

bral symptoms, puncture showed the diplococcus; the serum was used with almost immediate improvement. In this case we had difficulty in getting out enough fluid. We used 15 c.c., and at one time 30 c.c., without any trouble. The spinal fluid cleared up and no diplococci could be found, but the pneumonia continued and the patient was in the hospital for three weeks with pneumonia, without recurrence of spinal symptoms. In most of the cases the temperature rapidly dropped after treatment.

Dr. WILLIAM M. WELCH, Philadelphia: I have seen Flexner's serum used in a large number of cases of cerebrospinal meningitis in our Municipal Hospital and I regret very much that I have no exact data at hand to show the results. The members of the hospital staff feel sure that the death rate has been considerably reduced by the serum treatment. We have found that it is more useful in the true form of the disease, in which the *Diplococcus intracellularis* is present. But I rise more particularly to speak of an unfortunate result in one instance. The patient was a negro, who was admitted to the hospital with well-marked symptoms of cerebrospinal meningitis. Lumbar puncture was done, but no fluid flowed out. The direction of the needle was changed once or twice, but still no fluid was obtained. It was determined, however, to inject the serum. The patient was sitting in the upright position with his arms and chest resting on the foot of the bed; 15 c.c. were injected without any symptoms, but before the entire contents of the second bottle of 15 c.c. had been used the man complained of severe pain in his head; his head dropped forward and he became unconscious. The respirations were very slow, not more than eight or ten a minute. The breathing was stertorous. The patient never regained consciousness, and died within fifteen or twenty minutes. I should conclude from this experience that it is dangerous to introduce into the spinal canal any considerable amount of the serum without first withdrawing a similar amount of the spinal fluid.

Dr. GEORGE D. SCOTT, New York: It is important to lay particular stress on the fact that the *Diplococcus intracellularis* should be found before the employment of the serum. In the epidemic in New York City some time ago I saw a number of cases in the hospital and in private practice and it was a question often of an absolute diagnosis from lumbar puncture alone. In many cases a wrong diagnosis had been made, sometimes due to the fact that the bacteriologist was not so well informed as he might have been. I find that it is better to inject a small amount of serum more often and not to allow too much of the spinal fluid to escape at each injection. Bearing those points in mind, we can get more benefit from the serum as Dr. Flexner has stated.

Dr. F. S. CHURCHILL: I have had the same experience of injecting more serum than we have removed fluid. I would emphasize what Dr. Scott says that it is of the utmost importance to remember that the serum is a specific. I think I must have been misunderstood by one of the gentlemen. I did not say that the serum was of more value in the epidemic form. It is of no value whatever in any other form of the disease. It is a specific for epidemic cerebrospinal meningitis and that fact must be borne in mind. In regard to the unfortunate termination of the case reported from Philadelphia I do not know whether the fact that the patient was sitting up had anything to do with it, but I never have given it to a patient sitting up. In one case of a baby, two years old, where I had employed two successive doses, I used an anesthetic at the third injection. It was a mild case and the baby was pretty thoroughly conscious. I removed the fluid, injected the serum, withdrew my needle and the child seemed all right. But suddenly the breathing stopped and, though we worked with her nearly an hour, she died. In that case the fluid was almost sterile at the last examination. I think I should use gas another time instead of any other anesthetic. We must remember that the serum must be given as early as possible, it must be given in the spinal canal and it must be repeated until the organisms are not found in the spinal fluid.

Practice of Medicine.—The everyday internist can and must be able to practice medicine scientifically. The most important aid to this end is a clinical laboratory properly equipped.—O. I. Halbert, in *Texas State Journal of Medicine*.

HEMOLYSIS OF HUMAN AND RABBIT ERYTHROCYTES BY CROTALUS VENOM *

JOSEPH McFARLAND, M.D., AND PAUL G. WESTON, M.D.
PHILADELPHIA

The first studies of the hemolytic action of serpents' venoms seem to have been made by Stevens and Myers,¹ who found that when cobra venom was added to shed blood, *in vitro*, destruction of the corpuscles (hemolysis) and retardation of coagulation took place. They also found the hemolytic power of the venom inhibited by antivenomous serum.

