# A SPREADING FACTOR IN CERTAIN SNAKE VENOMS AND ITS RELATION TO THEIR MODE OF ACTION

By F. DURAN-REYNALS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

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The present investigation was undertaken to determine whether the venom of poisonous snakes contained a factor capable of increasing tissue and blood capillary permeability. A preliminary note on this subject has been published (1).

Substances which effect an increase in connective tissue and capillary permeability have been extracted from a number of biological sources. In normal mammalian tissues, the testis is particularly rich in this spreading factor (2). It has also been demonstrated in malignant tissues (3), in leech extracts (4), and in certain bacteria (5). The elaboration of such substances by certain species of bacteria is correlated with their ability to spread through or invade tissues, or to become rapidly disseminated *via* the blood stream. Thus the association of such spreading factors with noxious agents of one type or another tends to alter considerably their spread through the tissues, and eventually through the body as a whole. So doing, it may in the one instance result in an enhancement of a reaction, and in another act to diminish it, according to the factors involved amongst which are the nature of the spreading agent, and the native ability of the host to deal with it (6).

It is obvious that the presence of a spreading factor in snake venom would have an effect upon the sequence of events following introduction of it. In the present studies we have investigated the spreading factor content of the venom from several species of venomous snakes, of extracts of the supralabial glands of two species of harmless snakes, and of toad venom. In addition experiments are described which were aimed to differentiate the spreading factor from the toxic factor in snake venoms.

## *Materials and Methods*

*The Snake Venoms.*—Venom from 9 species of snakes was employed. In some instances fresh venom was available, while in others the dried preparations, either

purchased in the open market or obtained from foreign countries, was used.[1] The family and species of the snakes, and the original state of the venom, are indicated in Table I, together with the dilutions prepared for animal injection. Extracts of the supralabial glands from 2 species of harmless snakes were prepared, using 1 gm. of fresh gland and 9 cc. of saline, with subsequent dilution. The dried venoms were dissolved in distilled water to restore the original volume of the fresh secretion. The amount of water to be added was determined by the figures published by Noguchi (7). Subsequent dilutions were made with the addition of 0.9 per cent NaCl solution. When fresh venom was used it was diluted with 0.9 per cent NaCl solution.

*Toad Venom.*—Venom from 6 species of toads was used. These preparations represented the dried secretions of the skin glands. The names of the species will be found in the corresponding section. For use in these experiments, the dry venom was ground in a mortar with 0.9 per cent NaCl, and finally made up in the proportion of 1.0 gm. of dry venom to 49 cc. of saline solution. This suspension was centrifuged and the supernatant fluid used for injection.

*Infectious Agents.*—For testing the effect of the venoms on infections, vaccine virus, *Staphylococcus aureus*, and the bacillus of mouse typhoid II were used. The Levaditi strain of neuro-virus was secured from an infected rabbit testicle and prepared by the following method. 5 days after an intratesticular inoculation of neuro-virus suspension the animals were sacrificed, the testes removed and ground with sterile sand in 0.9 per cent saline (1 gm. testes to 9 cc. saline). After centrifugation, the supernatant fluid was diluted in various proportions for the test inoculations, as shown in Table III. The bacterial suspensions were the product of 24 hours growth on plain agar shaken up with 10 cc. of saline. Dilutions of the freshly prepared virus or bacterial suspensions provided the graded series of doses used in the tests.

*Testing of the Venoms.*—0.5 cc. of each of the venom dilutions was mixed with 0.25 cc. of diluted India ink (1:2) and injected into one flank of a rabbit. On the opposite side, a similar series was injected, into the corresponding locations, but using 0.25 cc. of saline solution in place of the ink. This was done so as to be able to judge the extent of the spread of the injected material by aid of the India ink on one side, and the severity of the lesion produced by the different dilutions of venom uncomplicated by the presence of the ink on the other side.[2] In addition

[1] We are indebted to Dr. C. H. Kellaway of the Albert and Eliza Hall Institute, Melbourne, Australia, and Dr. C. Picado of the San Juan Hospital, San José, Costa Rica, for supplying several of the dried venoms. The toad venoms were kindly supplied by Dr. G. H. A. Clowes and Dr. K. K. Chen of the Lilly Research Laboratories.

