so-called "fern-fronds" of the Palæobotanist really belonged to Spermophyta. It is at present impossible to say at what stage in the evolution of the Fern-Cycad phylum, the great change in reproductive methods came, whether it followed in the wake of general anatomical advance, or *vice versâ*. The discovery of further evidence as to the reproductive processes of these ancient plants may be expected to yield interesting results.

The authors are much indebted to Miss Marie Stopes for her valuable aid in the examination of the numerous sections in the Williamson and various other Collections.

Mr. James Lomax deserves high praise for his good judgment and skill in collecting and preparing the material for the investigation.

A full account of the fossils dealt with in the present note is in preparation, and will shortly be submitted to the Royal Society.

"On the Physiological Action of the Poison of the Hydrophidæ."

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It has long been known that the great group of the Hydrophidæ, or Sea-snakes, are poisonous, and cases of death produced by their bites have been recorded, for example, that in Sir Joseph Fayrer's work on the Poisonous Snakes of India, of the ship's captain bitten while bathing in the Bay of Bengal, with a fatal result. The fishermen on this coast are also well aware of the danger of the bites of these reptiles, and take such good care to avoid them, that deaths among them are quite uncommon as far as I can ascertain. Deaths, however, not very rarely occur among those employed in oyster fisheries in shallow water in some places on the Madras coast, owing to snakes being trodden on, so that a study of the nature of the poison of this class of snakes has a practical as well as a scientific side, and, as far as I can gather from the literature of the subject obtainable in Calcutta, it has not yet received much attention. During the last year I have been investigating the subject, and although the amount of poison I have been able to obtain has been very small, yet it has sufficed to allow of certain definite results being obtained, which will be summarised in the following paper.

The Collection of the Poison.

The Hydrophidæ are met with in large numbers all round the coasts of the Indian peninsula, and have been specially studied at Puri on the east coast in Orrisa. It was at this place that I obtained

my specimens, which are caught by the fishermen in their nets during the calm cold-weather months with a frequency which is in proportion to the number of fish taken. By small payments they were induced to bring them to a tank which I had constructed near the beach, in which they usually only lived a few days, although some survived several weeks. By making them bite on a watch-glass covered with a thin layer of guttapercha tissue stretched tightly across it, they eject the poison into the glass as clear drops free from all saliva. This is then dried over calcium chloride or strong sulphuric acid, and can then be kept indefinitely in dry well-corked glass tubes, without losing its potency. The snake which is met with in greatest abundance in Puri is the *Enhydrina Bengalensis*, measuring from three to five feet in length, and it has a thick body and a large head. This species also furnishes the largest amount of poison, and from this alone have I yet been able to obtain a sufficient quantity to allow of a considerable number of experiments being performed with it. That of four other species, belonging to three different genera, has also been obtained in small quantities, so that four out of the six genera of Indian Hydrophidæ have now been examined, and will be dealt with.

Appearance and Quantity Ejected.

When the clear watery drop of poison is dried, it forms white shining scales, freely soluble in water or normal salt solutions, and differing from the poisons of Cobra and Daboia by the absence of the yellow tinge of the latter. The only exception I have met with was a faint yellow tinge in the dried poison of a *Disteira cyanocincta*, the others having all been colourless.

The quantity of poison ejected at a single bite is of great importance in relationship to the deadliness of these snakes, and fortunately it is very small. In many of the smaller species it is often impossible to get a drop at all, but probably when free in the water they can eject more poison than when being held close behind the head, with consequent great limitations of their power of motion. The amount of dried poison obtained from a single bite of thirteen different fresh specimens of the Enhydrina was weighed, and the average quantity was found to be 0·0094 gramme, or almost one centigramme. This is very much less than that obtainable from a Cobra or a Daboia, for the average amount of poison (dried) obtained from a Cobra is, according to D. D. Cunningham, 0·254 gramme, or twenty-five times as much as is obtained from an Enhydrina. In fact, so small is the amount, that at the end of a season I had only been able to obtain about one-third of a gramme of the latter poison, and for most of which I am greatly indebted to Dr. Reid of Puri. The poison also appears to be slowly formed, as a week after a snake had been made to bite, it is usually

