Immunisation against serpents' venom and the treatment of snake-bite with antivenene / an address delivered by Professor Fraser on Friday, March 20, 1896.

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Publication/Creation

London : William Clowes, 1896.

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WEEKLY EVENING MEETING,

Friday, March 20, 1896.

Sir James Crichton-Browne, M.D. LL.D. F.R.S. Treasurer and Vice-President, in the Chair.

Professor Thomas R. Fraser, M.D. LL.D. F.R.S.

Immunisation against Serpents' Venom, and the Treatment of Snake-bite with Antivenene.

From a remote period of antiquity, there has been enmity between the human race and serpents, and, in a literal sense, man has bruised the head of the serpent, and the serpent has bruised the heel of man. This long-continued feud has not resulted in victory for either side. Venomous serpents still annually destroy the lives of tens of thousands of human beings, and, in self-defence, tens of thousands of serpents are annually slain by man.

The progress of knowledge has greatly increased the means for protecting mankind against the death-producing effects of many diseases; yet, although these means have been liberally employed in the contest against venomous serpents, none of them have hitherto been found sufficient.

The reality of the contest is appreciated when we find pervading medical literature from its earliest beginnings—from the time of Pliny and Celsus—to the present time, disquisitions on the treatment of the bites of venomous serpents, and lengthy descriptions of the numerous remedies, organic and inorganic, that have been used for this purpose. Although extended experience and the application of the scientific methods of the present day, have resulted in showing that each of these remedies had been recommended on insufficient grounds, we may hesitate in pronouncing their recommendation to have been premature, in view of the impossibility of waiting, in the presence of imminent dangers, until accurate demonstration has been obtained by the usually tardy and laborious processes of science.

Let me pause here for a few minutes to indicate the practical importance of a scientific demonstration of the value of any remedy that is used in the treatment of snake-poisoning.

When a serpent inflicts a wound, I need scarcely say that it is not the wound, but the venom introduced into it which causes the symptoms of poisoning, and the death that may result. This venom is now known to be a complex mixture, containing several non-poisonous as well as poisonous substances. The latter are not ferments, and have no power of reproducing themselves in the body, but they are substances that produce effects having a direct relationship to the quantity introduced into the body. This quantity in the case of each serpent varies with its size and bodily and mental

condition; with the nature of the bite—whether both fangs or only one has been introduced, whether they have penetrated deeply or only scratched the surface; and with other circumstances related to the serpent, such as whether it had recently bitten an animal or not, and thus parted with a portion or retained the whole of the venom stored in the poison glands.

A bite may, therefore, result in very little danger, or it may be rapidly fatal; but, in order to produce death, there must have been introduced into the tissues at least a certain quantity of venom, which is spoken of as the minimum-lethal quantity or dose. The minimum-lethal quantity for the animal bitten, again, is different for different species of animals, and different also for different individuals of the same species, the chief cause of difference between adult animals of the same species being the body weight of the individual, the quantity required to produce death being very exactly related to each pound or kilogramme of weight.

If even a minute fraction below the minimum-lethal has been introduced into the tissues by an effective bite, death will not follow, although serious and alarming symptoms will be produced of exactly the same kind as those which follow a bite which terminates fatally.

How then can we be assured, in any case of snake-bite in man, that a quantity of venom sufficient to produce death has been introduced? It is impossible to answer this question except by the result. If a quantity less than the minimum-lethal has been introduced, although the gravest symptoms may be produced, the patient will recover, whatever remedies are administered, provided, obviously, that the remedies have not been so injudiciously selected or used that they themselves, and not the insufficient quantity of venom, produce a fatal termination. The recovery of a patient after the introduction of less than the smallest quantity of venom capable of producing death, has thus too often been attributed to the remedies that have been administered; and, consequently, as, indeed, is exemplified in the treatment of many diseases, a large number of substances have acquired an unjust reputation as antidotes. The list of antidotes has, accordingly, become a very large one; but when their pretensions have been subjected to sufficient tests, the verdict is that all of them are valueless to prevent death when even the smallest quantity of venom required to produce death has been received by an animal.

Without entering into details, I will content myself with reproducing the opinion of Sir Joseph Fayrer, that, "after long and repeated observations in India, and subsequently in England, I am forced to the conclusion that all the remedies hitherto regarded as antidotes are absolutely without any specific effect on the condition produced by the poison."

But while medical practice and science, in each period of its development, has thus failed to protect man against this ancient enemy, legendary traditions, the tales of travellers and of residents among nations and tribes existing outside of the civilisation of the

time, at least suggest that, by means apart from the use of remedies, some measure of success may actually have been obtained.

Many of these legends and statements are probably of great significance, and, in connection with facts derived from experiment, which to-night I have to describe, they possess a deep interest.

We learn from these legends that from a remote period of time the belief has existed that a power may be acquired by man of freely handling venomous serpents, and even of successfully resisting the poisonous effects of their bites.

The Psylli of Africa, the Marsi of Italy, the Gouni of India, and other ancient tribes and sects, were stated to have been immune against serpents' bites, and this immunity has been explained on the supposition that serpents' blood was present in the veins of the members of these tribes and sects.

In more modern times and, indeed, at the present day, the same belief is expressed in the writings of many travellers. In 'A New and Accurate Description of the Coast of Guinea,' by William Bosman, published in 1705, an account is given of the great "reverence and respect" of the negroes for snakes, worshipped by them as gods; in connection with which the following statements are made. "But what is best of all is that these idolatrous snakes don't do the least mischief in the world to mankind; for if by chance in the dark one treads upon them, and they bite or sting him, it is not more prejudicial than the sting of millipedes. Wherefore the natives would fain persuade us that it is good to be bitten or stung by these snakes, upon the plea that one is thereby secured and protected from the sting of any poisonous snake" (p. 379).

At Southern Africa, the Rev. John Campbell, in 1813, observed that it was "very common among the Hottentots to catch a serpent, squeeze out the poison from under his teeth, and drink it. They say it only makes them a little giddy, and imagine that it preserves them afterwards from receiving any injury from the sting of that reptile" (p. 401).

Drummond Hay, in his work on Western Barbary, published in 1844, gives a description of the performances by members of a sect of snake-charmers, called the Eisowy, who freely handled, and allowed themselves to be bitten by serpents proved to be venomous by a rapidly fatal experiment performed on a fowl. At the termination of the exhibition, the Eisowy, apparently as a usual part of the performance, "commenced eating or rather chewing" a poisonous snake, "which, writhing with pain (to quote Mr. Hay's words), bit him in the neck and hands until it was actually destroyed by the Eisowy's teeth." He states that, on another occasion, at Tangier, a young Moor, who was witnessing the performance of a snake-charmer, ridiculed his exhibition as an imposture, and having been dared by the Eisowy to touch one of the serpents, the lad did so, was bitten by one of them, and shortly afterwards expired. In connection with my subject, a special interest is attached to the

account given by Mr. Drummond Hay, and repeated in its main features by Quedenfeldt in the 'Zeitschrift für Ethnologie' of 1886, of the origin of this Eisowy sect, and of the immunity which they claim. The founder, Seedna Eiser, was being followed through the desert of Soos by a great multitude, who, becoming hungry, clamoured for bread. On this, Seedna Eiser became enraged, and turning upon them he uttered a common Arabic curse, "Kool sim," which means "eat poison." So great was their faith in the teaching of the saint, that they acted upon the literal interpretation of his words, and thereafter ate venomous snakes and reptiles; and from that time they themselves and their descendants have been immune against serpents' bites (p. 65).

Dr. Honigberger, in his 'Thirty-five Years in the East,' published in 1852, relates the incident of a faqueer who was bitten by a serpent, and to whom he at once sent medicines which he judged likely to prevent the ill-effects of the venom. "On the same afternoon," he writes, "I visited him and found him in good spirits. I at first attributed the circumstance to the effect produced by the remedies I had sent him, but was surprised on hearing that he had not taken them, he being of opinion that the venom of the serpent was incapable of affecting him, inasmuch as he had often been bitten by serpents without having sustained any injury." On the suggestion of the faqueer, the same serpent, which had been caught and retained, was allowed to bite him again, and afterwards to bite a fowl. This fowl was taken home by Dr. Honigberger, and he found it dead on the following morning, "although the faqueer, who was bitten first, was quite well" (p. 135).

