolves but reprecipitates on standing.
Rabbit	" "	" "	Almost complete; fluid retains a faint color.	Partially soluble; large precipitate on standing.
Rat	" "	" "	Complete	Slightly soluble
Guinea-pig	" "	" "	None	" "
Dog	Complete hæmolysis	Complete hæmolysis	"	
Pigeon	Slight "	" "	Slight	Easily soluble.
Crotalus	No "	Incomplete "	"	" "

* The precipitate was easily soluble in different acids and alkalis.

Yet, since it is not only the hæmoglobin which is precipitated by venom, but the intracorpuscular globulins as well, it is not possible to describe the phenomenon of protection as if it depended wholly on a reaction with the former proteid. It is probable that all the precipitable proteids are acted upon and affect the result, although it seems not unlikely that the fixation of the hæmoglobin in an insoluble form is what directly prevents its diffusion out of the corpuscles into the surrounding medium.

In the course of this paper allusions to a change in the permeability of the corpuscles have been made. The question as to whether the so-called protected corpuscles are altered in their physical condition so as to exclude certain substances, which under normal conditions easily enter them, is pertinent. It is

to be considered whether the rendering of certain of the proteids insoluble is not associated with an obstruction to osmotic processes taking place between the corpuscles and the surrounding medium. If in the case of water and the protected corpuscles this fluid was successfully excluded from the corpuscles by an insoluble although superficial barrier, laking could not occur. However, many of the experiments described show that the absence of laking does not depend upon changed conditions of osmosis, but upon the formation of insoluble compounds.

A set of experiments was undertaken to determine whether, at the same time, the permeability of the corpuscles may not have been altered. For this purpose saponin was employed, since, as stated, Kobert has shown that saponin causes hæmolysis by virtue of the fact that it dissolves the lecithin of the corpuscles. Now, the failure of saponin to act upon the protected corpuscles could be due either to their impenetrability for saponin, or to the insolubility of the venom-hæmoglobin compound in the menstruum in spite of the change of the cells caused by a solution of the lecithin in saponin. The following experiment was devised with a view of determining the nature of the protection from saponin.

Twenty per cent cobra venom in salt solution was treated with washed horse corpuscles (5 cubic centimeters of venom plus 0.5 cubic centimeters of corpuscles) for three hours at the room temperature. The corpuscles were rendered water-protected and also non-hæmolyzable when treated with 2 per cent watery solution of saponin.

Two cubic centimeters of the venomized corpuscles were dropped into 150 cubic centimeters of distilled water; sedimentation was complete in twenty hours. The corpuscles were twice washed in the centrifuge with water. Examined microscopically they were entangled with venom granules. These corpuscles were protected perfectly both from water and 0.9 per cent salt solution. The sediment was mixed with an equal volume of 2 per cent solution of saponin, and the mixture was allowed to stand for ten minutes. The corpuscles were diluted and washed in water until all the saponin (determined by testing

with unprotected corpuscles) was removed. A thick suspension in water of the saponin-venomized corpuscles was now added separately to water and to 0.9 per cent saline solution (0.2 cubic centimeter of suspension to 2 cubic centimeters). The salt solution quickly caused laking, while, as was to be predicted, the water was without effect.

The interpretation of this experiment is probably as follows: The saponin enters the venom-protected corpuscles in spite of their fixation and takes up the lecithin, whereby the corpuscles are seriously damaged. However, as the venom-hæmoglobin compound is insoluble in water, no visible expression of this damage can occur. Since the venom-hæmoglobin compound is soluble in 0.9 per cent saline solution, it quickly dissolves in the latter whether it is capable of leaving the corpuscles or not. Unless the corpuscles have been previously injured, the dissolved hæmoglobin compound does not diffuse into the medium; but in view of the action of the saponin upon the corpuscles a sufficient injury has been inflicted to allow the hæmoglobin to escape. Venom protection of blood corpuscles does not seriously affect the permeability of the corpuscles, but it prevents, under many conditions, the diffusion of the hæmoglobin into the surrounding medium.