[2] This proved to be a wise precaution for it was noted that the lesions produced by the ink mixtures were smaller and less severe than those produced by the same dilutions of the venom without the ink. Probably some of the spreading factor is absorbed by the carbon particles, as demonstrated in other instances by Favilli and McClean (8).

a control injection was made consisting of 0.5 cc. of saline plus 0.25 cc. of ink. As a rule no more than 5 dilutions of venom were tested on the same animal.

The area of epidermis beneath which the ink particles had spread was measured at the end of 24 hours, and recorded in square centimeters. The character of the lesions produced was graded from extremely pronounced through intermediate stages to very mild. The extremely pronounced lesions were those which were very hemorrhagic, edematous, and necrotic. The very mild lesions were those which showed only erythema, which disappeared in 24 to 48 hours. The intermediate classifications were represented as gradations between these extremes. Essentially the same technique and nomenclature were used when testing the effect of the addition of venoms to bacterial and viral suspensions, injected intradermally.

### *The Relationship between the Spreading Factor and the Local and General Toxicity of Snake Venom*

In a preliminary survey we studied the effect on the rabbit of the fresh or dry venoms of 9 species of poisonous snakes as compared with the effect of extracts of supralabial glands of 2 species of harmless snakes.

*Experiment.*—Progressive saline dilutions of the venom or of the supralabial gland extract were injected intradermally in one side of rabbits in the amount of 0.5 cc. mixed with 0.25 cc. of India ink so as to be able to judge the extent of spreading. The same injections were repeated in the other side using saline solution instead of India ink. Each venom was tried on a separate rabbit. Details and results of the tests are given in Table I.

It is apparent from Table I that all the snake poisons have a more or less marked spreading power, and that extracts from the supralabial glands of harmless snakes do not. The bleb formed by the injection of the venoms flattens out immediately and the injected material spreads through the skin, causing a hemorrhagic and edematous lesion. It is also seen that the lesions produced by venoms from the Viperidae are larger and more severe than those produced by the venoms of the Colubridae family. The fact that 6 out of 10 of the rabbits injected with venoms of the latter group died within 15 hours, whereas none of those injected with the venoms of the Viperidae died, emphasizes the distinction between the locus of action of these two groups of venoms, in agreement with the early work of Flexner and of Noguchi (7) who found that venoms from the Viperidae (rattlesnakes) contain large amounts of locally acting toxins, and compara-

## TABLE I

*Spreading Power and Local and General Toxic Power of the Venoms and Supralabial Gland Extracts of Various Snakes*
*(Results after 24 Hours)*

| Type of snake | State of secretion and snake species | Area of spread and severity of lesion in rabbit skin injected with 0.50 cc. venom or extract plus 0.25 cc. India ink or saline | | | | | Spreading of 0.50 cc. saline plus 0.25 cc. India ink dilution (control) | Number of rabbits injected | Number of rabbits killed by venom |
|---|---|---|---|---|---|---|---|---|---|
| | | Dilutions of snake venom | | | | | | | |
| | | 1:100 | 1:1000 | 1:10,000 | 1:100,100 | 1:1,000,000 | | | |
| *Poisonous* | | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | | |
| Viperidae crotalinae | Dry *Crotalus adamanteus* venom (diamond rattlesnake) | 95.0 Very pronounced | 35.0 Mod. pronounced | 19.6 Mod. pronounced | 12.8 Mild | 7.6 Very mild | 6.3 | 5 | 0 |
| | Dry *Crotalus terrificus durissus* venom (rattlesnake) | 72.0 Very pronounced | 30.0 Pronounced | 18.2 Mod. pronounced | 8.1 Mild | 6.0 Very mild | 5.0 | 1 | 0 |
| | Fresh *Crotalus atrox* venom (western rattlesnake) | 70.0 Ext. pronounced | 30.0 Very pronounced | 19.9 Pronounced | 6.2 Mod. pronounced | 3.2 Mild | 2.6 | 2 | 0 |
| | Dry *Ancistrodon piscivorus* venom (water moccasin) | 69.0 Pronounced | 20.0 Mod. pronounced | 19.8 Mod. pronounced | 7.0 Very mild | 7.0 No lesion | 5.6 | 1 | 0 |
| | Fresh *Ancistrodon piscivorus* venom (water moccasin) | 50.0 Pronounced | 15.1 Mild | 8.0 Very mild | 7.0 No lesion | 7.4 No lesion | 7.6 | 1 | 0 |
| Colubridae proteroglypha | Fresh *Elaps fulvius* venom (coral snake) | 72.6 Mod. pronounced | 18.6 Mild | 12.9 Very mild | 12.4 Very mild | 8.0 Very mild | 8.4 | 1 | 1 (15 hrs.) |
| | Dry *Denisonia superba* venom (superb snake) | 46.0 Mod. pronounced | 20.0 Mild | 14.7 Very mild | 8.7 Very mild | 6.7 No lesion | 4.9 | 3 | 1 (15 hrs.) |
| | Dry *Naja tripudians* venom (cobra) | 11.5 Mod. pronounced | 10.5 Mild | 10.0 Very mild | 9.5 Very mild | 8.5 No lesion | 6.0 | 2 | 1 (2 hrs.) |
| | Dry *Acanthophis antarticus* venom (death adder) | | 14.5 Very mild | 10.9 Very mild | 7.3 No lesion | 6.1 No lesion | 7.2 | 3 | 2 (5 hrs.) |
| | Dry *Notechis scutatus* venom (black tiger) | | | Some spreading detected. No lesions produced | | | | 1 | 1 (2 min.) |
| *Harmless* | Fresh supralabial gland *Pityophis cateniferis* (pine snake) | 5.5 No lesion | 5.1 No lesion | | | | 5.1 | 1 | 0 |
| | Fresh supralabial gland *Elaphe quadrivittata* (chicken snake) | 10.0 | 5.0 | | | | 5.0 | 1 | 0 |