Nicholson, in his work on 'Indian Snakes' (1875), and Richards, in his 'Landmarks of Snake-poison Literature' (1885), also narrate instances, the latter with obvious disbelief in their reality, suggesting that snake-charmers may possess some means for protecting themselves against the bites of venomous serpents.

Many other examples might be quoted in which this suggestion is made. The attention which has been drawn to the subject during the last twelve months has prompted the publication of other instances, such as that related by Dr. Bawa, of a Tamil snake-charmer who, in the course of his performances, was bitten by a cobra without any effect, while an onlooker, foolishly repeating the performance, was bitten by the same cobra, and died in three hours; and the description given by M. D'Abbadie, in a recent issue of the Comptes rendus, of the custom, recently prevailing at Mozambique, of inoculating with serpents' venom, under the firm conviction that protection is thereby produced against the effects of serpents' bites.

It may be instructive to associate with these statements the belief that venomous serpents are themselves protected against the effects of bites inflicted upon them by individuals both of their own and of other species. On mere anatomical grounds, it is difficult to understand how serpents could escape the absorption of their own

venom through mucous surfaces, even admitting that absorption of venom does not occur in normal conditions of these surfaces. Venom must, however, be so frequently introduced into their bodies, in situations where absorption could not fail to occur, by the bites inflicted upon them by other serpents, that the conclusion seems inevitable that they possess some protective quality, without which, probably, no venomous serpents would now be in existence. Not only have many general observations been made in favour of this belief, but it has been supported by direct experiments, such as those made by Fontana of Tuscany more than a century ago, and by Guyon, Lacerda, Waddell, Kaufmann, and Sir Joseph Fayrer.

This, and other evidence, pointing to the existence of protection against venom, not only in serpents themselves, but also, in certain exceptional circumstances, in human beings, several years ago originated a wish to investigate the matter. It was obviously suggested that if protection occurs, it must be caused by some direct result of the absorption of venom; and, therefore, that its existence could be proved or disproved by experiment. In the former event, the first steps would already have been taken to obtain, by further experiments, results likely to be of value in the treatment of poisoning by serpents' venom, and, indeed, likely to be of suggestive importance in even the wider field of general therapeutics.

The general plan to be followed in the first stages of the investigation was obviously suggested by some of the statements I have reproduced; for they indicate that individuals might become accustomed to, or protected against the effects of serpents' bites, by the introduction into their bodies of a succession of doses of venom, no one of which, necessarily, at the beginning of the process, was so large as the minimum-lethal. A consideration also of the facts, proving the possession of protection on the part of venomous serpents themselves, indicated the same plan of procedure; for, equally obviously, these serpents, from an early period of their existence, must absorb venom from their own gradually-developing poison-glands, until, in the course of time, they had acquired sufficient protection to remain unaffected by the larger quantities which the now fully-developed glands would introduce into their bodies.

My first supplies of cobra venom were obtained in 1869, from the late Dr. Shortt, of Madras, and in 1879 from Surgeon-Colonel Moir, of Meerut. They were in very small quantity, but with them I was able to satisfy myself that, by a succession of minute doses, animals became able to receive the minimum-lethal dose without any distinct injury. At this point, however, the supply of venom failed, and the observations could not then be carried further. It became evident that until large quantities of venom had been obtained, definite results could not be hoped for.

It was not until several years afterwards that a sufficient supply had been gradually accumulated, by further small quantities received from Sir Joseph Fayrer, the Thakore of Gondal, and Dr. Phillips;

and by larger quantities from Sir William Mackinnon, Director-General of the Army Medical Department, and especially from Surgeon-Colonel Cunningham, of Calcutta, who for many years has been engaged with much success in the study of venoms and their antidotes. Within the last few months, and subsequently to the publication of some of the experimental results which had by this time been obtained, the India Office has also placed at my disposal a considerable quantity of venom, which had been collected by Dr. Hankin, of Agra, at the request of Dr. Cleghoorn, Surgeon-General with the Government of India.

But, besides these specimens of the venom of the cobra of India, I have also been fortunate in obtaining specimens of venoms from other parts of the world.

From America, Dr. Weir Mitchell, of Philadelphia—whose work on the chemistry and physiology of serpents' venom constitutes the great advance of the century on the venom of viperine serpents—has supplied me with the venom of three species of rattlesnakes, viz. *Crotalus horridus*, *C. adamanteus*, and *C. durrisus*, and also with a specimen of the venom of the Copper Head (*Trigonocephalus contortrix*).

From Australia, Dr. Thomas Bancroft, of Brisbane, has at various times sent specimens of the venoms of the black snake (*Pseudechis porphyriacus*), the brown snake (*Diemenia superciliosa*), and of a large unidentified snake of the Diamantina district of Queensland (probably a new species of Diemenia).

From Africa, the kindness of Mr. Andrew Smith, a distinguished naturalist of Cape Town, of Dr. Brook, of the Orange Free States, and of Dr. John Murray and Mr. Van Putten, of Cape Colony, has placed at my disposal small quantities of the venom of the puff adder (*Vipera arietans*), the night adder (*Aspidelaps lubricus*), the yellow cobra (*Naja haie*), and the "Ring Hals Slang" or "Rinkas" (*Sepedon hæmachates*).

In the meantime, however, the results of experiments on the inoculation of the toxines of diseases, as well as of proteid toxines of vegetable origin, had suggested to several observers that serpents' venom, because of its chemical analogies with several of these substances, might possibly be found capable, like them, of producing immunity against the effects of poisonous doses; and further important evidence has thus been obtained in favour of the reality of the protection to which I have referred.

Sewall, in 1886, undertook an investigation with the object of determining if immunity against the fatal effects of rattlesnake venom could be produced by the inoculation of repeated doses, each too small to produce ill-effects. The experiments were made on pigeons, and he succeeded in proving that immunity could be secured to the extent, at least, of protection against seven times the minimum-lethal dose. Kanthack made a similar series of experiments in 1891, which allowed him to conclude that rabbits may be accustomed to resist lethal doses

of cobra venom. Working with the venom of vipers, Kaufmann in 1891, and Phisalix and Bertrand in 1893, obtained experimental evidence of the possibility of producing a definite, though not high degree of resistance against the toxic effects of this venom. In the following year, Calmette, continuing some earlier observations which had led him to express the opinion that protection against snake venom could not be produced, published evidence confirming the results of previous investigators, but also showing that a higher degree of protection could be secured than they had obtained, for he succeeded in administering to each of several rabbits, within a period of eight months, a total quantity of from 30 to 35 milligrammes of venom.

In 1894, also, both Phisalix and Bertrand and Calmette obtained evidence of the power of the blood-serum of protected animals to counteract the effects of venom. Calmette at the same time claimed that hypochlorite and chloride of calcium were antidotes of considerable value; and in a later publication, he showed that the blood-serum of animals immunised by the administration of venom possesses a certain degree of antidotal efficacy against the toxines of several diseases.

In the case of many of the venoms which I have had the good fortune to obtain, the quantity at my disposal was not sufficient for experimental examination on the plan that seemed desirable, and, besides, the examination of each of them would require several months of work. The venoms that have as yet been used are four in number, those namely of the cobra of India (*Naja tripudians*), of the *Crotalus horridus* of America, of a large colubrine snake, probably a species of *Diemenia* from Queensland, Australia, and of the *Sepedon hæmachates* of Africa. They are, therefore, those of the most deadly of the poisonous serpents of Asia, America, Australia and Africa respectively; and, further, they are representative of the chief differences that occur in the composition and action of venoms, for they are derived from members of the two great groups of the colubrine and viperine serpents. My supply of cobra venom, however, being much larger than that of any of the others, this venom was chiefly used in the experiments.

An esssential preliminary to exact investigations with active substances must always be the determination of the activity of the substances. The only convenient method for doing this is to define the smallest dose capable of producing death for any given weight of animal—that is, the minimum-lethal dose. The venoms in their natural liquid state are unstable, and they are also inconstant in activity, mainly because of variations in the quantity of the water which they contain. Dried venoms have therefore been used in all the experiments. The cobra venom has, however, nearly always been received in the form of a dry solid; but when this was not so, it has been dried *in vacuo* over sulphuric acid.