Myers² later studied the hemolytic substance—"cobralysine"—of the venom and found it destroyed by heat. During these studies he found that the susceptibility of the erythrocytes to cobralysine *in vitro* bore no relation to the susceptibility of the animal to subcutaneous intoxication by venom.

Stephens³ found that the hemolytic constituents of different venoms were not identical.

Myers⁴ made further studies of venom hemolysins and serum antihemolysins.

Mitchell and Flexner⁵ and Flexner and Noguchi⁶ have further studied the phenomena of agglutination, hemolysis, leucolysis, and the germicidal activity of the blood serum. They found that the phenomena of agglutination appeared rapidly in favorable solutions, while in weak solutions a delay of some minutes up to an hour might be noted. Active agglutination took place in 0.2 per cent. solutions, while weaker ones either produce no change at all or show imperfect fusion. They studied corpuscles of the dog, rabbit, guinea-pig, sheep, ox, pig, necturus and frog. The phenomena of hemolysis were found to be complicated. Solutions of 0.2 per cent. were found most favorable for bringing out the hemolytic property, though the different venoms differed considerably, cobra venom being most active, water moccasin, copperhead and rattlesnake venoms less so in the order named. They also found that the corpuscles of different animals differed in their susceptibility to the action of the venom, those of the dog's blood being most easily affected, those of ox blood least so. The intermediate animals in order of susceptibility were sheep, guinea-pig, pig and rabbit. Solutions of 5 per cent. were needed to hemolyze the corpuscles of the ox. Heating to from 75 to 80 degrees C. for 30 minutes does not diminish the hemolytic action of any venom; heating to from 90 to 96 degrees diminishes the activity of rattlesnake venom; heating to 100 degrees C. for fifteen minutes diminishes the hemolytic activity of cobra, moccasin and copperhead venom a little. They believe that venoms contain various intermediate bodies, not all of these being absorbed by any one kind of corpuscles. Venom leucolysis was carefully studied and it was found that venoms contain principles that are agglutinating and dissolving for the white corpuscles of which the agglutinating principle may be identical with those for the erythrocytes. The leucolytic principle is distinct from the hemolytic principle. The several varieties of white cells show different susceptibilities to the action of the venom.

* Read in the Section on Pathology and Physiology of the American Medical Association, at the Sixtieth Annual Session, held at Atlantic City, June, 1909.

1. Brit. Med. Jour., Mar. 5, 1898, p. 621.
2. Jour. of Path. and Bact., 1899-1900, p. 415.
3. Jour. of Path. and Bact., 1899-1900, p. 273.
4. Trans. of the Path. Soc., London, Feb. 6, 1900.
5. National Academy of Sciences, 1901.
6. Jour. Exper. Med., March, 1902, vi, 3, p. 277.

Flexner and Noguchi⁷ found that venoms have a cytolytic action on many kinds of cells, probably because of a pleurality of the amboceptors they contain.

Kyes⁸ finds that the hemolysis by venom bears a definite relation to the lecithin content of the blood, by which the venom amboceptor is activated.

Noguchi⁹ found that the hemolytic principle of venom extended over a wider group of animals than the agglutinative principle, its activity on the bloods of different animals diminishing the more widely the vertebrata were departed from. Cobra venom contains the largest, crotoalus venom the smallest number of hemolytic units, while moccasin venom contains the greatest number of agglutinative units for such bloods. The mechanism of hemolysis is identical with that for the bloods of warm-blooded animals; complements are, therefore, present in all vertebrates and in many, at least, of the invertebrates. The heat liability of the venom agglutinins and hemolysins for cold-blooded animals agrees closely with that for warm-blooded animals.