tively small amounts of neurotoxins, whereas the reverse is true for the Colubridae (cobras).

*Independence of the Spreading and Toxic Effect of Snake Venom*

The injection of rattlesnake venom into the skin is followed almost immediately by a remarkably acute hemorrhagic necrosis. It might be argued that such a substance, by destroying all of the physiologic barriers to the spread of fluids in the tissues, would permit a spreading of any associated fluid or particulate matter. That this is not the case is shown by the following experiment in which the toxicity of the venom was markedly reduced by heating. The spreading factor from other sources has been shown to be comparatively thermostable (9).

*Experiment.*—4 samples of rattlesnake venom diluted 1:50 were heated for 25 minutes at 55°, 65°, and 95°, and for 5 minutes at 100° respectively. After eliminating the coagulated material by centrifugation progressive dilutions up to 1:1000 were prepared from each sample and 0.50 cc. of each dilution was mixed with 0.25 cc. of India ink or 0.25 cc. of saline. The ink mixtures were injected intradermally into one side and the saline mixtures into the other side of each of 4 rabbits. The results are given in Table II. This table gives also the average of 6 tests performed with unheated venom on separate rabbits for comparison.

The experiment shows that the venom heated at 65–100° has been largely deprived of its toxicity, but still retains the capacity to increase the permeability of the dermis.

The following experiment was carried out to find whether heated venom will increase capillary permeability.

*Experiment.*—2 rabbits were each injected intradermally with 0.5 cc. of each of 3 samples of rattlesnake venom, diluted 1:100 and heated 25 minutes at 65°, 85°, and 100° respectively. 2 more rabbits were similarly injected with dilutions of the unheated venom ranging from 1:5000 to 1:100,000. As a control, 0.5 cc. of saline was also injected into each of the 4 rabbits. Immediately after the intradermal injections, each animal received an intravenous injection of 4 cc. of a 0.1 per cent solution of T. 1824.[3] The intradermally injected venom began to spread at once and the localization of the circulating dye in the affected skin began immediately. Practically no localization of the dye occurred at the site of the saline injections during this time.

[3] A poorly diffusible azo dye which remains a long time in the blood stream. Manufactured now by Eastman Kodak Company under the name of Evans blue.

At the end of 24 hours the sites injected with the venom heated at 65°, 85°, and 100° appeared as dyed areas measuring 47, 25, and 17 sq. cm. respectively. The sites of the injection of the 1:5000, 1:10,000, 1:50,000, and 1:100,000 dilutions of the unheated venom showed large blue areas measuring 72, 58, 29, and 26 sq. cm. respectively. Mild hemorrhagic lesions were observed in the areas injected with venom heated at 65°, and in those injected with the unheated venom, diluted 1:5000 and 1:10,000.