impossible to get any further poison from it, even if it bite vigorously. Yet if made to bite a small fish immediately after ejecting his poison, the bite is fatal in a short time, showing how fatal a trace of it is. The largest amount of poison obtained at a single bite was 0·023 gramme. The other species mostly gave a smaller quantity than the Enhydrina.

Effect of Heat on the Poison.

On boiling a dilute solution of the poison, it becomes slightly opalescent. After being boiled for 15 seconds, two minimal lethal doses were recovered from, after slight symptoms had appeared, but four minimal lethal doses proved fatal in a somewhat longer time than with unheated poisons. After boiling for one minute, four minimal lethal doses were recovered from after only slight symptoms. Thus the poison is readily destroyed by boiling for a short time, but merely bringing it to the boiling point does not materially affect its strength. Some similar experiments with Cobra poison show that the latter is slightly more resistent to heat than is that of the Enhydrina.

Symptoms Produced by the Poison.

The following symptoms are common to all the species yet tested, no differences having been met with, except with regard to the exact amount of the minimal lethal doses in different animals, which will be dealt with presently. Briefly, the symptoms produced by the poison of the Hydrophidæ may be said to be identical with those caused by cobra venom, with one very important exception, namely, that the former venom has no appreciable action on the blood, which is a marked feature of cobra toxin. In the case of warm-blooded animals, such as rabbits, rats or birds, the symptoms produced by sea-snake poisons are as follows. When minimal lethal, or slightly supra-minimal lethal doses are given subcutaneously, there is always a long period before any symptoms of poisoning occur, the time varying in accordance with the dose from half an hour to several hours, in which respect it resembles Cobra, and differs markedly from Daboia venom. If large doses are given, the symptoms set in much earlier, and in that case death rapidly results. The symptoms are best studied by the use of small doses, when the first thing noticed is that the animal remains quietly in one position, and soon begins to show signs of drowsiness, closing its eyes at intervals. Next it begins to nod its head, but every now and then appears to wake up again and opens its eyes. In the case of birds—in which the symptoms can be best seen—the subject of the experiment next sits down on the floor of the cage, and although it can be made to stand up if disturbed, yet there is now evident commencing muscular weakness, and it can only walk with an

unsteady gait. By the time this stage is reached, it will be found that the animal is breathing more deeply than normal, while the number of respirations is also increased to a variable, but often considerable, degree. From this time the picture is one of progressive paralysis, affecting all the muscles of the body, and ending with respiratory convulsions. The animal nods more and more deeply, until the nose or beak touches the floor of the cage, only to be raised again with a jerky motion. It is now unable to stand upright, and the eyes remain closed. The respirations are now very deep and laboured, and in case of birds, the beak is half open, and gaping takes place with every inspiration, while the head is more and more lowered until its vertex instead of the beak rests on the floor, and the animal is unable to raise its head. Very soon after this stage of paralysis is reached, convulsions set in, and the respirations immediately fall very greatly in frequency, while they remain deep in character, although less regularly so than before, some being shallow, so that Cheyne-Stokes breathing is somewhat simulated. The convulsions recur, and soon respiration entirely ceases, but the heart continues beating for some time, usually two or three minutes in the case of warm-blooded animals, after the breathing has entirely ceased. When the convulsions commence, the animal rolls over on its side in a state of nearly complete paralysis. Every word of the above description of the symptoms produced by the poison of the Hydrophidæ is equally true of Cobra poisoning, so much so that if two animals are severally given minimal lethal doses of these two poisons, it is impossible to distinguish which animal has received which poison by the clinical symptoms produced, a fact which I have repeatedly demonstrated.