The hemolytic activity of the venoms of the Indian and Australian snakes, and the effects of the antivenomous serums, have been made the subjects of several papers by Lamb.¹⁰

Throughout the writings to which reference has been made either no mention, or very brief mention is made of the effect of the venoms, and particularly of the rattlesnake venom on human corpuscles. As the rattlesnake is the most common venomous snake of this country, it seemed to us that the effects of its venom on the blood of its most important, though fortunately infrequent victim, man, ought to be investigated, and we set to work accordingly. As in the course of our studies certain facts were brought out confirming or refuting the work of those that preceded us, we feel justified in publishing what we found.

In a previous paper¹⁰ we reported studies made about the same time on the agglutinating effect of crotoalus venom on human and rabbit erythrocytes.

The venom used in both series of experiments was obtained for us from the *Crotalus adamanteus* through a special collector in Texas. According to the directions given him, the freshly collected venom was spread out in a thin layer on the surface of clean dinner-plates and dried in the sun. In this way a considerable quantity of excellent dry venom was secured. When the venom was to be employed for making the hemolytic tests, the dry product was finely pulverized, dissolved in the least possible quantity of 0.85 per cent. sodium chlorid solution, filtered through fine filter paper and the filtrate evaporated. The resulting scales were again pulverized, placed in a sterile test tube, covered with chloroform and stood in the thermostat until the chloroform had evaporated through the cotton plug with which the tube was stoppered. Experiments showed that the venom so treated was unmodified as regards hemolytic or agglutinative activity. It was from the powder so prepared that the solutions for hemolysis were made.

The blood was secured from the tip of a thoroughly cleansed finger, the same blood (W.'s) being used in all the tests. By making a deep puncture with a somewhat broad lancet, from one to three cubic centimeters could be secured. The blood was caught in a sterile test tube, immediately defibrinated, then centrifugalized. The

serum was removed with a pipette to be used subsequently as serum complement, the corpuscles washed three times in sterile salt solution and finally made into a 5 per cent. suspension in 0.85 per cent. sodium chlorid solution.

In making the hemolytic tests, 1 c.c. of the corpuscular suspension was placed in each of a series of tubes suitable for use in the centrifuge, 0.5 c.c. of a mixture of one part of normal human serum (that removed with the pipette from the centrifuge tube as mentioned above) and two parts sterile saline solution and varying quantities of venom added. The mixture was shaken gently and enough saline solution added to bring up the volume to 2 c.c. The tubes were then stood in the thermostat at 37 degrees C. for one hour, when the first observation was made. They were then again gently shaken and placed in the icebox over night, after which the final observations were made by whirling the tubes for 5 minutes in the centrifuge and then examining the clear fluid for variations in the color tint.

In the tabulations we express the occurrence of hemolysis by the letter H, doubling or trebling the letters to express the degree. "H?" means doubtful hemolysis or a very faint trace. "HC" means complete hemolysis. We also exhibit the associated agglutination by the use of the letter A. The amount and character of the sediment is, in general, perhaps, a better index to the amount of hemolysis. In these experiments the sediments in tubes marked H were all the same, those in tubes marked HH were the same but different than those in tubes H or HHH, so that, whether the tints or sediments were used as indices, the results would be the same.

The quantity of venom has been expressed by some of those who experimented with the hemolytic reactions in the form of percentages, but, as these vary according to the final dilution of the mixture, and as after some experiments we were obliged to increase from a 2 c.c. to a 10 c.c. mixture, we believe it to be more accurate to express the quantity of venom in the form of grams of the dry venom in the final mixture.

TABLE 1.—TWO CUBIC CENTIMETER DILUTIONS

Grams dry venom of <i>Crotalus adamanteus</i> in total quantity of final mixture.	%		Normal human erythrocytes from defibrinated blood susp. in 0.85% NaCl sol., (5% susp.)	Normal human serum diluted with 2 vols. normal NaCl solution.	Results.
0.001 (.05)	plus	1 c.c.	plus	0.5 c.c. equals	A plus H
0.005 (.25)	plus	1 c.c.	plus	0.5 c.c. equals	A plus H
0.01 (.5)	plus	1 c.c.	plus	0.5 c.c. equals	A plus H
0.0125 (.625)	plus	1 c.c.	plus	0.5 c.c. equals	A plus HHH
0.03 (1.5)	plus	1 c.c.	plus	0.5 c.c. equals	A plus HHH
0.05 (2.5)	plus	1 c.c.	plus	0.5 c.c. equals	A plus HHHH
0.06 (3)	plus	1 c.c.	plus	0.5 c.c. equals	A plus HHHH
0.2 (10)	plus	1 c.c.	plus	0.5 c.c. equals	A plus H

