

AN ANTI-SERUM FOR SCORPION VENOM.

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MANY deaths occur every year in Egypt as the result of scorpion sting. These deaths are practically confined to Cairo and Upper Egypt, and are almost entirely among children. Unfortunately, owing to local conditions, it is almost impossible to obtain really accurate figures for this mortality, but some idea may be formed from the statistics given below of some of the larger towns.

The mortality in the country is probably considerably greater than in the towns, which have many European houses where the scorpion naturally does not find such favourable conditions for its existence as in the small mud huts of the villages.

The following are the statistics for some of the larger Egyptian towns, for a period of seven years, viz.: 1901—7¹.

Name of town	Population	Deaths from scorpion sting 1901—7	Per 1000 per annum
Cairo	600,000	153	0·036
Fayoum	35,000	29	0·120
Beni-Souef	19,000	18	0·130
Minia	25,000	15	0·080
Assiout	47,000	87	0·260
Sohag	15,000	33	0·320
Keneh	33,000	46	0·200
Assouan	13,000	63	0·640

Some of these figures are very high, notably in the case of Assouan, with 0·64 deaths per 1000 of the population, which represents 1·6% of the total death rate. The deaths occur almost entirely among children under 12 years of age though adults occasionally die.

¹ Births and Deaths in the principal towns of Egypt, 1901—7, Statistics Department of Public Health.

Apart from the mortality which it causes, the scorpion is a very serious pest, for even when the sting is not fatal it gives rise to very great pain, and often to severe collapse.

In the summer of 1906, Sir Horace Pinching, then Director General of the Egyptian Public Health Department, suggested to the writer the investigation of the venom of the scorpion, with a view to the possibility of preparing an anti-serum for use in Egypt.

Arrangements were made for the collection of scorpions in Upper Egypt, and as soon as sufficient of these were available, the immunisation of animals was commenced.

Historical.

Many scattered references to the effects of scorpion stings are found in the early literature of medicine, but the first systematic investigation of the venom appears to have been undertaken by Valentin (1876), Joyeux Laffuie (1882), and Paul Bert (1885). Later, the venom was more fully investigated by Wilson (1904), whose monograph on the subject includes a very complete account, not only of the character of the venom, but also of its effects on isolated nerve and muscle.

Preparation of scorpion toxin.

According to Wilson (1904, p. 11) of the numerous species of scorpion found in Egypt, only three are at all common, viz.:—

“(1) *Buthus quinque striatus*. Common throughout the country, more especially in Upper Egypt.

(2) *Prionurus citrinus*. Found in the desert near Cairo, and in the neighbourhood of Alexandria.

(3) *Buthus maurus*. A black scorpion which seems to be particularly common at Mariout to the west of Alexandria, but which is also found at Sakkara, south-west of Cairo.

Buthus quinque striatus is undoubtedly the common variety, and is generally thought to be the most dangerous; it is frequently found in houses and is the species in all probability giving rise to the numerous cases of scorpion sting said to be not uncommonly fatal in Upper Egypt.

The adult size of the three species mentioned is about the same, the maximum length in each case being about 10 cm.

Buthus quinque striatus is of a sandy yellow colour tending to

brown, the segments of the tail being roughly cylindrical in section, the telson or sting being relatively large.

Prionurus citrinus is of a greenish yellow colour, the tail segments being broad and carinated, and the sting well developed.

Buthus maurus is almost black on the upper surface, brown on the under surface of the abdomen, the comb-like sense organs attached to the abdomen just behind the posterior limbs being of lemon-yellow colour, and the tail segments broad with a deep median groove the lateral ridges of which are distinctly serrated, and the sting relatively small."

In collecting scorpion venom on a large scale it was impossible to make any determination of species, as the scorpions were obtained from various towns of Upper Egypt by means of a small reward given to people bringing them in to the government medical officers.

The latter, with a pair of scissors, snipped off the sting with the last joint of the tail. The stings and venom glands so obtained, after being dried for a day or so in the sun, were posted to Cairo, and there placed in dessicators over calcium-chloride where they were kept until required for use.

When a solution of the venom was required, the dried stings and venom glands were ground to a fine powder in a Turkish coffee mill, and added to a 0.8 % saline solution, in the proportion of one sting to 1 c.c. of saline solution.