Post mortem, after death from the poison of the Hydrophidæ, there is little or nothing noteworthy found. The seat of injection is free from extravasation of blood, and presents little or no serous effusion. The blood is of a dark colour, no doubt due to the respiratory paralysis. It is fluid on opening the heart, but rapidly clots when placed in a small test-tube, doubtless owing to the large amount of CO_2 gas in it. On standing it exudes serum, which is usually clear, but may be very slightly blood-stained, although very much less so than in the case of Cobra-poisoning under the same circumstances. There is no intravascular clotting to be found *post mortem* in the portal or other veins, as C. J. Martin first demonstrated in Pseudechis poisoning, and as occurs in acute Daboia poisoning as recently shown by Lamb. No other naked-eye changes have been found after death from sea-snake poisons.

In the case of cold-blooded animals, such as fish, which have frequently been used in these experiments, the symptoms are essentially the same in kind as in warm-blooded animals, although less easy to observe. After small doses there is the same long latent period, often

lasting for several hours. Sometimes temporary excitement with rapid motion may be observed for a short time, but more often the picture is simply one of slowly progressing paralysis. In most kinds of fish this is also very well shown by a gradually increasing difficulty in maintaining the upright position, the fish slowly turning over on one side and then swimming up into its upright position again, only to slowly sink on to its side once more. The respirations will now be found to be deeper than normal, although not as a rule quicker, but, on the contrary, they steadily slow down from the beginning of the symptoms to the end without any marked increase in the rate. This paralysis of all the muscles and of the respirations steadily progresses until convulsions set in, to be immediately followed by a very rapid failure of the respirations both in number and depth, so that they become difficult to detect, and death soon follows. The heart will be found beating some time after the breathing has ceased, and no extravasation of blood or other noteworthy change is found *post mortem*. Here again the symptoms are precisely similar in poisoning of fish by Cobra venom.

The Potency of the Poison.

By working out the smallest fatal doses of the poison per kilogramme of weight in different animals, and comparing them with those obtained by former workers for other snake venoms, we shall be able to estimate the potency of that now being dealt with. This has been done in the case of the poison of the Enhydrina by means of numerous experiments carried out with the mixed dried venom of a number of these snakes, with the following results. At the same time comparative experiments were also carried out with fresh dried Cobra venom for comparative purposes. White rats were first tested, and 0·07 milligramme per kilo weight was found to prove fatal, but smaller doses were sometimes recovered from. In the case of Cobra poison 0·5 milligramme per kilo was necessary to produce death, while Lamb in Bombay found the fatal dose of this poison for rats to be 0·33 milligramme. It is evident then that the poison of the Enhydrina is several times as potent as is Cobra venom on rats. In the case of rabbits only a few experiments have been performed, but 0·04 milligramme per kilo proved fatal in under four hours in one case, while in another 0·01 milligramme per kilo produced no symptoms but loss of appetite; but on giving a second dose of 0·02 milligramme per kilo five days later (the animal having fully recovered from the first dose in one day), death resulted in a few hours. On the other hand, Elliot found the minimal lethal dose of Cobra venom for rabbits to be 0·7 milligramme per kilo weight, so that it is evident that these animals are many times as suscep-

tible to the poison of Enhydrina as to that of Cobra, the former poison being some twenty times as potent for them as the latter—a remarkable difference.