TABLE 2.—TWO CUBIC CENTIMETER DILUTIONS

0.01 (5)	plus	1 c.c.	plus	1.0 c.c. equals	A plus O
0.04 (2)	plus	1 c.c.	plus	1.0 c.c. equals	A plus H

Table 1 shows that increasing additions of venom result in increasing hemolysis until a certain point is reached. Beyond this point added venom diminishes the hemolytic activity, presumably because of excess of amboceptor units in the venom. Table 2 shows that the same disturbance results from excess of complementary units; when 1 c.c. of the serum dilution, instead of the 0.5 c.c. employed in Table 1, is used, hemolysis is greatly diminished. Thus in Table 1, 0.01, 0.03 and 0.05 grams of venom produce marked hemolysis, but in Table 2, 0.01 causes no hemolysis and 0.04 very slight hemolysis.

Whether these results depend on the Neisser-Wechsberg phenomenon seems to us to be problematical.

7. Univ. Penn. Bull., July-August, 1903.

8. Berl. klin. Wochenschr., 1903, xli, 21, 57, 82.

9. Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government in India, No. 16, 1904, and No. 17, 1905.

10. Read at the meeting of the American Association of Pathologists and Bacteriologists, Boston, April 9-10, 1909.

We soon found it impracticable to continue to work with 2 c.c. mixtures, because of the difficulty of dissolving the necessary quantity of venom in the small quantity of fluid used. We were, therefore, obliged to change to a 10 c.c. mixture. In this mixture we deal with the same number of corpuscles, the same quantity of serum and the same quantity of venom, though, of course, the percentage of venom is changed by the dilution. This made us abandon the percentage expression and make use of figures indicative of the actual weight of dry venom added to the mixture.

Table 3 shows the details of experiments made with the 10 c.c. mixture for comparison with those in Table 1 in which 2 c.c. mixtures were employed. It will be seen that the results are the same. This was to be expected, as the proportions were the same. The percentages of venom are, however, entirely different.

TABLE 3.—TEN CUBIC CENTIMETER DILUTIONS

Grams of dry venom of <i>Crotalus adamanteus</i> in total quantity of final mixture.			5% susp. of normal human erythrocytes from defibrinated blood in 0.85% NaCl solution.	Normal human serum diluted with 2 vols. normal NaCl solution.	Results.
0.01	(0.1)	plus	1 c.c.	plus 0.5 c.c.	equals A plus H
0.03	(0.3)	plus	1 c.c.	plus 0.5 c.c.	equals A plus III
0.05	(0.5)	plus	1 c.c.	plus 0.5 c.c.	equals A plus IIIH
0.07	(0.7)	plus	1 c.c.	plus 0.5 c.c.	equals A plus IIIHH

TABLE 4.—TEN CUBIC CENTIMETER DILUTIONS

Corpuscles from citrated blood.			Citrated plasma.		
0.01	plus	1 c.c.	plus	0.5 c.c.	equals A plus II
0.03	plus	1 c.c.	plus	0.5 c.c.	A plus II
0.05	plus	1 c.c.	plus	0.5 c.c.	equals A plus IIIH
0.07	plus	1 c.c.	plus	0.5 c.c.	A plus IIIH

In our paper on the agglutination of the corpuscles, we have shown that it makes considerable difference in the rapidity with which the reaction takes place whether defibrinated or citrated blood is used to furnish the corpuscles.

In investigating this problem with reference to hemolysis we used the following technic:

The blood from the finger tip was permitted to flow into a sterile tube containing sterile sodium citrate solution (2 per cent. in 0.85 per cent. sodium chlorid solution). The fluids were well mixed, then centrifuged, the supernatant fluid removed with a pipette to be used in lieu of the serum ordinarily employed and the corpuscles washed three times as usual, then made into a 5 per cent. suspension.