The resulting suspension was agitated by means of a mechanical stirrer for one or two hours, and then centrifuged. The supernatant opalescent fluid was at first filtered through a Berkefeld or Chamberland candle, but later it was found more convenient to clarify it by the addition of a little aluminium sulphate and lime water in order to get rid of any spores that might not have been removed by the centrifuging. It was then placed in stoppered bottles with a little chloroform, and kept in the ice-safe.

In grinding large numbers of the glands, it is advisable to have a closed mill, as the resulting powder even in the smallest quantity is exceedingly irritating to the nasal mucous membrane, giving rise to violent coryza.

Prepared by the above method, the venom was found to be very stable, and the method is more practical than extraction by glycerine, particularly when the venom is to be employed for immunisation of animals, as before use the chloroform is easily removed by allowing filtered air to bubble through the solution.

As a certain amount of decomposition had often occurred in the tails before they became dry, the solution obtained was far from being a pure solution of venom; but the various methods of purification which were tried (e.g. precipitation by alcohol, ammonium sulphate, etc.) were found either to be too complicated for practical use, or to result in too great a loss of the venom, and as any products of decomposition remaining in the solution were not present in sufficient quantities to cause inconvenience the simpler method was adhered to.

General characteristics of the venom.

The venom is soluble to some extent in distilled water, i.e. it may be extracted from the glands by rubbing them with water, but as was pointed out by Wilson (1904, p. 13) it is much more completely extracted by normal saline. It is also soluble in glycerine.

Like the bacterial toxins, it is precipitated by saturating its solution with ammonium sulphate, or by pouring the solution into excess of alcohol.

The venom passes through a Chamberland filter without great loss.

In one experiment, the venom glands from 400 scorpions were powdered and shaken with 400 c.c. of 0·8 % saline solution, and the suspension allowed to stand over night in the ice-safe. Next morning the supernatant opaque fluid was tested on young pigeons and 0·3 c.c. was found to kill a pigeon of 250 grammes in two hours.

The fluid was then divided into portions which were filtered through Chamberland and Berkefeld candles respectively.

The Chamberland filtrate killed a pigeon of 250 grammes in a dose of 0·5 c.c. in 80 minutes, while of the Berkefeld filtrate almost 1 c.c. was required. This experiment was repeated with similar results. It is interesting to note that the toxin appears to pass through the close grained porcelain Chamberland filter more easily than the comparatively porous Berkefeld, but it is possible that this is accounted for by some absorption in the case of the Kiesselguhr filter.

Compared with the bacterial toxins, scorpion venom is very stable, and can be kept in solution in the ice-safe for many months with only a slight loss of toxicity.

It is unaffected by drying, and as was shown by Wilson, is very resistant to putrefaction, and is not affected by heating to 100° C. for short periods, but it is destroyed if the heating is continued from 12 to 13 minutes.

One cubic centimetre of scorpion extract, which killed a guinea-pig of 400 grammes in 20 minutes, when given subcutaneously after being heated for an hour at 90° C. killed an animal of the same weight in $1\frac{3}{4}$ hours.

As has already been mentioned, chloroform and glycerine appear to have no action on the venom, but it is very susceptible to certain other chemicals: e.g. ammonia and iodine.

Very small quantities of iodine render the venom quite inert, as is shown in the following experiment, where 0·5 c.c. of scorpion extract was mixed with varying quantities of ordinary Gram's solution, and after standing for one hour, injected subcutaneously into guinea-pigs of from 340 to 360 grammes weight:—

Guinea-pig	Mixture used	Effect
No. 1	0·5 c.c. scorpion extract with 0·2 c.c. Gram's solution (Mixture showed faint iodine tint.)	Lived. No symptoms.
No. 2	0·5 c.c. scorpion extract with 0·1 c.c. Gram's solution (Mixture colourless.)	Lived. No symptoms.
No. 3	0·5 c.c. scorpion extract with 0·075 c.c. Gram's solution (Mixture colourless.)	Died in 60 minutes.
No. 4	0·5 c.c. scorpion extract with 0·05 c.c. Gram's solution (Mixture colourless.)	Died in 45 minutes.
No. 5	0·5 c.c. scorpion extract with 0·02 c.c. Gram's solution (Mixture colourless.)	Died in 30 minutes.

As the venom of one scorpion is represented by 1 c.c. of the extract, this quantity is completely neutralised by 0·2 c.c. of Gram's solution, corresponding to two thirds of a milligramme of pure iodine.

Susceptibility of various animals to the toxin.