A larger number of experiments have been carried out with birds, pigeons and fowls. These also bear out the former ones in proving the far greater potency of the poison of the Enhydrina over that of the Cobra or other poisonous snake yet examined. In the case of pigeons the minimal lethal dose, 0·05 milligramme per kilogramme, always proves fatal, while in fowls the fatal dose is 0·04. These figures may be compared with those obtained by D. D. Cunningham in his numerous experiments with Cobra venom on fowls, for which he found the minimal lethal dose to be 0·5 milligramme per kilo, so that the poison of the Enhydrina for birds is at least ten times as potent as is Cobra venom, which goes far towards neutralising the effect of the much smaller dose of poison ejected by the Enhydrina as compared with the Cobra. Taking the minimal lethal dose of the Enhydrina for warm-blooded animals as 0·05 milligramme per kilo, the fatal dose for an average man of 70 kilogrammes would be 3·5 milligrammes, or about one-third of the average amount of venom ejected by a fresh full-grown specimen of this, by far the most commonly met with, kind of snake in the Bay of Bengal. There is good ground, then, for the belief in the deadliness of the Hydrophidæ.

The Minimal Lethal Dose for Fish.

It is well known that it is necessary to give many times as large a dose of Cobra venom, in proportion to the weight of the animal, in order to kill cold-blooded animals as is required for destroying the life of warm-blooded animals. Now there is no doubt that the Enhydrina live on fish, and I have been able to ascertain that they can swallow those of considerable size. One specimen of Enhydrina after being handled in the process of taking poison vomited a piece of half-digested fish, which on comparison with complete fish of the same kind was found to have certainly been a foot or more in length, while it was over 2 inches in depth. Such a fish could not have been swallowed if it had not first been killed, or at least paralysed to a marked degree. It is of interest, then, to ascertain the minimal lethal dose of these snakes against fish, and to compare it with that of the Cobra. As I have not been able to find accurate records of the effect of Cobra venom on fish, I have also ascertained this by a series of experiments, using the hardy Mud-fish (*Saccobranchus fossilis*), which lives for weeks in a small vessel of water. It was found that 25 milligrammes per kilo of Cobra venom had to be given to be certain of causing death, although sometimes a slightly smaller dose was effective. Thus fifty times as much Cobra venom is required to

kill a fish as is sufficient to kill a warm-blooded animal—a very marked difference. On testing the same species of fish with the poison of the Enhydrina, it was found that 0·5 milligramme per kilo of freshly dissolved poison was always fatal, and sometimes a smaller dose caused death. Thus the dose of this sea-snake poison required to kill fish was but ten times as much as the minimal fatal dose for warm-blooded animals, that is, considerably less than we found to be the case with Cobra poison. In other words, the poison of the Enhydrina is much more deadly than is Cobra venom for fish, even allowing for the greater potency of the former for warm-blooded animals, so that it appears to be specially adapted for the needs of the Sea-snake, which lives on fish, being in all about fifty times as potent for fish as is Cobra venom. This great concentration of the poison may be of considerable advantage to the reptile when dealing with such active prey as fish in their own element. This special affinity of the poison for fish was even more marked in the case of some of the other species tested. Thus, that of a single species of the *Disteira cyanocincta* was fatal to pigeons in doses of 0·5 milligramme per kilo, being thus considerably weaker than that of the Enhydrina, but only 1 milligramme per kilo was required to kill fish, that is but twice as much as was needed to kill birds. Similarly with the *Disteira viperina* the minimal lethal dose for pigeons was 0·5 milligramme, and for fish only 0·75, or but very little more. Again, the poison of the *Hydrophis cantoris* for both pigeons and fish was just the same as the last-mentioned species. Lastly, the poison of the *Hydrus platurus* killed pigeons in doses of 0·075 and fish in one of 0·25 milligramme per kilo, being thus very deadly for both cold and warm blooded animals. The above include four out of the six genera of Hydrophidæ found in Indian waters, so that, although the poison obtained from the last four species was from single specimens, and therefore cannot be taken as more than approximately accurate, yet they suffice to prove that the Hydrophidæ as a class secrete very virulent poisons, which are specially poisonous to fish. It is also worthy of note that the two genera which proved to be most deadly to warm-blooded animals, namely, the Enhydrina and the *Hydrus platurus*, are just the two which the fishermen at Puri said were the most dangerous ones, as the accuracy of their statement points to actual experience in the human subject of their deadliness having been handed down among them. Some of the smaller species, however, probably do not eject sufficient poison to prove fatal, to adults at any rate, and hence are not so much dreaded by the fishermen. It will also be observed that the poison of the *Enhydrina Bengalensis* is the most potent of those so far tested, while it also yields the greatest amount of poison, with the exception, perhaps, of the *Disteira cyanocincta.*

Effect of the Poison on other Cold-blooded Animals.