TABLE II

*Discrimination of the Spreading Factor of Snake Venom from the Local Toxic Factors as Demonstrated by Heating the Venom from a Rattlesnake (Crotalus adamanteus)*

*(Results after 24 Hours)*

| Degree and time of heating of the venom solution | Area of spread and severity of lesion in rabbit skin injected with 0.05 cc. venom + 0.25 cc. India ink or saline | | | | Area of spread of 0.5 cc. saline + 0.25 cc. India ink (control) |
|---|---|---|---|---|---|
| | Dilutions of snake venom | | | | |
| | 1:50 | 1:100 | 1:500 | 1:1000 | |
| | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| 100° for 5 min. | 17.8<br>Slight erythema | 11.5<br>Slight erythema | 9.0<br>No lesion | 11.0<br>No lesion | 2.7 |
| 85° for 25 min. | 50.0<br>Slight erythema | 44.5<br>No lesion | 9.7<br>No lesion | 7.8<br>No lesion | 6.7 |
| 65° for 25 min. | | 45.6<br>Mild | 22.5<br>Very mild | 20.0<br>Very mild | 7.8 |
| 55° for 25 min. (average of 2 tests) | | 100.0<br>Very pronounced | 45.0<br>Pronounced | 30.0<br>Mod. pronounced | 5.3 |
| Unheated (average of 6 tests) | 125.0<br>Extremely pronounced | 95.0<br>Very pronounced | 45.0<br>Pronounced | 30.0<br>Mod. pronounced | 6.3 |

The experiment shows that the venom rendered practically atoxic by heating or dilution still retains its ability to increase the permeability of the blood capillaries, and thus allows the rapid escape of a circulating dye into the tissue spaces of the injected site.

In order to eliminate the complicating factor of the vascular reactions (hemorrhage and edema) brought about by the toxin, injections of venom were made into excised skin. It is known that the spreading factor from other sources exerts its action in the skin of the dead rabbit (10).

*Experiment.*—2 rabbits were killed by air embolism. Then one received in one side three 0.50 cc. injections of rattlesnake venom in dilutions of 1:50, 1:100, and 1:1000, mixed with 0.25 cc. of India ink. The skin of the second rabbit was excised, nailed down to a board and injected as above. As a control, the usual saline and India ink mixtures were injected. The bleb formed by the venom mixtures disappeared as promptly as when injected into the living animals.

After 24 hours the areas of ink spread where the venom was injected were from 3 to 6 times larger than the control areas. In the excised skin the difference was less pronounced although quite clear.

The experiment shows that when snake venom and India ink were injected into the skin of dead rabbits the mixture spread through the tissue. The areas of spread were smaller than those which obtain in the living animal, and naturally, in the skin of the dead animal none of the edema and hemorrhage usually produced by the venom was present.[4]

Intravenous injection of venom was used as another means of eliminating the local toxicity of the venom. It has been previously shown (2, 5) that spreading factors from testicle and bacteria, when injected into the vascular system, bring about an increase of permeability of the whole skin.

*Experiment.*—Mixtures of 0.50 cc. of saline plus 0.25 cc. of India ink were injected intradermally into each of 3 rabbits. The familiar discoid, convex bleb about 4 sq. cm. was formed in every case. 2 of the animals were then injected intravenously with 0.02 gm. of dry rattlesnake venom dissolved in 1 cc. of saline, while the third rabbit was left as a control. The injection of venom was followed by immediate signs of respiratory disturbance and the animals died in about 40 to 50 minutes. 4 more intradermal injections of ink were made at intervals while they were still alive and up to 2 hours after their death, and the resultant spreads were measured 5 hours after death. In every case the spreading was observed to involve an area 3 or 4 times as large as the bleb formed in the control rabbit. No gross evidence of tissue damage was observed in these areas.

Thus the spread of an India ink suspension in the skin is enhanced when the rabbit is inoculated intravenously with snake venom. The permeability of the skin has been obviously increased by the cir-

[4] The fact that final spreads in the living animal are much larger than in the dead one suggests that liquid from the circulating blood aids the spreading. An action of the spreading factor on blood capillaries must be thought of in this connection.

culating venom. Not improbably the latter passes into skin recently injected with ink as not elsewhere.