Experiments were made with it on several animals—as the frog,

guinea-pig, rabbit, white rat, cat, and the innocuous grass snake of Italy (*Tropedonotus natrix*). Very considerable differences were found to occur in the minimum-lethal dose for each of these animals. For the guinea-pig, the minimum-lethal dose per kilogramme was ·00018 grm.; for the frog, ·0002 grm.; for the rabbit, ·000245 grm.; for the white rat, ·00025 grm.; for the cat, somewhat less than ·005 grm.; and for the grass snake, the relatively large dose of ·03 grm.* Cobra venom thus takes a position among the most active of known substances, rivalling in its lethal power the most potent of the vegetable active principles, such as aconitine, strophanthin or acokantherin.

These facts having been ascertained, attempts were next made to render animals proof against lethal doses, by administering to them a succession of gradually increasing non-lethal doses. These were, for the first few doses, in some of the experiments, one-tenth of the minimum-lethal, in others one-fifth, in others one-half of the minimum-lethal, and in others almost as great as the minimum-lethal. At varying intervals the doses were repeated, and by-and-by gradually increased, until the actual minimum-lethal had been attained. The subsequent doses by gradual increments exceeded the minimum-lethal, and after five or six times the minimum-lethal had been reached, it was found that the increments could be increased so that each became twice, four times, and latterly even five times the minimum-lethal, and still the animal suffered little, and, in many cases, no appreciable injury.

This brief statement, however, does not represent the experimental difficulties that were encountered. It describes the course of events in the altogether successful experiments. Non-success, however, was frequent, and many failures occurred before experience indicated the precautions and conditions that are necessary for success.

Serpents' venom exerts what may broadly be described as a duplex action. It produces functional disturbances unassociated with visible structural changes, and it also produces obvious structural changes. The latter are of a highly irritative character, causing intense visceral congestions in the lungs, kidneys, and other organs, and when the venom is given by subcutaneous injection, on all the structures of the skin and subjacent parts. There are apparently also some definite changes produced in the blood, with regard to which several important facts have been discovered by Dr. Martin, of the University of Sydney, and by Surgeon-Colonel Cunningham, of Calcutta. Irritative effects are obviously produced by cobra venom, even in non-lethal doses, and with greatly increased virulence by doses that exceed the

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* Guinea-pig, nearly $\frac{1}{5}$ millig.
Frog, $\frac{1}{5}$ „
Rabbit, nearly $\frac{1}{4}$ „
White rat, $\frac{1}{4}$ „
Kitten (6 weeks), 2 millig.
Cat, 5 „
Grass snake, 3 centig.

minimum-lethal; but, in respect to this action, the other three venoms used are greatly more active than the venom of the cobra. Evidence was obtained to indicate that in the process of immunisation a diminution occurs in the intensity of these local actions; but this diminution does not proceed so rapidly as that in the unseen functional or other changes which are the more direct causes of death; and, further, the local irritative changes, after having been produced, are slower to disappear than the unseen functional disturbances. Until these facts had been appreciated, and, indeed, even with the adoption of precautions suggested by them, frequent failures occurred. The apparently contradictory results, accordingly, were obtained of the production, by gradually increasing doses, on the one hand, of a protection against quantities much above the minimum-lethal, so perfect that no apparent injury was caused; and, on the other hand, when the intervals of time separating successive doses had been too brief, of an intolerance so decided that death was produced by the last of a succession of gradually increasing doses, no one of which was so great as the minimum-lethal. The latter unfortunate event was frequently displayed in frogs and guinea-pigs, and attempts to carry immunisation in them to a high point usually resulted in failure.

Notwithstanding these difficulties, however, such gratifying results have been obtained as that rabbits could at last receive, by subcutaneous injection, so much as ten, twenty, thirty, and even the remarkable quantity of fifty times the minimum-lethal dose, without manifesting any obvious symptoms of poisoning.

Almost the only observable phenomena were a rise in the body temperature, which continued for a few hours after the injection, and which contrasts with the fall that occurs after the administration of even non-lethal doses, in non-protected animals; and a loss of appetite, which usually, though not invariably, occurred, and was probably the cause of a temporary fall in weight during the day or two days succeeding each injection. On the other hand, during the process of successful immunisation, the animals increased in weight, fed well, and appeared to acquire increased vigour and liveliness.

It is marvellous to observe these evidences of the absence of injurious effects, and even of the production of benefit in an animal which, for instance, has received in one single dose a quantity of venom sufficient to kill, in less than six hours, fifty animals of the same weight, and in the course of five or six months a total quantity of venom sufficient to destroy the lives of 370 animals of the same species and weight (Fig. 1, p. 10).

With the cobra venom I have also immunised cats and white rats, both by subcutaneous and by stomach administration; but the significance of the latter method of administration will be afterwards considered. A horse has also been immunised; and I have to express my obligations to Principal Williams and Prof. W. Owen Williams for granting me the accommodation of their establishment, and to

Mr. Davis, also of the New Veterinary College, for much valuable assistance.

Following the same plan of research with the three other venoms, it was found that for rabbits the minimum-lethal dose per kilogramme of the Diamantina venom is ·0015 grm.; of the venom of *Sepedon hæmachates*, ·0025 grm.; and of the venom of Crotalus, ·004 grm.* The Crotalus venom was, in its purity, altogether comparable with the cobra venom; and the determinations, therefore, show that cobra venom is sixteen times more powerful than Crotalus or rattlesnake venom. This venom, as well as the two others, however, much exceed cobra venom in the intensity of their local action. When death is produced by Crotalus venom, the subcutaneous tissues become

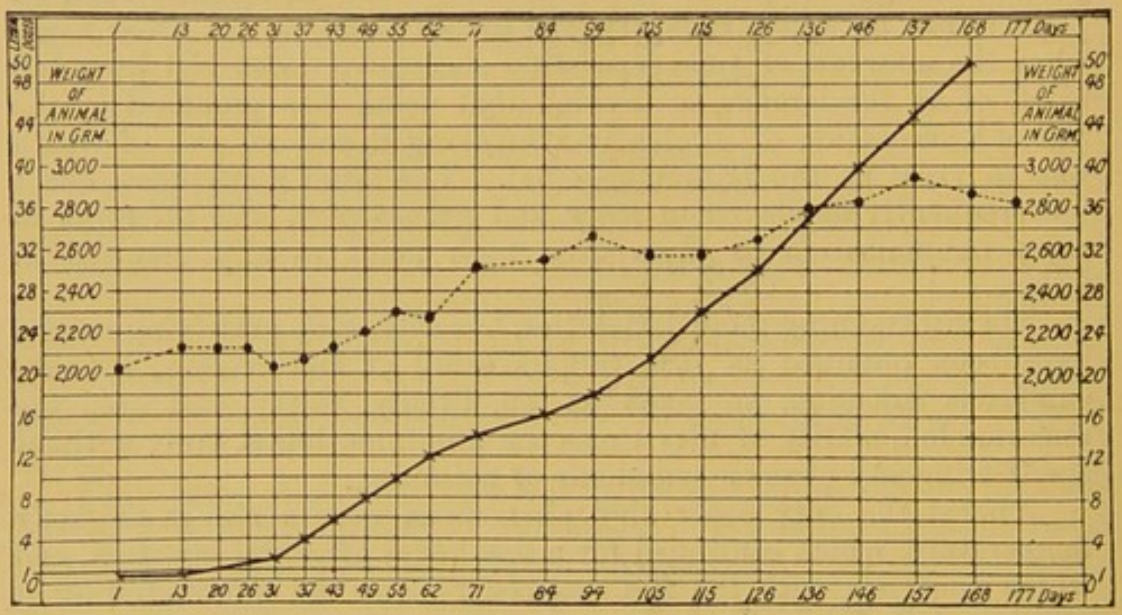

FIG. 1.—Immunisation of a rabbit against 50 times the minimum-lethal dose of cobra venom. The crosses connected by the continuous line represent administrations of venom. The dots connected by the interrupted line represent the weights of the animal.

extensively infiltrated with a large quantity of blood and of blood-stained serum, the underlying muscles are reduced to an almost pulpy blood-stained substance, and decomposition occurs very soon after death. Similar changes in the subcutaneous tissues, but to a rather less degree, are caused by the Diamantina venom, and in addition, hæmaturia, or more probably hæmoglobinuria, was invariably produced by lethal and by large non-lethal doses. I mention these circumstances to indicate the perfection of the protection which is produced by the administration of successive gradually increasing doses; for they can be so adjusted that a dose of the Diamantina

* Diamantina venom, 1½ milligramme.
Sepedon hæmachates, 2½ „
Crotalus horridus, 4 „

venom, even fifteen times larger than the minimum-lethal, may be administered without producing more than an inconsiderable degree of local destructive effect.