One c.c. of the suspension was placed in each of a series of tubes, varying quantities of venom were added, and to each was added a quantity of the citrated plasma shown by calculation to be the equivalent of the 0.5 c.c. of the serum mixture ordinarily employed. Thus the conditions, after dilution to 10 c.c., were identical with the other experiments except that the corpuscles had been acted on by the sodium citrate and a small quantity of sodium citrate remained mixed with the plasma. The experiment in Table 4 shows that the hemolysis is slightly less when the citrated corpuscles are employed for the test. Thus the effect of the citrate is to diminish hemolysis and to retard agglutination, using defibrinated blood as a standard.

In order to compare our results with some known quantity, we next made a series of tests in which rabbits' corpuscles instead of human corpuscles were employed.

Table 5 shows the hemolytic effect of the *crotalus* venom on rabbit erythrocytes from defibrinated and citrated blood. As in the human blood, there is a dimi-

nution in the amount of hemolysis when the citrate is employed. It will also be observed that the defibrinated rabbit corpuscles are about ten times as sensitive to the hemolytic action of the venom as are the citrated corpuscles.

TABLE 5.—TEN CUBIC CENTIMETER DILUTIONS: RABBIT

Grams of dry venom of <i>Crotalus adamanteus</i> in total quantity of mixture.			5% susp. of normal erythrocytes from defibrinated blood in 0.85% NaCl solution.	Normal rabbit serum diluted with 2 vols. of NaCl solution.	Results.
0.0000125	plus	1 c.c.	plus 0.5 c.c.	equals	O plus II
0.000125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus II
0.00125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus III
0.0125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus IIIH
0.125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus IIIHH

			RABBIT: 5% susp. of normal erythrocytes from citrated blood in 0.85% NaCl sol.		
0.0000125	plus	1 c.c.	plus 0.5 c.c.	equals	O plus O
0.000125	plus	1 c.c.	plus 0.5 c.c.	equals	O plus O
0.00125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus H
0.0125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus HH
0.125	plus	1 c.c.	plus 0.5 c.c.	equals	A plus IIIH

Flexner and Noguchi found that when venom was heated to from 75 to 80 degrees C. the hemolytic properties were not affected. As no mention was made of the state in which the venom was heated, we decided to try whether the fact was true both of pulverized dry venom and of venom in solution.

To test the effect of heating it in the dry state, a small quantity was spread on the bottom of a small Erlenmeyer flask and floated on a water bath which was kept for 30 minutes between 75 and 80 degrees C. The powder was then dissolved and hemolytic tests made on both human and rabbit corpuscles. The results of the experiments are shown in Tables 6 and 7. When Table 6 is compared with Table 5, it becomes evident

TABLE 6.—VENOM POWDER HEATED DRY FOR 30 MINUTES AT 75° TO 80° C.

Grams of dry venom of <i>Crotalus adamanteus</i> in total quantity of final mixture.			5% susp. of rabbits' erythrocytes from defibrinated blood in 0.85% NaCl solution.	Normal rabbit serum diluted with 2 vols. 0.85% NaCl solution.	Results.
0.002	plus	1 c.c.	plus 0.5 c.c.	equals	A plus II(?)
0.01	plus	1 c.c.	plus 0.5 c.c.	equals	A plus H
0.03	plus	1 c.c.	plus 0.5 c.c.	equals	A plus III

TABLE 7.—VENOM POWDER HEATED DRY FOR 30 MINUTES AT 75° TO 80° C.

			5% susp. of normal human erythrocytes from defibrinated blood in 0.85% NaCl solution.		
0.002	plus	1 c.c.	plus 0.5 c.c.	equals	O plus II(?)
0.01	plus	1 c.c.	plus 0.5 c.c.	equals	O plus II (slight)
0.03	plus	1 c.c.	plus 0.5 c.c.	equals	A plus H (slight)

that heating the venom, even in the dry state, produces a marked diminution of the hemolytic activity, for in Table 5 hemolysis is distinct in 10 c.c. mixtures when only 0.0000125 grams of venom are added, though in Table 6 hemolysis begins only after 0.002 grams of the heated venom have been added. The same result was obtained with both defibrinated and citrated serums.