*Enhancement of Virus and Bacterial Infections by the Spreading Factor*

We have previously shown (2, 5, 6) that under certain conditions, the spreading factor greatly enhances experimental infections in the skin. These experiments were repeated with rattlesnake venom.

*Experiment.*—Samples of 0.5 cc. of venom were mixed with 0.5 cc. of dilutions of vaccine virus or a suspension of *Staphylococcus aureus* or bacillus of mouse typhoid II. Each resulting mixture of infectious agent and venom was injected

TABLE III

*Enhancement of Virus and Bacterial Lesions by the Injection with Rattlesnake Venom (Crotalus adamanteus) Deprived of Toxicity*

| 0.50 cc. venom or saline injected along with 0.50 cc. of suspension of the infectious agent | Area of lesions produced by infectious agent injected along with venom or saline | | | | |
|---|---|---|---|---|---|
| | Results after 8 days | | | Results after 2 days | |
| | Vaccine virus 1:1000 | Vaccine virus 1:100 | Vaccine virus 1:10 | Staphylococcus | Mouse typhoid |
| | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Venom 1:50 heated at 100°C. | 7.4 | 12.4 | 34.7 | 23.6 | 16.5 |
| Saline solution (control) | 7.5 | 12.9 | 21.7 | 18.0 | 8.4 |
| Venom 1:50 heated at 75°C. | 30.8 | 75.9 | 68.2 | 42.4 | 20.8 |
| Saline solution (control) | 19.3 | 42.0 | 34.0 | 18.0 | 8.4 |
| Unheated venom that has lost its toxicity 1:1000 | | | | 69.2 | |
| Saline solution (control) | | | | 18.0 | |

intradermally on one side of a rabbit. As a control, the infectious agent alone was injected on the other side. In order to eliminate the disturbing toxic factor of the venom, it was heated at 75° for 25 minutes or at 100° for 5 minutes. We also used a 1:1000 dilution of venom which we knew had lost most of its toxicity by long standing in the ice box. The results are given in Table III.

Venom heated at 75°, as well as unheated venom that had lost its toxicity on standing, markedly enhanced both bacterial and virus infections. Venom heated at 100° also enhanced the infections but to a much lesser extent than the other 2 preparations. Therefore, from the point of view of its effects on infection the spreading factor from rattlesnake venom behaves like the spreading factor from testicle and from invasive bacteria.

### *Action of Antivenom Serum on the Spreading Power of Snake Venom*

Experiments were undertaken in an attempt to reveal with antitoxin the extent of the linkage between spreading and toxic factors in snake venom. The antigenicity of the spreading factor from testicle, and from invasive bacteria had previously been investigated (11, 12).

*Experiment.*—3 rabbits were immunized against rattlesnake venom by an intradermal injection of 1 cc. of it, diluted 1:50, repeated twice later at weekly intervals. These injections produced the familiar local hemorrhagic lesions which, however, healed promptly. 10 days after the last injection the sera of the rabbits was found to possess a high titre of precipitins against rattlesnake venom. They

TABLE IV

*Partial Suppression of the Spreading Power and Local Toxicity of Rattlesnake Venom (Crotalus adamanteus) in the Immunized Rabbit*

| Dilutions of venom | Amount of venom injected | Venom plus 0.25 cc. India ink or 0.25 cc. saline. Area of spread and severity of lesion | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Immune rabbit | | | | Normal rabbit (control) | | | |
| | | 40 min. | 2 hrs. | 5 hrs. | 24 hrs. | 40 min. | 2 hrs. | 5 hrs. | 24 hrs. |
| | *cc.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| 1:1000 | 0.50 | 6.9 | 12.5 | 18.0 | 15.0 | 7.7 | 12.5 | 18.0 | 20.0 |
| | | Slight erythema | Very mild | Mod. pronounced | Mod. pronounced | Mild | Mod. pronounced | Mod. pronounced | Pronounced |
| 1:100 | 0.50 | 6.3 | 13.2 | 16.1 | 21.8 | 8.8 | 16.4 | 25.0 | 57.0 |
| | | Very mild | Mod. pronounced | Mod. pronounced | Pronounced | Mod. pronounced | Pronounced | Pronounced | Very pronounced |
| 1:10 | 0.25 | | 12.5 | 23.1 | 39.2 | | 13.5 | 36.5 | 64.0 |
| | | | Pronounced | Pronounced | Pronounced | | Very pronounced | Very pronounced | Very pronounced |

were now injected intradermally with dilutions of this venom mixed with either India ink or saline. Care was taken to choose sites in the skin which had not been previously injected. At the same time 3 normal rabbits were injected with the same venom dilutions. The resulting spreads and the character of the lesions in both groups of animals were recorded. In order to simplify the presentation of data, results from one control and one experimental animal are shown in Table IV. The other two pairs gave entirely similar results.