Most of the vertebrates which have been tested, with certain exceptions, are susceptible to scorpion venom, though the degree of susceptibility shows considerable variation in the different species.

The *horse* is highly susceptible: in one case 1 c.c. of a toxic extract, corresponding to the venom of one scorpion caused very severe illness, with marked salivation. The *goat* is considerably less susceptible, the subcutaneous injection of a dose of 5 c.c. of a similar extract into a young

animal only causing it pain, profuse salivation and straining: the next morning the symptoms had completely disappeared. The *dog* is susceptible, but the susceptibility appears to vary in different individuals. The *common fox* was found by Wilson to be susceptible. The *mongoose* is very resistant, but not completely immune. The *guinea-pig* is very highly sensitive to the venom, young animals of 300 grammes being killed by 0.05 c.c. of the extract (i.e. 1/20 of the total poison of a scorpion). The *rabbit* is more resistant than the guinea-pig. 1 c.c. of the extract being required subcutaneously to kill an animal of 1000 grammes. The *white rat* and *white mouse* are highly susceptible. *Mus alexandrinus* and *Mus musculus* are both susceptible. The *pigeon* is very susceptible: 0.17 c.c. of the toxic extract being sufficient to kill a bird of 240 grammes. A *tortoise* (*Testudo graeca*) weighing 1275 grammes was injected subcutaneously with 2 c.c. of somewhat weak extract and found dead next morning. A large *lizard* (*Uromastix spinifex*) showed very marked symptoms after an injection of 3 c.c. (Wilson). *Frogs* and *toads* were found by Wilson to show marked symptoms after a large dose of the poison.

Animals immune to the poison.

A very interesting observation was made by Wilson who showed that certain desert animals, which live under conditions which must constantly bring them into contact with scorpions, possess a high degree of immunity to scorpion venom.

This he showed to be the case with the following:

- Desert rat. (*Gerbilus pyramidum*.)
- Jerboa. (*Jaculus jaculus*.)
- Fennix Fox. (*Vulpes zerda*.)
- Zarilla. (*Ictonia lybica*.)
- Hedgehog. (*Erinaceus auritus*.)
- Varanus cinereus*.

In this connection it is interesting to note that the *Acomys cahirinus* (Prickly mouse) which is very common in native houses, shows a very marked immunity to the venom, a dozen of 0.5 c.c. of a toxic extract (corresponding to half the total venom of a scorpion) gives rise to no symptoms, whilst the ordinary mouse (*Mus musculus*) and rat (*Mus alexandrinus*) are very susceptible to the poison.

A non-poisonous snake (*Zamenis*) which received 2 c.c. of scorpion extract showed no symptoms of any kind and remained well.

Action of the toxin on susceptible animals.

This will not be gone into here, as the physiological action of the venom has been investigated by Wilson, who, in his monograph, gives very complete details not only of the symptoms in animals, but also of the action of the toxin on isolated muscles and nerves, as well as the post-mortem appearances.

He summarises the symptoms as follows, in order of their appearance.

- (a) Local irritation.
- (b) Muscular twitching, chiefly confined to the head and neck.
- (c) Jumping movements.
- (d) Lachrymation.
- (e) Milky orbital secretion. Nasal secretion, and salivation.
- (f) Prolonged muscular spasms; most marked in the hind limbs, but affecting the muscular system generally.
- (g) Erection of hair; especially on the fore part of the body and face, to which it gives a peculiar swollen appearance over the jaws.
- (h) Passage of liquid faeces (often absent).
- (i) Erection of penis, and discharge of semen.
- (j) Apparent paralysis; the animal lying on the side, abdominal muscles usually tense; and breathing shallow; expiration prolonged.
- (k) Symptoms of asphyxia; blueness of mucous membranes. Convulsions; intermittent gasping respiration.
- (l) Cessation of respiratory movements. Gradual slowing and stoppage of heart-beat.

Talaat (1904) concludes that the toxin acts on the nerve centres, especially the medulla and spinal cord, and in support of this view he gives the following experiment:—

“A guinea-pig of 750 grammes was stung by a scorpion and died after 70 minutes, after showing all the symptoms of acute poisoning. The medulla and spinal cord were removed and allowed to macerate for 7 days in 25 c.c. of glycerine. After this time 1 c.c. of the glycerine extract injected intraperitoneally into a guinea-pig whose weight is not stated was found to cause death with typical symptoms in 60 minutes.”