I have not yet been able to test any extensive series of other cold-blooded animals to see if they are equally susceptible to the poison of the Hydrophidæ as fish are, but in one instance a frog weighing 30 grammes was injected with a dose of 0·2 milligramme per kilo, with the result that it showed well-marked symptoms of paralysis, but eventually recovered, so that it would appear to have been about as susceptible as fish. Some harmless snakes were injected with noteworthy results. Thus, two specimens of the *Coluber fasciolatus* were injected with doses of 10 and 50 grammes per kilo respectively, with the poison of the Enhydrina, with no ill effect, and the former received a second dose of 50 milligrammes per kilo three days after the smaller dose, equally without effect. Here we have a harmless colubrine snake withstanding 100 times the fatal dose for a fish and 1000 times that for a warm-blooded animal. Further, two specimens of the harmless green Whip-snake (*Dryophis mycterizans*) were tested, but in this species 25 milligrammes per kilo in one instance, and 15 in the other, each produced death in less than two hours, so that a smaller dose would nearly certainly have been fatal. This opens up a large question which must await further investigation.

The Physiological Action of the Poison on the Blood.

The striking similarity of the symptoms produced by the poison of the Hydrophidæ and by Cobra venom leads one to expect a similarity of action on the blood. The researches of Cunningham have shown that Cobra poison has a very marked power of dissolving the red corpuscles of the blood and also in reducing its coagulability, and, contrary to the views of Lauder Brunton and Fayrer, he holds that these blood changes are the essential features of the action of the poison, and not its action on the nervous system, as held by the latter authors. Experiments have been carried out to test the effect of the poison of the Hydrophidæ on the blood, with unexpected and important results. Taking first the poison of the Enhydrina, with which most of the observations have been made, and remembering that it is ten times as potent for warm-blooded animals as is Cobra venom, we may compare the action of the two poisons in dissolving the red corpuscles of the warm-blooded animals, the blood of pigeons and of the human species having been used in the experiments. The method of mixing the poison in different degrees of dilution with a minute measured drop of blood, and counting the number of corpuscles with a hæmocytometer before, and at varying periods after, the addition of the venom was adopted. The poisons were always dissolved in isotonic salt solutions, and equal quantities of blood in the same salt solution,

but without the addition of the venom, used as controls. These control solutions showed no dissolution of the red corpuscles after twenty-four hours. From 5 to 10 cubic centigrammes of blood were added to from $\frac{1}{2}$ to 1 c.c. of the isotonic solution of the poison, varying strengths of the latter being tested in this way. Pigeon's blood is specially well suited for these experiments, as the bodies of the corpuscles are dissolved while the nuclei remain visible. It was found that a 1-in-1000 solution of Cobra venom (1 milligramme in 1 c.c.) produced a very rapid solution of the red corpuscles, which had all disappeared in seven minutes. A 1-in-20,000 solution took a much longer time to produce complete dissolution, namely two and a half hours. In the case of human blood a 1-in-10,000 solution of cobra venom dissolved the whole of the red corpuscles in from fifteen to thirty minutes, while one of a strength of 1 in 20,000 took about one hour to do so. A 1-in-100,000 solution had very much less effect, having produced only a slight diminution in the number of the red corpuscles within one hour's time. The white corpuscles were not dissolved by the venom in the strengths used.