Experiments have also been made by which it has been demonstrated that when an animal has acquired a resistant power over the minimum-lethal dose of one venom, that animal is also able successfully to resist the lethal action of a dose above the minimum-lethal of other venoms. To a rabbit protected against cobra venom, a dose above the minimum-lethal of Sepedon venom has been administered; to rabbits protected against Crotalus venom, doses above the minimum-lethal of Diamantina and of cobra venoms have been given; to rabbits protected against the Diamantina venom, doses above the minimum-lethal of Crotalus and Sepedon venoms have been given; and in each case the animal has recovered, and but few symptoms of injury were produced. At the same time, in other experiments, indications were obtained that animals protected against a given venom are capable of resisting the toxic effect of that venom more effectually than the toxic effect of other venoms.

The experiments have not yet proceeded sufficiently far to show for what length of time the protection conferred by any final lethal dose may last. It has been discovered, however, that protection lasts for at least a considerable period of time, even when the last protective dose has not been a large one. For example, to a rabbit which had last received four times the minimum-lethal dose of cobra venom, twice the minimum-lethal dose was administered thirty-four days subsequently; while to another rabbit, which had last received twice the minimum-lethal dose of Crotalus venom, the same dose of this venom was administered twenty days subsequently, and in each case the second dose failed to produce any toxic symptom.

Having thus succeeded in producing a high degree of protection in animals against the toxic effects of serpents' venom, the blood-serum of these animals was, in the next place, collected for the purpose of testing its antidotal properties. In this portion of the investigation, the method followed was essentially the same as that described in a communication made by me to the Royal Society of Edinburgh in 1871, on "The Antagonism between the Actions of Physostigma and Atropia," as it appeared to be the most direct method for obtaining accurate knowledge of the value of an antidote.

A few preliminary experiments were, however, early made with the serum of animals in whom the protection had not been carried to a high degree, and they were sufficient to show that antidotal properties are possessed even by this serum. It soon became apparent that in order to obtain some reasonable approximation to constancy in the conditions of the experiments, it was necessary that the serum should be in such a state that it would remain unchanged during at least several weeks. It was found that this could be insured, without any appreciable loss of antidotal power, by drying the freshly-separated serum in the receiver of an air-pump over sulphuric acid.

A perfectly dry and easily pulverisable solid is thus obtained from which a normal serum can readily be prepared as required, by dissolving a definite quantity of the dry serum in a definite quantity of water. The dry substance is on the average equivalent to about one-tenth of the weight of the liquid serum. I have found that, without any special precautions, it retains its antidotal power unimpaired for at least a year, and it is probable that it may be kept unchanged for an unlimited period of time.

To this antidotal serum, whether in the dry form or in solution, I have given the name "Antivenene," a name which, notwithstanding etymological objections, has the advantages of brevity and freedom from ambiguity.

The experiments now to be described were made with antivenene derived from a horse which had last received a dose of cobra venom estimated to be twenty times the minimum-lethal. On some previous occasions I have stated the results of observations on the antidotal value of the blood-serum of rabbits which had last received thirty and fifty times the minimum-lethal, respectively. The antivenene obtained from cats and white rats has also been examined. The special interest, however, is attached to antivenene derived from the horse, that it is more likely than any others to be used in the treatment of snake-bite in man.

The experiments were so planned as to obtain in different conditions of administration as exact a definition as possible of the antidotal power of the antivenene. In the meantime, four series of experiments have been undertaken on rabbits. In one series the venom was mixed outside of the body with the antivenene, and immediately thereafter the mixture was injected under the skin of the animal; in the second series the venom and antivenene were almost simultaneously injected into opposite sides of the body; in the third series the antivenene was injected some considerable time before the venom; and in the fourth series the venom was first injected, and thirty minutes afterwards the antivenene.

In the experiments of the *first series*, the doses of cobra venom administered were the minimum-lethal, one-and-a-half the minimum lethal, twice, thrice, four times, five times, eight times and ten times the minimum-lethal. In the case of each dose of venom, experiments were made with different quantities of antivenene, until the smallest quantity required to prevent death was discovered. In order to render it certain, in this and in the other series, that a lethal dose had been administered in the experiments with the so-called minimum-lethal, the minimum-lethal indicated by previous experiments was not used, but instead of it a slightly larger dose (·00025 instead of ·00024 gramme per kilogramme).

When this certainly lethal dose, capable of producing death in three or four hours, was mixed with the antivenene, and the mixture injected two minutes afterwards, under the skin, it was found that so small quantities were sufficient to prevent death as ·001 cc.,

·0008 cc., ·0005 cc., and ·0004 cc. (1/1000, 1/1500, 1/2000, and 1/2500 of a cc., for each kilogramme of the weight of animal; with ·0003 cc. (1/333) per kilogramme, however, the animal died. The antivenene was therefore found to be so powerful as an antidote, in the conditions of these experiments, that even the 1/2500 part of a cubic centimetre, equivalent to about the one-hundred-and-fiftieth part of a minim, acted as an efficient antidote, while even with the one-two-thousandth part of a cubic centimetre not only was death prevented, but there was almost no symptom of poisoning produced. In the experiments of this series with one-and-a-half the minimum-lethal dose, recovery occurred when the doses of antivenene were ·32 cc., ·3 cc., ·28 cc., ·25 cc., and ·24 cc. per kilogramme; but ·23 cc. and ·2 cc. failed to prevent death. In the experiments with twice the minimum-lethal dose, recovery occurred when the doses of antivenene were ·5 cc., ·4 cc., and ·35 cc.; but ·3 cc. and ·2 cc. failed to prevent death. In the experiments with thrice the minimum-lethal dose, a dose capable of producing death in less than two hours, recovery occurred when the doses of antivenene were ·7 cc. and ·65 cc.; but death occurred with ·6 cc., ·55 cc., and 5 cc. With four times the minimum-lethal dose, recovery occurred with 1·5 cc., 1·3 cc., and 1·2 cc., and death with 1 cc. With five times the minimum-lethal dose, recovery occurred with 2·5 cc., 2·2 cc., 2 cc., 1·8 cc., and 1·5 cc.; but death with 1·3 cc. With eight times the minimum-lethal dose, recovery occurred with 2·6 cc. and 2·5 cc.; but death with 2·4 cc., 2·3 cc., and 2 cc. And even the enormous dose of ten times the minimum-lethal failed to produce death, or any important symptoms, when it had previously been mixed with 3·5 cc. and 3·4 cc. of antivenene for each kilogramme of animal; and it only succeeded in producing death, although not until the lapse of several hours, when the doses of antivenene were 3·3 cc., 3·2 cc., ·3 cc., and 2·5 cc. per kilogramme.

These results show a remarkable, an almost directly proportional accordance in the increment required in the dose of antivenene for each increment in the dose of venom. In the diagram, the comparatively straight direction of oblique line separating the fatal from the non-fatal experiments is noteworthy, considering that the conditions of the experiments, in regard both to the animals and the substances used, could never be absolutely the same. Indeed, from twice the minimum-lethal dose of venom upwards, the addition of little more than ·3 cc. per kilogramme represents the addition in the quantity of antivenene required for each addition of a minimum-lethal dose of venom. Apparently the antivenene is able in this proportion to prevent death from almost any lethal dose of venom, however large it may be (Fig. 2, p. 14).