Now in the above experiment the animal that was stung by the scorpion did not die until 70 minutes after the sting, showing that it

could not have received more than two or three times the minimal lethal dose of the venom: the glycerine extract of the medulla and the spinal cord however, according to the test on the second guinea-pig, contained at least 25 minimal lethal doses, which would mean that an actual increase of the venom had taken place in the body.

There does not appear to be any evidence of the fixation of the scorpion venom by the central nervous system as was shown by Wassermann to occur in the case of tetanus toxin. This is seen in the following experiment:—

The brain of a healthy guinea-pig was rubbed down to an emulsion in a mortar with as small a volume of normal saline as possible, and the following mixture made:—

2 c.c. scorpion extract,
5 c.c. brain emulsion, and
3 c.c. normal saline.

The mixture was kept at 37° C. for an hour, at the end of which time it was centrifuged, and the supernatant fluid decanted. The deposit of brain substance was washed once with fresh saline, and again centrifuged, and the deposit made up to the same volume as the original supernatant liquid. The supernatant fluid from the first centrifugalisation and the washed deposit of brain substance were then tested on guinea-pigs.

Supernatant fluid.

No. 1.	Guinea-pig: 2 c.c. intraperitoneally.	Died in 90 mins.
No. 2.	„ „ 4 c.c. „	„ „ 45 „

Washed deposit.

No. 3.	Guinea-pig: 2 c.c. intraperitoneally.	Lived, no symptoms.
No. 4.	„ „ 4 c.c. „	„ „ „

A further experiment was made in which a young guinea-pig (350 grammes in weight) was inoculated with 2 c.c. of scorpion extract (T. 19) which represented over 20 M.L.D. Death occurred in 19 minutes. The medulla and spinal cord were at once emulsified in 5 c.c. normal saline, and the whole mixture injected intraperitoneally into another guinea-pig. This animal remained well, and showed no symptoms.

Action of the venom on the blood.

Unlike snake venom, scorpion venom appears to have no effect on the coagulability of the blood, nor does it appear to have any haemolytic action on the red blood corpuscles.

The corpuscles of the ox, sheep, guinea-pig, rabbit, and pigeon were tried, but showed no trace of haemolysis. This is in accordance with the observations of Paul Bert, and Wilson.

Flexner and Noguchi (1902) showed that snake venom has no action on certain red blood corpuscles if these are thoroughly freed from serum, and that the haemolysis which takes place, when serum is present, must be due to the complement like action of some substance in the serum. Calmette (1902) showed that this substance, unlike ordinary serum complement, is not destroyed by heating to 62° C., and Kyes was able to identify it as a lecithin. Kyes (1903) then made experiments with scorpion venom, and found that this venom which was only slightly haemolytic for guinea-pigs' corpuscles, and without any action on other corpuscles, on the addition of lecithin became haemolytic for all the corpuscles on which it was tested, and almost equally for all, being about 20 times weaker than cobra venom.

He was also able by the usual method to prepare qualitatively a typical lecithide of the scorpion venom. Kyes does not mention the species of scorpion with which these experiments were made, but states that they were obtained from the Botanical Gardens of Buitensorg. These experiments of Kyes have been repeated by the writer with the mixed venom of the Egyptian scorpions, but the results so far have been quite negative as regards the complement-like action of lecithin, as is shown by the following experiments, made with washed guinea-pigs' corpuscles.

Action of scorpion venom, with and without lecithin, on guinea-pig corpuscles.

Guinea-pigs' red blood corpuscles three times washed with normal saline solution. Scorpion venom filtered through Berkefeld candle.

Merk's "Ovolecithin" 1 1/2%	Scorpion toxin	Scorpion toxin+lecithin
	No trace of haemolysis	No trace of haemolysis
1 c.c.	" "	" "
0.5 c.c.	" "	" "
0.2 c.c.	" "	" "
0.1 c.c.	" "	" "
0.05 c.c.	" "	" "
0.02 c.c.	" "	" "
0.01 c.c.	" "	" "

In this experiment the presence of lecithin appears to have no action in producing haemolysis. The same experiment was repeated with rabbits' red blood corpuscles with similar negative results.

It was then thought that possibly the lecithin was at fault, and the experiments were repeated with *afga lecithin* as recommended by Kyes, but again with negative results.