Let us now compare these data with those obtained with the poison of the Enhydrina, bearing in mind the much greater potency of the latter as compared with Cobra venom. The poison of the Enhydrina was mixed in the same way as above described with the blood of pigeons and with human blood, in strengths of 1 in 1000, with the result that at the end of one or two hours there had been no appreciable dissolution of the red corpuscles. On testing again several hours later, slight dissolution was found to have taken place, and by this time the solution also showed naked-eye evidence of commencing hæmolysis. After having been kept at room temperature (from 70° to 80° F.) for twenty-four hours the dissolution appeared to be complete, but, on examination with the microscope, a few red corpuscles were still found to be undissolved, showing that even after this lapse of time the hæmolytic change was not quite complete. The poison of the *Disteira cyanocincta* and the *Hydrophis cantoris* were also tested in the same way with precisely similar results, namely, that a strength of 1 in 1000 had no appreciable hæmolytic effect at the end of one hour, but caused nearly complete dissolution at the end of the course of twenty-four hours. This is about the same effect as is brought about by a solution of Cobra venom of a strength of 1 in 100,000, although Cobra venom has a potency of only one-tenth that of the poison of the Enhydrina. Thus we find that in proportion to its potency the poison of the Cobra has about 1000 times as great a hæmolytic effect on the red corpuscles of warm-blooded animals as has that of the Enhydrina. We have already seen that the latter poison produces no blood-stained effusion at the site of the injection of a fatal dose, evidently on account of the strengths used

having no hæmolytic action, for the solutions employed for the small animals experimented on were 1 in 10,000 or less. If we work out the amount of poison required to dissolve a certain amount of the blood of a pigeon, for example, we find that it takes about 200 times a fatal dose to dissolve 1/2000th part of the bird's blood in twenty-four hours, calculating this fluid to be one-thirteenth of its body weight. It is obvious, then, that ordinary fatal doses of the poison of the Hydrophidæ can have no appreciable hæmolytic effect, and that death cannot be attributed, even in a partial degree, to its action on the blood of the animal killed by it. This can also be demonstrated by another method of experiment, namely, by counting the number of the red corpuscles before the administration of the fatal dose of the poison, and again immediately after death. This I have done several times, with the result of showing that no dissolution of the red corpuscles resulted from the action of a lethal dose of the Enhydrina poison. For example, a fowl's blood was counted, and 3,190,000 red corpuscles per cubic millimetre were found. A lethal dose of Enhydrina poison was then injected subcutaneously, which proved fatal in just one hour, when the blood count showed 3,120,000 red corpuscles in the same quantity of blood.

Next we have to deal with the action of the poison on the coagulability of the blood. In the case of Cobra venom marked changes are produced, as shown by D. D. Cunningham, and this point has recently been studied by Lamb. The virus has the action of reducing or totally destroying the clotting power of the blood when mixed with it in small quantities. I have made a few observations on this point with the following results. Wright's tubes were used, the solution of the poison being first drawn up into them, and then an equal quantity of the blood drawn up and quickly mixed with the venom solution in the mixing chamber, and blown down into the tube again, and the conditions as regards clotting examined in a series of such tubes at given intervals. The clotting time, when mixed with an equal quantity of the normal salt solution (in which the venom was also dissolved) of a rabbit, having first been found to be three minutes, those of different strengths of Cobra venom in normal salt solution were found to be as follows: when a 1-in-10,000 solution was added the coagulation time was seven and a half minutes; with 1-in-1000 solution it was twenty minutes, and with a 1-in-200 one the blood was still quite fluid after twenty-four hours, its coagulability having been completely destroyed. On testing the effect of the poison of the Enhydrina in a similar manner it was found that a 1-in-1000 solution had no effect in reducing the coagulability of the blood, which still clotted solid in three minutes; when a 1-in-200 solution was added the blood still clotted in five minutes, showing only a slightly reduced time with the same strength, which in the case of Cobra venom had completely

destroyed the clotting power, and this, too, it must be remembered, in spite of the Enhydrina poison being ten times as powerful as that of the Cobra. It is evident, therefore, that the poison of the Enhydrina has no appreciable effect in ordinary dilute minimal lethal doses on the coagulability of the blood, while, as a matter of fact, we have already seen that such doses do not produce any loss of the clotting power of the blood. This was also the case when fifty times a minimal lethal dose of the venom was injected into the vessels of rabbits with the result of causing death in about six minutes.