*Experiment.*—Dilutions of rattlesnake venom at 1:100, 1:1000, and 1:10,000 were respectively added to an equal volume of either normal serum or serum from a rabbit partly immune to the venom. The mixtures were allowed to remain in contact for 6 hours at 37°C. and overnight in the ice box. In those mixtures containing immune serum flocculation occurred, whereas the control mixtures remained clear. 4 rabbits were injected intradermally on one side with 0.50 cc.

of each of the immune serum mixtures (previously shaken), together with either 0.25 cc. of India ink or saline, and on the opposite side with the normal serum mixtures plus India ink or saline. The results obtained in every case showed that the mixtures containing venom plus immune serum spread the ink much less and produced milder lesions than those containing normal serum. Details from tests on 2 of the rabbits are shown in Table V.

These two experiments show that the specific antiserum neutralizes both the toxic factor and the spreading factor in snake venom, and that this neutralization takes place *in vitro* as well as *in vivo*.

TABLE V

*Partial Suppression of the Spreading Power and Local Toxicity of Snake Venom (Crotalus adamanteus) by the Specific Antitoxin (Results after 24 Hours)*

| | Area of spread and lesions produced by 0.50 cc. of mixture of venom and sera plus 0.25 cc. of India ink or saline | | | | | |
|---|---|---|---|---|---|---|
| | Immune serum | | | Normal serum | | |
| Dilutions of venom... | 1:100 | 1:1000 | 1:10,000 | 1:100 | 1:1000 | 1:10,000 |
| | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Rabbit 1 | 35.5 Pronounced | 19.3 Mod. pronounced | 11.0 Mild | 68.4 Very pronounced | 26.2 Pronounced | 14.2 Mod. pronounced |
| Rabbit 2 | 29.8 Mod. pronounced | 17.9 Mod. pronounced | 9.1 Mild | 62.0 Very pronounced | 22.0 Mod. pronounced | 12.5 Mod. pronounced |

## *Studies on the Venom from Toads*

In the light of the above observations it was of interest to know whether spreading factors are associated with other animal poisons. The secretions from the parotid and other skin glands of toads were next studied from this point of view.

*Experiment.*—The dried secretions of the skin glands of 6 species of toads were each extracted with saline in the proportion of 1:50. After centrifugation 0.5 cc. of each extract was injected intradermally into one rabbit, along with 0.25 cc. of India ink or 0.25 cc. of saline. Control mixtures were injected using saline instead of venom. The results obtained were as follows:

The poisons of 3 species, *Bufo bufo*, *B. viridis viridis*, and *B. arenarum*, were found to be entirely devoid of spreading factor. Local lesions were absent or

extremely mild. However, the general toxicity which these poisons are known to possess was shown in our tests, since each rabbit injected died within a few hours. The poisons from 3 other species, *Bufo formosus*, *B. alvarius*, and Ch'an Su, proved to have a moderate amount of spreading factor, the areas of skin over which the ink extended being 2 to 4 times the control area. 2 of the materials produced a moderate local skin reaction. The third, that of *B. alvarius*, gave rise to no evident lesion. The general toxicity proved less with these last 3 venoms, the rabbits recovering after some intoxication.

These experiments show that the venoms of toads contains very little spreading factor or none, despite the fact that they are extremely potent poisons for the circulatory and nervous systems.[5]

## DISCUSSION

It seems apparent from the above experiments that the venom from many snakes contains a factor capable of increasing the permeability of connective tissue and blood capillaries. Of considerable interest is the finding that the venom from various species of snakes differs considerably in spreading power, and apparently does so independently of its toxicity. Thus the spreading power is great in the Viperidae (rattlesnake) family, and relatively scant in the Colubridae proteroglypha (cobra) family. These two groups are characterized by the local action, and the neurotropic action, respectively, of their venoms. The secretions of the skin glands of toads are very toxic, and yet are almost devoid of any spreading factor. They also have very little local effect upon the tissues. Taking the facts together it would appear that a relationship exists between local tissue action and spreading factor content. This relationship may be analogous to that which has been discussed in connection with bacterial agents (5, 6). A distinction was drawn between invasive bacteria, which