These results are in marked contrast with those that occur when an antidote acts because of its physiological properties, and they alone suggest that the antidotism is rather the effect of a chemical than of a physiological reaction. The indications obtained with

doses of twice the minimum-lethal and upwards cannot, however, be carried down to the minimum-lethal dose. The quantity of antivenene required to prevent death from this dose is much less than might have been anticipated when the results of experiments with larger doses are considered. Thus, it appears that while ·35 cc. of antivenene per kilogramme is required to prevent death from twice the minimum-lethal of venom, the minute quantity of the 1/2500th of a cc., or nearly 1000 times less (·0004 as compared with ·35 cc.), is sufficient to prevent death from a little more than the minimum-lethal dose of venom. It is apparent that this minute quantity of antivenene does not render inert the whole of the minimum-lethal dose. All that is required, in order that the minimum-lethal dose

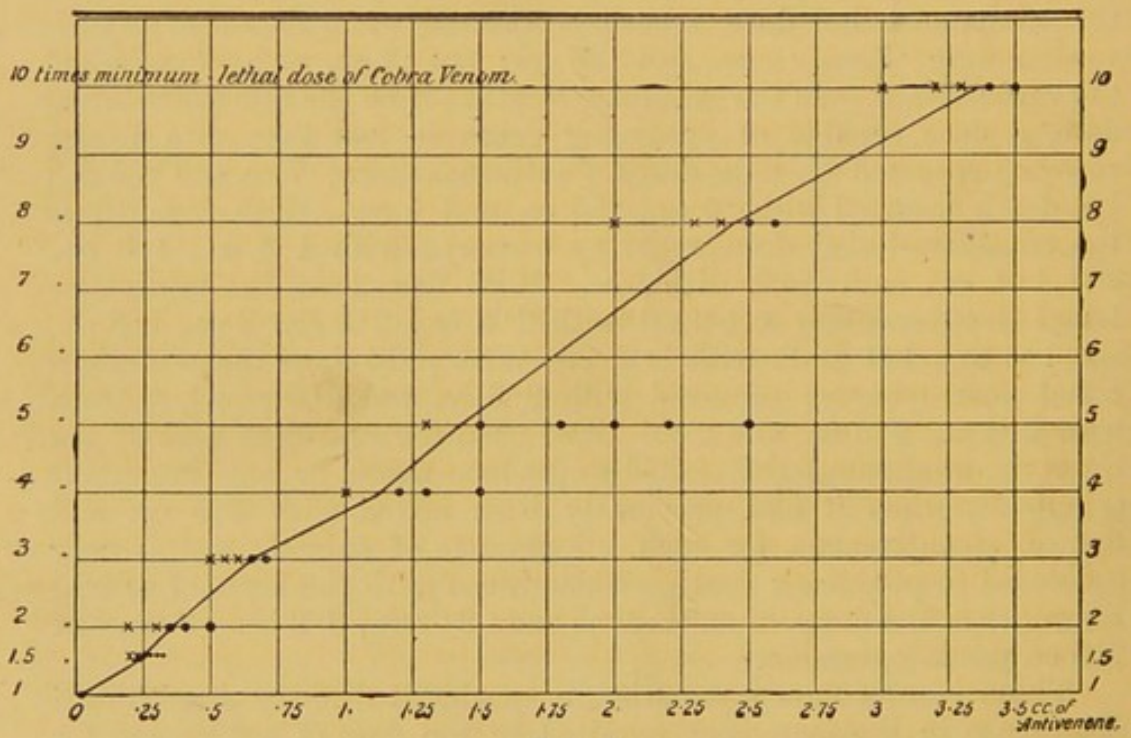

Fig. 2.

should not produce death, being that only a minute portion of it should be rendered inert; for, if this dose be the actual minimum-lethal, the rendering inert of any portion of it, however minute, will prevent the remainder from causing death.

In the *second series*, experiments with the antivenene of the horse have been completed only with one-and-a-half the minimum-lethal dose of venom. When this dose was injected into the subcutaneous tissues of one side of the body, and, immediately thereafter, a dose of antivenene into the subcutaneous tissues of the opposite side, it was found that antivenene in doses of 3 cc. and 3·3 cc. per kilogramme failed to prevent death, but that 3·5 cc. and 3·6 cc. per kilogramme were able to do so.

In the *third series*, experiments have been made with the minimum-

lethal, one-and-a-half the minimum lethal and twice the minimum-lethal dose of cobra venom. With the first of these doses, recovery occurred with ·5 cc., ·45 cc., and ·42 cc.; but death with ·4 cc., ·3 cc., and ·25 cc. of antivenene, administered thirty minutes before the venom. With one-and-a-half the minimum-lethal of venom, 2·9 cc. and 2·7 cc. of antivenene were able to prevent death; while 2·6 cc., 2·5 cc., 2·3 cc., and 2 cc. each failed in doing so. With twice the minimum-lethal dose of venom, recovery occurred when the doses of antivenene were 5 cc., 4·5 cc., and 4 cc.; but 3·9 cc., 3·8 cc., 3·5 cc., 2·5 cc., and 2 cc. were insufficient to prevent death.

In the *fourth series*, where the results give the truest indications of the antidotal value of antivenene in the actual treatment of snake-poisoning, it was found that recovery occurred in the experiments in which ·8 cc., ·7 cc., and ·65 cc. per kilogramme of antivenene was injected thirty minutes after an assuredly minimum-lethal dose (·00025 per kilo.) of venom; but that the antivenene was insufficient in quantity to prevent death when ·6 cc. or any smaller quantity was administered. In this series, further, it was found that 3·4 cc. and 3·2 cc. per kilogramme of antivenene were sufficient doses to prevent death after one-and-a-half the minimum-lethal dose of venom, but that 3 cc., 2·8 cc., and 2·5 cc. per kilogramme were insufficient. In a corresponding series of experiments made with the antivenene derived from rabbits which had last received thirty and fifty times the minimum-lethal dose of cobra venom, it was found that 5 cc. per kilogramme of this antivenene was the smallest dose by which death could be prevented in an animal which had received twice the minimum-lethal dose of venom thirty minutes previously.

Attention is conspicuously drawn by these facts to the remarkable difference in the dose of antivenene which is required to prevent death when it is mixed with the venom before administration, as contrasted with the doses required when the two substances have not previously been mixed together. Restricting attention to the experiments in each series in which the dose of venom was the same—to the experiments with one-and-a-half the minimum-lethal dose, for instance—it appears that in order to prevent death, when this dose was mixed with antivenene before administration, only ·24 cc. of antivenene is required; whereas, when both substances were injected simultaneously, but under the skin at different parts of the body, the required dose of antivenene is 3·5 cc.; when the antivenene was injected thirty minutes before the venom, it was 2·7 cc.; and when the venom was injected thirty minutes before the antivenene, it was 3·2 cc. per kilogramme.

It is impossible to consider the great difference between the dose of antivenene required when the two substances, though in each case simultaneously administered, are, in the one case, mixed together before injection, and in the other not so mixed, without again having the suggestion originated that the antidotism is the result of chemical, and not of physiological reactions.

This suggestion receives a further support from the fact, observed in several experiments, that the longer before their administration the two substances were allowed to remain together after they had been mixed, the greater is the antidotal efficiency of the antivenene. Thus, while 1·3 cc. per kilogramme of antivenene, mixed with five times the minimum-lethal dose of venom, was followed by death when the two had been mixed together five and also ten minutes before administration, this mixture was, on the other hand, followed by recovery when the interval before the administration was extended to twenty minutes. In order to obtain uniform and comparable results in the first series of experiments, it was therefore found necessary to adhere, in all the experiments made with the larger doses of venom, to a time limitation of not more than ten minutes before the mixed substances were injected.

I have also administered cobra-antivenene thirty minutes after a dose one-twelfth larger than the minimum-lethal of the venoms, respectively, of the *Sepedon hæmachates*, the *Crotalus horridus*, and the Diamantina serpent; and the animals experimented on have recovered when the dose of cobra-antivenene was not smaller than 1·5 cc. per kilogramme. This successful result is all the more remarkable when the intensely destructive effects produced by even smaller doses of each, but especially of two, of these venoms is recollected.