An attempt was then made to prepare a scorpion lecithide, using the method employed by Kyes. The poison of about 80 scorpions was used. The stings were ground, extracted with saline, the extract filtered through cotton wool, and then evaporated to dryness over sulphuric acid. The dried extract was ground in a mortar with distilled water, and mixed with about half its volume of 15% *afga lecithin* in chloroform, and kept over night in the incubator, but from time to time being shaken up. The next morning this was centrifuged, the clear chloroform layer pipetted off and poured into 6 to 7 times its volume of pure ether. Not the slightest trace of precipitate was formed.

The difference between these results and those of Kyes may be due to the different species of scorpions used, as in his paper Kyes does not state what scorpion he worked with.

The venom does not appear to have any marked action on the coagulability of the blood.

Action on the mucous membranes.

As has already been stated the powdered glands are intensely irritating to the nasal mucous membrane, so much so, that before a well-closed mill was used for grinding the glands, it was found necessary to apply a layer of cotton wool to the nostrils to act as a filter during the grinding process.

The toxic extract of the glands (1 c.c. representing the venom of one scorpion) seems however to have very little irritant action upon the conjunctiva, as it did not give rise to any obvious redness, when dropped into the conjunctival sac in the rabbit or guinea-pig.

Anti-venom. Historical.

In most countries where scorpions are common, persons are found who handle these animals quite freely and without fear. It is somewhat difficult to know whether this is due to any actual immunity to the venom, or merely to the knowledge of the creature's habits, and a certain skill in handling.

The above does not refer to the Arabs who exhibit scorpions in the streets of Cairo for the entertainment of tourists, as in this case the scorpions will generally be found to have the points of their stings cut off, so that they are practically harmless.

It seems however that individuals do exist who have a true acquired immunity. Schinz (1891) states that in German S. W. Africa a number of the population render themselves immune to scorpion poison. Wilson (1904, p. 38) referring to this matter says:—

“In India, South Africa, and probably Egypt, a certain number of individuals among natives have methods of rendering themselves immune to the venom. The two men from whom I have obtained scorpions handle them without any fear; one of them informed me that he was unaffected by scorpion stings, as he had accustomed himself to the venom by pricking the skin from time to time with the sting removed from a dead scorpion; he stated that he carried the stings with him when on a journey, since if he failed to use them for any length of time he would lose the protection conferred upon him. I have never seen this man stung by a scorpion; whether the process would be sufficient to confer immunity is difficult to say: I find however that a small guinea-pig may be killed by pricking the skin with a sting recently removed from a scorpion, even if care be taken to avoid expressing any venom from the gland. It is probable therefore that there is always a certain quantity around the point at which the venom ducts open.

From this it may be regarded as probable that pricking the skin with a sting from a dead scorpion would introduce a small amount of venom into the system, and no doubt gradually confer immunity.”

The natural immunity of certain cold-blooded animals has already been referred to, and the scorpion is known to be immune to his own venom.

It is a popular belief that when a scorpion finds himself in a position where death appears inevitable, that he commits suicide by stinging himself, and that this can easily be demonstrated by placing a scorpion on any flat surface, and surrounding him with a ring of burning spirit. After running about, and finding no way of escape, he is said to curl his tail over and sting himself in the body.

This question was investigated experimentally by Bourne (1887) in Madras, who worked with a large species of Indian scorpion and showed that not only was the so-called suicide a myth, but that the scorpion is immune to scorpion venom.

Metchnikoff (1901, p. 344) confirmed this, and in addition made

the very interesting observation that the blood of the scorpion is distinctly anti-toxic as regards scorpion venom, and to so great a degree that one-tenth of a cubic centimetre (.1 c.c.) of scorpion blood is sufficient to protect a mouse against a dose of the venom which would cause death in half an hour.

This anti-toxic power of the blood he found to be the same in both *Scorpio afer* and *Androctonus*.

He points out that this is the only example known of an anti-toxin occurring in an invertebrate, and discusses the question as to whether the anti-toxic power of the blood must be regarded as natural and innate or as having been acquired during life.

Metchnikoff (1901, p. 384) also showed that the blood of the crayfish, when injected into mice, protects the latter against doses of scorpion venom given from a few minutes to an hour later; a most interesting fact, seeing that the crayfish itself is highly susceptible to the venom.

Kobert (1902) refers to the scorpion poison as giving rise to an antibody when injected, but gives no references.

Calmette (1905, 1907) found the venom of the *Scorpio occitanus* was neutralised by the serum of animals immunised against snake poison, and states that Metchnikoff has verified the fact.