[5] Besides snakes and toads, insects were also studied as possible sources of spreading factor (1). Their whole bodies were ground and extracted and for this reason the results obtained deserve only brief mention. It was found that extracts of spiders, bees, wasps, and mosquitoes injected together with India ink into the rabbit skin brought about areas of spread several times larger than the control even when used at high dilutions. Spider extract was the most active in this respect. On the other hand extracts of non-poisonous insects as, *e.g.* crickets, grasshoppers, dragon-flies, etc., were inactive. These preliminary results are only suggestive of a possible association of the venom with a spreading factor in the poisons.

liberate spreading factor, thus aiding their spread through the tissues, and virulent bacteria, which are devoid of spreading factor and rely upon other means to maintain and augment their status as pathogens. The locally acting rattlesnake venom containing spreading factor may be likened in this regard to *Clostridium welchii*, and toad venom may be likened to *Clostridium tetani*.

The relationship between the spreading factor and the toxins of snake venoms is not yet understood. The experiments conducted with heated venom, and those performed upon the excised skin, indicate that the two factors can be dissociated. On the other hand the specific antiserum of animals immunized with venom inhibits or suppresses both. In this connection it is of interest that extracts of the cells of the poison gland of the rattlesnake contain very little toxin, or spreading factor, but that both are present in the secretion of the gland (13).

## CONCLUSIONS

The venom of several species of poisonous snakes acts to spread India ink through the skin as do the spreading factors procurable from certain tissues and elaborated by invasive bacteria. The factor is most abundant in the venom of the Viperidae (rattlesnake) family and relatively scant in the venom of Colubridae proteroglypha (cobra) family, and it is absent from toad venom. Extracts of the supralabial glands of harmless snakes contain only negligible amounts of the factor.

Rattlesnake venom heated at 65° to 100° loses a large proportion of its toxicity but retains the ability to spread ink.

Rattlesnake venom that has lost its toxicity on standing or on heating markedly enhances the infection produced by bacterial or virus suspension in the rabbit skin.

Antivenine serum inactivates both the toxic and spreading factors of venom.

## BIBLIOGRAPHY

1. Duran-Reynals, F., *Science*, 1936, **83**, 286.
2. Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99**, 6; *J. Exp. Med.*, 1929, **50**, 327. Hoffman, D. C., and Duran-Reynals, F., *Science*, 1930, **72**, 508; *J. Exp. Med.*, 1931, **53**, 387. See general review, Duran-Reynals, F., *Ann. Inst. Pasteur*, 1936, **57**, 597. McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045.

3. Duran-Reynals, F., and Stewart, F. W., *Am. J. Cancer*, 1931, **15**, 2790. Boyland, E., and McClean, D., *J. Path. and Bact.*, 1935, **91**, 553.
4. Claude, A., *J. Exp. Med.*, 1937, **66**, 353.
5. Duran-Reynals, F., *J. Exp. Med.*, 1933, **58**, 161. McClean, D., *J. Path. and Bact.*, 1936, **42**, 477. Bronfenbrenner, J., and Sulkin, S. E., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1419. Pradhan, M. G., *Brit. J. Exp. Path.*, 1937, **18**, 90.
6. Duran-Reynals, F., *J. Exp. Med.*, 1935, **61**, 617. Thomas, R. M., and Duran-Reynals, F., *J. Exp. Med.*, 1935, **62**, 39.
7. Noguchi, H., Snake venoms, an investigation of venomous snakes with special reference to the phenomena of their venoms, *Carnegie Institution of Washington*, 1909.
8. Favilli, G., and McClean, D., *J. Path. and Bact.*, 1934, **38**, 153.
9. Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1937, **65**, 661.
10. McClean, D., *J. Path. and Bact.*, 1931, **34**, 459.
11. Duran-Reynals, F., *J. Exp. Med.*, 1932, **55**, 703.
12. McClean, D., *J. Path. and Bact.*, 1936, **42**, 477.
13. Duran-Reynals, F., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 763.