The antivenene derived from rabbits which had been protected to the extent that they had last received fifteen times the minimum-lethal dose of the Diamantina venom has also been tested against the Diamantina venom itself. When the two were administered together, after having been mixed *in vitro*, this antivenene in a dose of 0·5 (1/20) cc. per kilogramme was able successfully to antagonise slightly less than one-and-a-half the minimum-lethal dose of the venom; but ·025 (1/40) cc. per kilogramme failed to do so.

In the experiments which I have hitherto described, and, indeed, apparently in all others made in this new subject of serum therapeutics, protection has been produced, and the antidotal properties of the antitoxic blood-serum have been tested, by the subcutaneous, or, less frequently, by the intravenous injection of the venom or other toxic substance. No endeavour seems to have been made to discover how far the same effects, or what effects, may be produced by stomach administration.

Anticipating that results of an interesting nature might be obtained by this method of administration, I have adopted it for the introduction of both antivenene and venom into the body, and the results have even exceeded my anticipations.

The plan followed was the simple one of mixing the substances, previously dissolved in water, with a small quantity of milk, and allowing white rats, which had not received any food for several hours previously, to drink this milk. In the meantime, I will briefly describe only those experiments in which antivenene was thus

administered, reserving, for a few minutes, a description of the results that were obtained when the venom itself was used.

The first experiments were made with the object of determining if, by repeating the process followed in the production of immunity, with the exceptions that the administrations were by the stomach, and that antivenene was substituted for venom, an animal could be protected against the poisonous effects of venom. With this object, a white rat received on alternate days during several weeks, doses of antivenene, which were gradually increased from 1 to 10 cc. per kilogramme, and then, by subcutaneous injection, one-and-a-half the minimum-lethal doses of cobra venom; with the result that death was not produced. Other white rats received 10 cc. per kilogramme on each of four days, and on the fifth day 15 cc. per kilogramme of antivenene, and still recovery took place when one-and-a-half and one-and-three-quarters the minimum-lethal dose of venom was injected under the skin. To other white rats, 10 cc. and 15 cc. of antivenene were given by the stomach, on two successive days, and on the second day, one-and-a-half the minimum-lethal dose of venom, and the result also was that death was prevented. It was thus suggested that a single administration of antivenene might be as efficacious as a succession of administrations; and accordingly, the antidotal efficiency of single doses of 7 and of 10 cc. per kilogramme was tested, in some instances three hours, in others two days, and of 15 cc. three days before one-and-a-half the minimum-lethal dose of venom was subcutaneously injected; and in all cases the animals recovered. When, however, 5 cc. per kilogramme of antivenene was thus administered three hours before, and 10 cc. per kilogramme three days before, one-and-a-half the minimum-lethal dose of venom, the animals died.

The experiments have not as yet been carried further, but I hope to continue them so that the limits of the antidotal power of the antivenene, and the duration of the protection after single doses of antivenene, may be defined. Enough has, however, been done to prove that the stomach administration of antivenene, equally with its subcutaneous administration, confers protection against lethal doses of serpents' venom, and to justify the use of antivenene by the former and more convenient method for the purpose of securing protection for, at least, a period of several days after a single administration of the protecting antidote.

The facts hitherto narrated are sufficient to establish that the protection acquired by animals as a result of the administration of venom is not chiefly, or even to any important degree, caused by the venom having produced a tolerance by accustoming the body, as it has been expressed, to the presence of the venom—although a certain degree of this protection may possibly be due to such accustoming—but rather to the presence in the body, as a result of the introduction into it of venom, of a definite substance having antivenomous qualities. Notwithstanding the powerful protective and antidotal action of this

substance (antivenene) against serpents' venom, it is instructive to find that it is itself almost devoid of any physiological action, for even very large quantities may be injected under the skin without producing any other physiological reaction than a moderate degree of irritation in the neighbourhood of the injection. How then are we to explain the operation of this physiologically inert substance in protecting an animal against even fifty times the minimum-lethal dose of venom, or by a single administration of it, in saving an animal from death after there has been introduced into its body more than twice the quantity of venom that is required to kill it? When an answer has been attempted to be given to this question in discussions in the wider field of the serum therapeutics which deals with the toxines of diseases, the answer has been found either in the destructive power of phagocytes upon microbes and their toxines, or in the theory that the toxine elaborates from the blood the antidotal antitoxine, which, whether thus originated or separately introduced into the body, confers upon the body a resisting power which enables it to oppose successfully the injurious action of the toxines.

These answers cannot solve the problem in so far as snake venom is concerned. Phagocytosis cannot, of course, operate *in vitro* in solutions which are free from organised structures. Even when solutions of venom and antivenene, mixed together *in vitro*, have been inserted into the body, it is incredible that the increase in the quantity of antivenene by the 1/500th part of a cubic centimetre could cause such an increased proliferation of leucocytes as to prevent a lethal dose of venom from producing death, whereas a dose only the 1/500th part of a cubic centimetre smaller would be unable to do so. Further, there is no observable increase of leucocytes when much more than these infinitesimal quantities of antivenene have been administered to an animal.

In view of many of the facts that have to-night been stated, the "resistance of tissues" theory is also untenable. It is opposed, for instance, by the fact that so great a quantity of antivenene as ·42 cc., or nearly $\frac{1}{2}$ of a cubic centimetre, per kilogramme is required to prevent death when given thirty minutes before a lethal dose of venom, whereas, for the same dose of venom, only ·0004 cc., or the 1/2500th part of a cubic centimetre, or nearly the 1/1000th part of the former dose is sufficient, when it is mixed with the venom before administration, and in circumstances, therefore, which are much less favourable for the production by the antivenene of this supposed increase in the resistance of the tissues.

As I have already pointed out, however, a chemical theory, implying a reaction between antivenene and venom, which results in a neutralisation of the toxic activities of the venom, is entirely compatible with the observed facts.

The experiments which I have described to-night indicate that, with some limitations in the largest quantities, the greater the quantity of venom that has been introduced into the body in the process of

producing protection, the greater is the anti-venomous power of the blood-serum, and therefore the larger is the production of the anti-venene. While not an actual proof, this circumstance is at the same time in harmony with the supposition that the antivenene may actually be a constituent of the venom itself. The difficulties encountered in the separation by chemical methods of the several constituents of venom are so great, that it is not probable that the only proof or disproof of this supposition will soon be obtained by chemical analysis. Some physiological experiments which I have made seem, however, to go a long way in supplying the demonstration, which in the meantime has not been obtained from chemistry.

With the object of determining, in the first place, if the still disputed statement is correct, that serpents' venom is inert, or nearly so, when introduced into the stomach of an animal, cobra venom was administered, in a series of gradually increasing doses, to a cat, until finally it had received a single dose eighty times larger than the minimum-lethal; and to each of six white rats, single doses corresponding to 10, 20, 40, 300, 600, and 1000 times the minimum-lethal, if given by subcutaneous injection. Although no poisonous symptoms were produced in the animals by even the largest of these enormous quantities, it was found that the cat had so far been protected, that it could afterwards receive, by subcutaneous injection, one-and-a-half the minimum-lethal dose of cobra venom, without any other injury than some localised irritation at the seat of injection; and that the white rat, into whose stomach 1000 times the minimum-lethal dose had been introduced by one administration, survived perfectly, when seven days afterwards slightly more than the minimum-lethal dose of venom was injected under the skin.

It was also found that the blood-serum of the cat was definitely antivenomous, and the curious further fact was ascertained that her progeny had acquired protection through the milk supplied by the protected mother, thus supplying a scientific foundation for a half-admitted conviction, expressed by Wendell Holmes throughout his 'Romance of Destiny,' in regard to the heroine Elsie Venner.

These significant facts have been extended in a number of other experiments on white rats. In one group of experiments, each animal received, by stomach administration, 500 times the minimum-lethal, if given subcutaneously; and, as before, no toxic symptoms were observed. On the day following this administration, three of the animals received subcutaneously one-and-a-half the minimum-lethal dose of the same cobra venom, and they all recovered. In one of the other three animals, however, death was caused by this dose, when it was injected only three hours after the stomach administration; in a second, when this dose was injected two days after the stomach administration; and in the third, when nearly twice the minimum-lethal was injected twenty-four hours after the stomach administration.