When human corpuscles were employed the effect of heating the venom was much less marked. Thus when Table 3 is compared with Table 7 it will be seen that the unheated venom produces hemolysis when quantities of 0.01 gram and upward are added, and becomes marked when 0.03 grams are reached, though when the

heated venom is added the hemolysis effected by 0.01 is very slight and with 0.03 is still very slight. Experiments made with citrated blood showed the same result.

It thus becomes apparent that there is a difference in the hemolytic activity of the heated and unheated venoms for rabbit and human corpuscles.

This led us to conjecture that the hemolytic factors for these different corpuscles might be different, but this we were unable to prove.

When the venom was first dissolved and then heated, the heating being accomplished in the same manner, by placing the solution in a small Erlenmeyer flask and floating it on a water bath kept for 30 minutes at between 75 and 80 degrees C., a much more pronounced destruction of the hemolytic power was brought about.

The results are shown in Table 8. When human corpuscles are exposed to unheated venom solutions, as shown in Table 1, hemolysis begins when 0.001 grams are added: when the venom solution is heated, when 0.002 grams are added. If, however, rabbit corpuscles are used, the results are entirely different, for when the unheated solution is employed, hemolysis begins (Table 5) at 0.0000125 grams, but when the solution is heated at 0.002 grams. Practically the same diminution of hemolysis takes place whether defibrinated or citrated blood is used.

TABLE 8.—VENOM SOLUTIONS HEATED FOR ONE-HALF HOUR AT 75° TO 80° C.

Grams of dry venom of <i>Crotalus adamanteus</i> in total quantity of final mixture.		5% susp. of normal human erythrocytes in 0.85% NaCl solution.	Normal human serum diluted with 2 vols. of saline solution.	Results.
0.00001	plus	1 c.c. plus	0.5 c.c. equals	O plus O
0.0005	plus	1 c.c. plus	0.5 c.c. equals	O plus O
0.001	plus	1 c.c. plus	0.5 c.c. equals	O plus O
0.002	plus	1 c.c. plus	0.5 c.c. equals	O plus H (?)
5% susp. of normal rabbits' corpuscles from defibrinated blood in 0.85% NaCl solution.				
0.0002	plus	1 c.c. plus	0.5 c.c. equals	O plus H?
0.0005	plus	1 c.c. plus	0.5 c.c. equals	O plus H?
0.001	plus	1 c.c. plus	0.5 c.c. equals	O plus H?
0.0015	plus	1 c.c. plus	0.5 c.c. equals	O plus H

We observed an interesting change in the hemoglobin of the corpuscles not hemolyzed.

If the corpuscles from a tube containing venom and serum in quantities too small to effect hemolysis be collected by centrifugalization and suspended in fresh 0.85 per cent. sodium chlorid solution, the corpuscles remain unchanged. If again collected and again suspended in fresh saline solution, a change may or may not be seen; but if the operation be repeated a third time the corpuscles undergo immediate hemolysis in the salt solution.

In the course of some immunization experiments that turned out badly, we prepared a venom solution by dissolving the dry venom in 100 parts of 0.85 per cent. sodium chlorid solution and then saturating it with chloretone. This prevented bacterial contamination and the toxic properties of the venom were preserved for considerable time. On using this solution for a hemolytic test, we found complete hemolysis in 30 minutes when it was not expected. We then tried a saturated solution of chloretone in the normal salt solution, and found that the antiseptic itself caused rapid and complete hemolysis when in saturated solution. We were thus led to abandon the use of chloretone for preserving the venom solutions.