SUMMARY.

Washed blood corpuscles of certain species of animals in a concentration of about 5 per cent suspended in salt solution containing above 4 per cent of cobra venom undergo changes in their resistance to certain physical and chemical agents. They become non-hæmolyzable by water, ether, saponin, and quite strong solutions of lecithin, provided always that the excess of venom has not been entirely removed. On the other hand, certain acids and alkalis, excepting ammonia, lake the venomized corpuscles more easily than they lake normal corpuscles. Venom solutions of 2 per cent and less exert no protective property upon blood corpuscles, but they induce changes in the corpuscles whereby they are rendered more easily laked by the same physical and chemical agents.

The changes in the corpuscles upon which this protection relies can be set aside by repeated washings of the venomized corpuscles in 0.9 per cent salt solution. When the protection is removed the corpuscles are left in a state of diminished resistance to injurious agencies.

The substance in venom upon which the protective action depends is not destroyed by the temperature of 95° C., although at this temperature a part of the venom is coagulated. The protective body is found in the coagulated portion; while the venom hæmolysin is contained, in full activity, in the clear filtrate. The agglutinin for red corpuscles disappears at 75° C.; hence, the protection of corpuscles by venom depends neither upon the hæmolysin nor the agglutinin.

Blood corpuscles absorb the hæmolysin from concentrated solutions of venom as readily as from weaker solutions. This fixed hæmolysin can be brought into action, under suitable conditions, by the addition of complements. But the hæmolysin is unable, in the presence of complements in excess, to cause laking of the corpuscles unless the protection produced by the venom has first been set aside.

Cobra venom forms a precipitate with blood serum when comparatively free of salts or when diluted with water; it forms a precipitate with a watery extract of blood corpuscles, and with the separated globulins, hæmoglobin and globin of the blood. The precipitates are insoluble in water, but dissolve in weak acids and alkalis. The stroma of the red corpuscles causes no change in venom solutions.

Protection of the corpuscles against hæmolysis depends upon the formation by venom of a water-insoluble compound with certain of the corpuscular constituents, chiefly probably with hæmoglobin. There is no reason to believe that the permeability of the corpuscles is greatly altered.

The restoration of the susceptibility of the corpuscles to the ordinary laking influences is accomplished by dissolving the venom-proteid compound. Since this compound is soluble in acids and alkalis and even in a large excess of salt solution, restoration is easily effected. Corpuscles which have been pro-

tected and again rendered hæmolyzable are less resistant to injurious influences than normal corpuscles, resembling in this respect corpuscles which have been treated with relatively weaker solutions of venom. Venom, therefore, always exerts a direct injurious influence upon blood corpuscles; but strong solutions of venom may obscure this injurious effect through the predominance of the protective action which they display.

Not all blood corpuscles are susceptible to the protective action of venom, and different corpuscles display varying degrees of capacity for protection. Dog corpuscles are not protected by cobra venom, and watery extracts of dog corpuscles, as well as pure oxyhæmoglobin and globin of this animal, are not precipitated by venom solutions.

Crotalus and moccasin venoms possess this protective property, but in less degree than cobra venom.

I wish to express my gratitude to Professor Flexner for his encouragement and for many valuable suggestions during the execution of this work.

ADDENDA.

After the completion of this study the paper of Sacharoff and Sachs (*Münchener med. Wochenschrift*, 1905, lii., 297) on the hæmolytic action of photodynamic substances came to my attention. I therefore tested the protected corpuscles of the rabbit by exposing them directly to sunlight in the presence of eosin. It was found that the protected corpuscles are not subject to the hæmolytic action of this florescent substance; while the corpuscles which have had the protection removed by means of salt solution are readily hæmolyzed by eosin when exposed to the sun's rays. In keeping with the other experiments upon the injurious effects of smaller and non-protective doses of venom, it was also found that venomized but unprotected corpuscles are more readily hæmolyzed by eosin in the light than the normal corpuscles.

Another fact may be added. The supervenomized corpuscles have become insusceptible to tetanolysin.

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