In a second group of experiments, a dose of cobra venom equivalent to 1000 times the minimum-lethal by subcutaneous injection was

introduced into the stomach. On several occasions in which this had been done, an injection under the skin of one-and-a-half the minimum-lethal dose of venom made, in some experiments, two days, and in others three days afterwards, resulted in the recovery of the animals. As was anticipated, this large quantity introduced into the stomach, conferred immunity against only certain lethal doses of venom, and, for each lethal dose capable of being rendered innocuous, only within certain definable intervals of time.

The extraordinary result was thus obtained that serpents' venom introduced into the stomach in large quantity—in a quantity, which, if injected under the skin, would be sufficient to kill 1000 animals of the same species and weight—while it failed to produce any definite symptoms of poisoning, nevertheless produced complete protection against the lethal effect of doses of venom more than sufficient to kill the animals. There is a probable significance, further, in the general resemblance between the results of these experiments and those already described in which antivenene, and not venom, was introduced into the stomach. The bearing of these facts is obvious upon discussions relating to the production of immunisation against the toxines of diseases and to the origin of the antidotal qualities of the blood-serum used in their treatment. It is difficult to account for them otherwise than by supposing that the venom while in the stomach had been subjected to a process of analysis, by which the constituents which are poisonous had failed to be absorbed into the blood, or had been destroyed in the stomach or upper part of the alimentary canal, while the constituent or constituents which are antivenomous, or rather antidotal, had passed into the blood, in sufficient quantity to protect the animals against otherwise lethal administrations of venom. I confidently anticipate that this natural process of analysis will, by-and-by, be successfully repeated outside of the body by chemical methods.

It is further to be observed that by stomach administration a degree of protection was acquired in a few hours against lethal doses, such as cannot be attained until after the lapse of several weeks by the method of injecting under the skin a succession of gradually increasing doses of venom. In circumstances, which are no doubt exceptional, the application of this method may therefore acquire some practical value.

Early this evening, I had occasion to point out that the leading facts connected with immunisation or protection, now being advanced as scientific novelties, had apparently been ascertained and practically applied for centuries by savage and uncultured tribes and sects in various parts of the world. In regard to the results I have last described, also, I discover that I have been anticipated by a long-existing and even now prevailing practice of unlearned savages. I have found in the *Lancet* of 1886, an interesting note by Mr. Alford Bolton, containing the following: "The most deadly snakes here are the puff-adders, the yellow cobra capellas, the horn-snakes, and the

night adders. Whilst frequently hearing of horses and cattle rapidly succumbing to the bites of these snakes, it appeared strange that the natives themselves, who mostly ramble about the Veldt almost naked, seldom or never appeared to suffer any further inconvenience from the bites of poisonous snakes than would be usual from any accident which would cause a local inflammation; and, on close inquiry, I found that the natives in Bushmanland, Namaqualand, Damaraland, and the Kalahari, are in the habit of extracting the poison-gland from the snake immediately it is killed, squeezing it into their mouths, and drinking the secretion, and that they thereby appear to acquire absolute immunity from the effects of snake-bites." He proceeds to describe the native treatment of snake-bite, and then adds: "Having a month ago seen a native named Snellsteve, who is a snake-poison drinker and collector, put his hand into a box containing two yellow cobras, and several horn- and night-adders, in doing which he was severely bitten, and has never since suffered anything more than a little pain, such as might be caused by any trivial mishap, I feel I can no longer refuse to believe in the efficacy of the snake virus itself as a remedy against snake-poison." Among several communications which I have recently received on the subject, is one from Dr. Knobel, of Pretoria, who writes that when a boy he came into frequent association with a Bushman shepherd, who informed him that he had for years been in the habit of swallowing small quantities of the dried venom-glands of serpents, and he averred that by doing so he obtained protection against serpents' bites, for he had often been bitten without any other ill effect than that an irritable wound was produced. He stated that the swallowed venom of the cobra produced greater protection than the venoms of less poisonous serpents; and that not only was this benefit produced by the swallowing of venom, but that there was also produced an exciting intoxication, differing from that of Indian hemp in so far that the venom always produced the same degree of intoxication with a definite quantity, however frequently it was taken, while the effects of the Indian hemp were gradually lessened by repetition. Another correspondent, Dr. Laurence, of Cape Colony, writes that a Kaffir boy, "aged about twenty-five years, frequently brings me for sale snakes of all kinds. . . . I have frequently seen this boy take hold of some most deadly snakes, especially the well-known puff-adder, which he will allow to bite him with impunity. Yesterday, I obtained from him what he states as the reason why the poison did not harm him. When a little boy, while walking in the Veldt, a puff-adder fastened on his leg. He shook it off, calling to his father, who a few minutes after killed the puff-adder and removed the poison glands. He then made small paper pellets and dipped them in the poison, and administered one occasionally to the boy, who stated that that cured him. He expressed his willingness to let any snake bite him."

Several other letters I have received describe similar events, and also confirm the statement of Dr. Knobel, that serpents' venom

produces intoxicating effects in man, evidences of which have been observed in many of theexperiments made by me on the lower animals.

The results of the experiments in which the venom was introduced into the stomach, probably also afford an explanation of the protection enjoyed by certain snake-charmers, as well as by other individuals who claim to be protected, whether members of special sects or not; for although inoculation of the venom is apparently sometimes practised by them, and protection is no doubt assisted and maintained by the bites, which with impunity they frequently receive, they are known also to swallow the venom or the dried poison-glands containing it.

These experiments also seem to throw a new light upon the clearly established protection possessed by venomous serpents against their own venom. They suggested the importance of determining if the blood-serum of venomous serpents contains, as does that of artificially protected animals, an actual substance possessing antivenomous properties.

In order to arrive at some definite conclusions on this subject, I last year obtained from India several living specimens of the Hamadryad (*Ophiophagus elaps*), a serpent of greater size and more aggressive disposition than the cobra, and reputed to be as deadly as it. From the blood of several of these serpents a serum was separated, which when dried gave a product having the same physical characters as the antivenene from artificially protected animals. It was tested against cobra venom, both when mixed with rather more than a minimum-lethal dose, and also when injected thirty minutes after this lethal dose of cobra venom. In the former case, ·25 cc. per kilogramme of this natural antivenene prevented death; and, indeed, so perfectly antagonised this certainly lethal dose that no decided symptoms of poisoning were manifested. In the latter case, 5 cc. per kilogramme was found to be a sufficient quantity to prevent death. I hope by-and-by to extend these observations by testing the antidotal power of this serum against the venom of the actual Hamadryads from whose blood it had been separated.

A determination of this kind has, however, been made with the blood-serum and venom of the Australian black snake (*Pseudechis porphyriacus*), a deadly serpent whose bite produces intense destructive changes, not only at the place where it has been inflicted, but also in the blood and in many of the organs of the body. When the blood-serum and the venom of this serpent were mixed together outside of the body, and then injected under the skin of a rabbit, it was found that half a cubic centimetre per kilogramme of the blood-serum was sufficient to prevent death from rather more than the minimum-lethal dose of venom.

Notwithstanding the obliging co-operation of the India Office, I have not yet succeeded in obtaining the blood-serum of the cobra, but it may safely be anticipated that it also will be found to possess antivenomous properties.

It has thus been shown that venomous serpents themselves possess a definite substance in the blood-serum which is capable of protecting them against their own venom, and the venom of other serpents. The results of the experiments made by stomach administration of venom, supply at the same time an explanation of one, at least, of the methods by which this substance is introduced into the blood. This natural antivenene, however, is apparently not so powerfully antidotal as the antivenene obtained by the process of artificial protection.

The foregoing statements, although referring mainly to observations on the lower animals, have, probably in every particular, a very direct bearing upon both the prophylaxis and treatment of snake-poisoning in man.

Some little consideration of the details of the application of the antivenene and the employment of auxiliary measures may, however, be serviceable; and, equally of practical service, some consideration of the probable limitations to the capacity of antivenene as an antidote.

In the meantime, I cannot adduce any actual experience of its use in human beings, as although a considerable quantity, both in the liquid and dry state, was last summer sent to India, and a smaller quantity to Africa, no opportunity for using it as an antidote has as yet occurred in the districts to which it had been sent.