As human blood in quantities sufficient to furnish enough corpuscles for making hemolytic tests is not

easily obtained, we were interested to determine how long the corpuscles, once suspended in the saline solution, might be available for use. Suspensions (5 per cent.) of both human and rabbit corpuscles were, therefore, prepared and kept in the icebox, being subjected to hemolytic tests from time to time. We found that during 24 hours there was very little change, but that after 36 hours hemolysis took place much more rapidly than before.

From this we came to the conclusion that the corpuscular suspensions should be prepared every day and not kept over, and the experiments we report were always made on freshly secured blood.

In order to study the effects of crotalus venom on the leucocytes, we made use of the sediments collected by centrifugating the tubes in which hemolysis had taken place. This was spread on slides and stained by the Romanowsky method. All these observations were, therefore, made on corpuscles that had been in contact with the venom in the icebox for 24 hours, and do not form very satisfactory data.

We found that the small lymphocytes stained well and appeared quite normal even in tubes to which as much as 0.07 grams of venom had been added.

Large lymphocytes were apparently unaffected by quantities of venom less than 0.03 grams per 10 c.c. of fluid. They were not found in tubes containing 0.03 grams and upward.

The polymorphonuclear neutrophils showed the most marked changes, and are, therefore, probably the most susceptible to the cytolytic action of the venom. Even small quantities of the venom produced marked karyolysis with a hyaline change of the cytoplasm. This is an interesting observation which may have some bearing on the invasion of the tissues by bacteria which so quickly takes place after venom injection.

The eosinophiles seemed to resist the venom very well. In no case, even up to the addition of 0.07 grams to the 10 c.c. mixture, were they completely destroyed, though the nuclei refused to stain. The granules were never destroyed. No basophilic cells were found in any of the sediments.

CONCLUSIONS

1. *Crotalus* venom is capable of hemolyzing red corpuscles of both man and the rabbit in the presence of serum or of citrated plasma.
2. When an excess of the venom, or an excess of the serum is present, hemolysis diminishes.
3. In the properly proportioned mixtures, hemolysis takes place regardless of the extent to which the mixtures may be diluted by the addition of salt solution.
4. Defibrinated blood furnishes corpuscles more sensitive to the venom than citrated blood.
5. Heating dry venom diminishes its hemolytic activity for human corpuscles a little, for rabbit corpuscles a great deal.
6. Heating solutions of venom diminishes its activity for human corpuscles a little, for rabbit corpuscles a great deal.
7. Rabbit corpuscles are much more susceptible to the hemolytic effect of the venom than human corpuscles.
8. Corpuscles in suspension should not be kept from day to day for venom experiments.
9. The small lymphocytes and eosinophiles resist the venom better than the large lymphocytes and polymorphonuclear neutrophils.

ABSTRACT OF DISCUSSION

DR. D. H. BERGEY, Philadelphia: Presumably, from the manner in which the experiments have been conducted, the hemolysis brought about by the venom is due to the presence in it of an amboceptor; and the addition of serum to the mixture of venom and blood supplies the complement. How do the authors of the paper explain the disappearance of hemolytic power, since we know that amboceptors are supposed to be thermostable?

DR. E. C. L. MILLER, Detroit: Is there any especial explanation for the fact that venom acts in proportion to the absolute quantity present and not in proportion to the dilution, as it forms, in this respect, an exception to the ordinary rule?

DR. JOSEPH MCFARLAND, Philadelphia: When we found that it was necessary to change from a mixture containing only 2 c.c. to one containing 10 c.c., the question of the relation of proportion to quantity arose, and we discovered that, as shown by a comparison of tables, the increase in quantity had no effect at all on the action of the venom. Then I suggested that it would be interesting to try still higher dilutions, which we did; and we found no change in the behavior of mixtures of 250 c.c., the largest amount used, hemolysis taking place in this the same as in smaller quantities. I cannot answer Dr. Bergey's question quite so positively. Just what the loss of hemolytic power in the heated venom indicates, I do not know. It might indicate that the amboceptors were in a different class from the ordinary bacteriolytic or hemolytic amboceptors. I scarcely think, however, that this would be the case. I believe that it is true that heating disturbs all amboceptors somewhat and that the virulence is diminished in proportion to the amount of heat and the length of time in which the heating takes place.