Nicolle and Catouillard (cited by Calmette, 1907, p. 293) on the other hand found that the same anti-venom was inactive against the venom of the scorpion occurring in Tunis. (*Heterometrus maurus*.)

Talaat (1904) working under Ruffer at the Medical School in Cairo immunised three goats with scorpion poison and found that the serum of these animals was capable of neutralising the venom.

Preparation of anti-toxin.

For this purpose horses were employed. The horse is very highly susceptible to scorpion venom, although the degree of susceptibility varies considerably in different individuals. One horse which received the poison of only one scorpion subcutaneously, showed very marked symptoms. The site of the injection was obviously very painful, and the animal stood with the tail arched, and the hind legs were often stretched out stiffly, presenting in fact the typical picture of an early case of tetanus.

For this reason in commencing the immunisation, the venom was usually mixed with Gram's solution of iodine, this mixture causes no symptoms, but appears to create a certain degree of immunity, so that later when the venom is given alone it is better supported.

The amount of iodine in the mixture was gradually diminished, and after a few injections a small dose of the venom without iodine was given. The injections were repeated at intervals of about a week, gradually increasing the dose, until about 500 c.c. (corresponding to the venom of 500 scorpions) was reached. The animals were then allowed an interval of rest from 14 days to a month, and then bled. The whole process of immunisation is exactly similar to the immunisation of the horse against tetanus toxin. The injections were always given intramuscularly.

When large doses were given, they always caused very severe symptoms, viz. :—pain at the site of inoculation, profuse salivation, repeated straining, with passage of urine and faeces, and great restlessness and sweating. The symptoms come on a few minutes after the injection, and last several hours. Next morning the animal, as a rule, looks perfectly well, and shows nothing but some swelling at the site of the inoculation, or in the brisket.

The horses were weighed once a month, so as to have some check on their general condition; after bleeding the blood was whipped, centrifuged, and filtered through a Berkefeld filter after the addition of 0·5% carbolic acid. (Ehrlich's mixture.)

In order to obtain a good yield of serum it was unfortunately found necessary to whip the blood, as in the case of the first two horses immunised, on allowing the blood to clot, there was practically no separation of serum. Whether this was due to the treatment of the animals, or was merely an individual peculiarity of the two animals in question was not settled.

Testing the serum.

When these experiments were begun there was rather a scarcity of guinea-pigs in the laboratory, so the earlier tests were made on pigeons. The serum and venom were mixed and injected into the muscles of the breast. This method gave fairly good results, but there was always a risk of some of the mixture exuding out of the needle puncture: therefore as soon as guinea-pigs were available in sufficient numbers they were employed exclusively. Both subcutaneous and intraperitoneal injections were tried, but the latter were found to give more constant results.

The strength of the serum obtained so far from two horses whose immunisation is fairly well advanced is practically the same in both

animals; 2 c.c. of the serum being required to neutralise 1 c.c. of the scorpion extract (corresponding to the venom of one scorpion), i.e. about 10 minimal lethal doses for a guinea-pig of from 500 to 600 grammes, when given intraperitoneally.

As 1 c.c. of scorpion extract represents approximately 2 milligrammes of the venom, one cubic centimetre of the serum neutralises 1 milligramme of the venom. It is hoped however, that by continuing the immunisation, a higher value will be obtained.

As the toxic value of scorpion venom is approximately the same as that of cobra venom (Wilson) it is interesting to note that the anti-toxic value of anti-scorpion serum is of the same order of magnitude as in the case of anti-cobra sera, where 1 c.c. was found by Lamb and Hanna (1902) to be capable of neutralising about 0.7 mgm. of the pure cobra venom.

Prophylactic action of the serum.

The serum has a powerful prophylactic action. A guinea-pig weighing 690 grammes received 6 c.c. of somewhat weak anti-scorpion serum intraperitoneally, and a quarter of an hour later 0.5 c.c. of a scorpion extract (corresponding to 3 M.L.D.), the animal remained well and showed no symptoms.

Curative action of the serum.

The curative action of the serum naturally varies enormously with the method of administration of the venom and the serum. If the venom is given intraperitoneally and followed after an interval of half an hour by an injection of serum also intraperitoneally, the results are most striking, as is shown in the following table.

Curative action of scorpion anti-venom on guinea-pigs.

Venom given intraperitoneally followed by the anti-serum also intraperitoneally, half an hour later.