But, first, let me say in regard to the altogether unsatisfactory experience of the use of medicines, ordinarily so-called, that I am not prepared to take the extreme position that no good can be done by their employment. While the evidence shows that no one of the very large number of those that have been recommended as antidotes is able, in any conditions of administration, to prevent death after the reception of even the smallest lethal dose of venom, it still may be that, by the physiological effects which they produce, they may assist any efficient antidote, such as antivenene, in preventing death; and also, by prolonging life, increase the opportunity for a more thorough use of this antidote. In this category I would especially place medicines which increase excretion, such as diaphoretics and diuretics; many of the rapidly acting stimulants of the circulation, such as alcohol and the old snake-remedy, ammonia; and stimulants of respiration, such as atropine and strychnine, the latter of which is enthusiastically championed by Dr. A. Mueller, of Sydney. And not only medicines, but also any measures that are available for these purposes, including artificial respiration, so distinctly indicated as a probably valuable therapeutical application in snake-bite by Fayrer and Brunton, which, though shown by the Indian Snake Commission to be incapable of preventing death when alone trusted to, was also shown to possess the valuable auxiliary power of prolonging life.

The first measure, however, that is usually and properly taken in the treatment of snake-bite, is to restrict, as far as is possible, the

absorption of the venom into the blood-vessels, from the place into which it has been injected by the poison-fangs, by separating this place from the more central parts of the body by a tight ligature. The efficiency of this measure, preventive rather than curative, is fortunately aided by the circumstance that snake-bites are most usually inflicted at parts to which a ligature can conveniently be applied; for in fifty-four cases collected by Wall, the part in nearly 89 per cent. of the cases was on the arms or legs. The ligature having been applied, whenever it is possible to do so, the next measure to adopt is to open up with a knife, to a considerable depth, the minute though deep punctures made by the fangs, and then to apply suction to the wound. Justification is found for this procedure in the fact, demonstrated by experiment, that notwithstanding the rapidity with which venom may be absorbed, a portion of it still remains for a considerable time in the tissues immediately surrounding the wound. This has been clearly demonstrated by both Kaufmann and Wall. The suction may be produced by the mouth, and in the absence of more effective apparatus this ready method would be serviceable, while it is attended with danger to the operator only in the infrequent occurrence of fissures or abrasions of the mouth. It is, however, more effectively and without any risk accomplished by a suction pump, such as the most useful pump invented by Mr. Andrew Smith, of Cape Colony, which I now show.

These steps having been taken, antivenene should be injected into the tissues at and near the wound and, also, under the skin above the ligature; and the ligature should not be removed until at least half an hour after a sufficient quantity of antivenene has been injected under the skin above it.

But the important question has yet to be answered, What is a sufficient quantity? The whole tenor of my remarks to-night has been to show how necessary it is to bear in mind that there is a definite relationship between the dose of venom received and the dose of antivenene required to antagonise it, and that this relationship also varies with the conditions of the administration of the antivenene, and, especially, with the interval of time that elapses between the reception of the venom and the administration of the antivenene.

In snake-bite in man it is impossible to estimate the dose of venom which has been injected, for the nature of the symptoms in the patient cannot give the information even approximately. In searching for a solution of this problem, several facts may be taken into consideration from which assistance may be obtained. And, firstly, what is the probable quantity of venom that a serpent injects into a wound? Some data for answering this question have, very kindly, been obtained for me by Brigade-Surgeon Lieut.-Colonel Cunningham, of Calcutta. Taking nine adult cobras, healthy and vigorous, he collected from each the venom ejected at a single bite,

dried and weighed each collection separately, and sent me the weights. They are as follows :—

(1) 0·726 gramme.	(4) 0·114 gramme.	(7) 0·239 gramme.
(2) 0·262 „	(5) 0·132 „	(8) 0·306 „
(3) 0·115 „	(6) 0·113 „	(9) 0·253 „

The total venoms yield an average of 0·255 gramme for each bite ; but, if the exceptionally large quantity stated in the first figure be excluded, the average for the remaining eight becomes ·195 gramme. It must also be considered that these quantities were obtained in the most favourable conditions for securing the total quantity ejected at a single bite, whereas in actual practice the conditions are less favourable for the insertion of the total available venom into the tissues of the victim.

Reverting now to determinations of the minimum-lethal dose for the lower animals, we find that if the minimum-lethal dose for the cat be adopted as being the same as that for man, the total quantity of dry cobra-venom required to kill a man of ten stones weight would be ·317 gramme, which is considerably more than the quantity, judging from the above averages, that a cobra is usually able to eject during a single bite. It would therefore appear necessary to assume that the minimum-lethal dose per kilogramme for man is smaller than for a cat; but, as it is probably greater than for a rabbit, we may for convenience assume that it is twice that dose. In this case, the smallest quantity required to produce death in a man of ten stones would be about ·0317 gramme, which, however, seems to be considerably less than the quantity which a fresh cobra has at its disposal. Applying now the facts that have been stated in the series of experiments where the smallest quantity of antivenene required to prevent death when injected thirty minutes after twice the minimum-lethal dose was determined, it will be recollected that that quantity is 5 cc. per kilogramme of animal. Taking this as a basis for the dose of antivenene, in order to prevent death in man from the estimated minimum-lethal dose of cobra-venom, so considerable a quantity as 330 cc., or about 11½ ounces, of antivenene would be required, if the antivenene be injected not much longer than thirty minutes after the bite had been inflicted. This, though a large, is by no means an impossible dose, and it could, without much inconvenience, be introduced under the skin at several parts of the body.

On the other hand, the estimate which I have adopted of the minimum-lethal dose for man may be too high a one, and if it should prove to be nearer that for the rabbit, then the quantity of antivenene required to prevent death, if administered half an hour after the snake-bite, would be reduced to about four ounces. It is also to be recollected that if dry antivenene be used, it may be dissolved in a much smaller quantity of liquid than is required to restore it to its original bulk.

As to the probability, in a fatal snake-bite, of the quantity of venom received by the victim being only about, and not much in excess of, the minimum-lethal dose, it would appear that, in many cases, even so large a dose is not introduced; for general experience indicates that the majority of persons who are bitten actually recover, whatever treatment is adopted. Sir Joseph Fayrer also shows, in his classical 'Thanatophidia,' that in 64 per cent. of fatal cases of snake-bite in India, the victims survived the infliction of the bite for periods of from three to twenty-four hours; and this duration of life implies that the dose of venom received could not have been much greater than the minimum-lethal.

It must be admitted, however, that even for the minimum-lethal dose of venom, the quantity of antivenene required to prevent death in man is probably inconveniently large, especially if, in the treatment, reliance is placed solely upon the administration of antivenene, to the exclusion of all or several of the auxiliary measures to which I have referred. It is desirable, also, that the antivenene treatment should be a practical one, not only for doses of venom which do not much exceed the minimum-lethal, but also for the considerably larger doses that are occasionally introduced in snake-bite.

To attain this object, further work is required in order that there may be obtained an antivenene even more powerful than that whose antidotal capabilities I have described.

I am not sanguine that this will be accomplished by carrying to a higher degree the process of artificial protection in animals. A comparison of the antivenene of rabbits which had last received thirty times the minimum-lethal dose of cobra venom with that of other rabbits which had last received fifty times that dose, has shown that the latter has but little antidotal advantage over the former, and has suggested that, in the process of artificial protection, the saturation point of the blood for antivenene is reached before the possible maximum non-fatal dose of venom has been administered.

I would anticipate with more hope the results of endeavours to separate the true antivenomous principles from the inert constituents of the blood-serum with which they are mixed; and although the required chemical manipulations are attended with many difficulties, some success has already been obtained in effecting this separation.

In the foregoing remarks, it has, however, been shown that even with the antivenene whose properties have been described, human life may be saved in a considerable, if not in a large, proportion of the cases of snake-bite which would otherwise terminate in death. The attainment of this result is a satisfactory one; for the mortality from snake-bite is large, and is not restricted to the 20,000 deaths which annually occur in India, but includes additional thousands in all the tropical and sub-tropical regions of the world.

[T. R. F.]

LONDON:
PRINTED BY WILLIAM CLOWES AND SONS, LIMITED, STAMFORD STREET
AND CHARING CROSS.

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