DR. D. H. BERGEY: I believe that this is true, but do not think that it is true to the extent that it has been found in your experiments.

DR. MCFARLAND: The disturbance affected about half its virulence in this case.

SOME POINTS OF CONTACT BETWEEN NEUROLOGY AND ORTHOPEDIC SURGERY *

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It is the purpose of this paper to call attention to a few of the more recently understood conditions which produce symptoms for which either the neurologist or the orthopedic surgeon may be consulted and where frequently the methods peculiar to both specialties are of advantage in bringing about relief. It is not proposed to discuss the many problems in which the two specialties have been for long correlated, such as the treatment of the infantile or spastic paralyses, as there is little new to add to such subjects, even if the time were sufficient, and in those which are considered the time is so short that only a brief mention of them is possible. It is the hope, however, that the statements here made may serve as suggestions which will lead to a more thorough investigation of the conditions so that an even better understanding of their various phases may be obtained. The honor of being permitted to speak before such a body as this is fully appreciated, and it is my earnest hope that that which is presented may be of sufficient value to justify the amount of time allotted.

SPINAL LESIONS

In a considerable number of lesions of the spine, other than tuberculous, the inflammatory swelling or the osseous thickening which is a part of such diseases

frequently causes pressure on or irritation of the nerve roots and results in symptoms referred to the portion of the body that the irritated nerve supplies. These symptoms may consist of pain, at other times areas of hyperesthesia or anesthesia, or even at other times true paralysis, the exact symptoms depending on the special nerve involved and the amount of pressure or irritation present.

Such a condition may result from the involvement of any region of the spine, but naturally in the regions of the greatest mobility, the cervical and lumbar, they will be most often met. The symptoms present in the given case must, therefore, depend very largely on the region of the spine involved. In the cervical region the symptoms will naturally be referred to the neck or arms or to the areas supplied by the cervical or brachial plexuses. In the dorsal region, aside from the symptoms present at the seat of the disease, the pain referred along the course of the intercostal nerves is the most common. In the lumbar region the referred pain may show in any part supplied by the lumbar and sacral plexuses, the low back or abdomen, the pelvis or perineum, or any part of the legs.

Whenever such spinal conditions exist the referred symptoms, those which most often lead to the consultation of the neurologist, are almost invariably either entirely on one side or at least much more marked on one side than on the other. With the dorsal or lumbar spinal lesions the unilateral manifestation is almost always the case, but in the cervical region, while one side is always worse, nevertheless both sides are usually somewhat involved.

The symptoms are always, if the disease is at all acute, aggravated by sudden movement of the affected part, such as in coughing or sneezing, slipping or any unguarded motion. With lesions in the lumbar or in the cervical spine the pain is commonly worse at night because of the opportunities offered for sag of these portions of the spine with the relaxation of the muscles, such as takes place during sleep.

Of the special diseases which may cause symptoms of this sort it is not possible in so short a time to do more than briefly to mention them, and the reader must refer to the various articles published elsewhere for further details. The most common, however, are the chronic infectious arthritis and the hypertrophic arthritis, but it should be remembered that any disease in which there is inflammatory swelling or thickening about the vertebral articulations may result not only in symptoms referred directly to the seat of the disease, but also to symptoms referred to other parts, owing to the pressure on or irritation of the nerve roots. With the two most common conditions, the infectious arthritis is the more acute, and the local symptoms are the most pronounced. There is more local swelling, which in the cervical spine can often be felt, and the muscular spasm causing the limitation of the motion is much more marked than with the hypertrophic arthritis. The referred pains are more sudden in their development and more acute, both in regard to the intensity of the pain as well as the duration. The recovery in such a type of disease should be correspondingly rapid and is usually complete.

With the hypertrophic arthritis the process is slow in its onset and there may be an entire absence of subjective symptoms at the seat of the disease. The referred symptoms have so little else to show for them that the real nature of the trouble is apt to be overlooked unless the examination be most thorough. In this type not only is the onset slow, but the recovery is correspond-

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