Animals receiving serum				Controls (no serum)			
Weight of guinea-pig	Venom in c.c.	Anti-serum in c.c.	Result	Weight of guinea-pig	Venom in c.c.	Anti-serum	Result
460 grs.	0.20	2.0	Recovered	460 grs.	0.20	—	Died in 4 hours.
510	0.25	2.0	Recovered	540	0.25	—	Died in 3 h. 20 m.
500	0.30	2.0	Recovered	480	0.30	—	Died in 1 hour.

Thus the guinea-pig which has received a dose sufficient to cause death in an hour may be saved by an intraperitoneal injection of serum given half an hour after the venom, i.e. at a time when it is seriously ill.

If the venom is given subcutaneously, followed by the serum also given subcutaneously after an interval of half an hour, the results are not so striking. The animal can be saved from the effects of one certain minimal lethal dose, but if larger quantities of the venom are given, there is only a retardation of the time of death.

This is shown in the following table:—

Curative action of scorpion anti-venom on guinea-pigs.

Venom given subcutaneously, followed by anti-serum also subcutaneously, half an hour later.

Animals receiving serum				Controls (no serum)			
Weight of guinea-pig	Venom in c.c.	Anti-serum in c.c.	Result	Weight of guinea-pig	Venom in c.c.	Anti-serum	Result
530 grs.	0.15	2.0	Recovered	520 grs.	0.15	—	Died in 7h. 30m.
465	0.20	2.0	Died in 5 h. 25 m.	470	0.20	—	Died in 3h. 30m.
480	0.30	2.0	Died in 2 h. 45 m.	500	0.30	—	Died in 47 mins.

It was thought that possibly better results might be obtained by giving the serum intraperitoneally, but this method did not show any marked difference in the results, as is shown below:—

Curative action of scorpion anti-venom on guinea-pigs.

Venom given subcutaneously, followed by the anti-serum given intraperitoneally, half an hour later.

Animals receiving serum				Controls (no serum)			
Weight of guinea-pig	Venom in c.c.	Anti-serum in c.c.	Result	Weight of guinea-pig	Venom in c.c.	Anti-serum	Result
530 grs.	0.15	2.0	Recovered	530 grs.	0.15	—	Died in 5h. 30m.
430	0.20	2.0	Died in 9 hrs.	470	0.20	—	Died in 2h. 30m.

It should be noted that these experiments were made with the serum of horses which had not advanced very far in their immunisation, so that their serum was not so powerful as that obtained later, the anti-toxic power of which was at least double that of the serum used for these tests.

Action of heat on scorpion anti-toxin.

Scorpion anti-toxin is somewhat resistant to heat, and is not destroyed by heating to 70° C. for 10 minutes.

Connection between scorpion venom and snake venom.

Calmette working with scorpions from Tunis and Egypt, found that their venom was neutralised by anti-cobra serum; 3 c.c. of the serum neutralising 1 mgm. of the dried venom; a dose which when mixed with normal horse serum, killed a control guinea-pig. These experiments have been repeated by the writer, using the mixed venom from Upper Egypt scorpions, and Calmette's snake venom; three series of experiments were made on guinea-pigs, but in no case could any protective effect be noted.

Use of the serum in man.

During the past summer a certain quantity of anti-scorpion serum has been issued to the government medical officers in Cairo, and to the hospitals of Upper Egypt for the treatment of cases of scorpion sting, and a number of reports on the subject have been received. The number of cases, however, concerning which full details are available is not yet sufficient to allow of any conclusions as to the effect on the mortality, particularly, as it is very difficult to obtain any reliable statistics as to the percentage of deaths in untreated cases.

Out of 23 cases in the town of Cairo which were treated with serum, only one death occurred. This was in a child two years old, who was not seen until two hours after having been stung. The child then received 5 c.c. of serum, but unfortunately the only serum available at the moment was a somewhat weak one, over a year old.

The general impression gained by those who have used the serum is very favourable, and almost all the reports note a very striking effect on the severe pain of the sting.

Conclusions.

1. The immunisation of suitable animals with scorpion venom gives rise to the production of an anti-venom.
2. This anti-venom is capable of neutralising the venom when mixed with it *in vitro*, and also acts both prophylactically and curatively in animals.

3. The venom is not fixed by the central nervous system, as is the case with tetanus toxin.
4. Calmette's anti-venine could not be shown to have any neutralising effect on the venom used.
5. Employed curatively in man, the serum appears to have a very marked effect on the intense pain following the sting.

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