The above experiments show that the poison of the Hydrophidæ has no appreciable action on the blood of animals, which can in any way account for the symptoms and fatality caused by it, yet it kills with precisely the same symptoms as are produced by Cobra venom, and, as we shall see presently, there are good reasons for believing that it has a special action on the nervous system. It will be evident at once that this furnishes a very strong argument in favour of the view that Cobra venom also kills through the nervous system, as held by Lauder Brunton and Fayrer, and not through the blood, as maintained by Cunningham. It is also of special interest to observe that although the action of the poison of the Hydrophidæ on the blood is practically a negligible quantity in its lethal effects, yet it still persists to a slight, but easily demonstrable, degree; for if it so persists in the Sea-snake, it may also persist in a still greater degree in the case of the Cobra without being a very active agent in the lethal effects produced by that poison, which kills through the nervous system as does that of the Hydrophidæ. In this connection it is interesting to observe that all through the poisonous snakes we find evidence of an action on the blood and on the nervous system in different degrees. Thus, beginning with the Viperine snakes, we first have the *Vipera Russellii*, which appears to be the purest blood poison of the known venomous snakes, killing by producing intravascular clotting in large doses, and the opposite effect of total loss of coagulability in repeated sub-minimal lethal ones. Then we come to the class of Pit-vipers, of which the rattlesnake of America has been most closely investigated by Weir Mitchell and Reichert. They also found a very marked effect on the blood, apparently similar to that produced by the Daboia, but, combined with this, we have a marked paralytic effect on the nervous system, and especially on the respiratory centre, for the authors mentioned conclude that although death may occur through the effect on the blood, yet they add "There can be no question, however, that the respiratory centres are the parts of the nervous systems most vulnerable to the poison, and that death is commonly due to their paralysis." Leaving the Viperine snakes and passing on to the poisonous Colubrines, we first come to the Australian species, so ably studied by C. J. Martin, namely, the Pseudechis, and we find again a combination of the two effects to

such a marked degree that, when the venom is administered intravenously, death results from intravascular clotting, as in the Viperine snakes, while if minimal lethal doses are given subcutaneously death results through paralysis of the respiratory centres. Next we come to the Cobra, another Colubrine snake, and here we find the nerve symptoms quite predominate, although some considerable effect on the blood in the form of reduction of coagulability and dissolution of the red corpuscles still survives, although it now takes quite a secondary position to the effect on the nervous system. Lastly, we have the Hydrophidæ, which, morphologically considered, are but colubrines modified for an aquatic existence, and here we find a practically pure nervous poison, although there still persists a trace of action on the blood if strong solutions of the venom are employed, although it can have no actively poisonous effect. The very slight action found, however, may be of some value to the snake in the following way. We have seen that a 1-in-200 solution of the Enhydrina poison has a slight retarding effect on the clotting power of the blood, which would doubtless be more marked in still more concentrated solutions, so that it is highly probable that the pure poison would have the effect of preventing the clotting of the blood at the point of injection of the poison, and so allow of its more ready absorption into the circulatory system through the patent vessels severed by the fang. This will account for the extreme rapidity of the absorption of the poison of the Cobra, for Fayrer showed long ago that if immediately after a dog has been bitten by this snake the fold of skin punctured is raised and freely excised, still the animal dies of the poison. The survival of some degree of action on the blood in the case of the Cobra and the Hydrophidæ, although not in itself an important element in directly causing the death of the animal, may nevertheless be of service in causing the venom to be more rapidly absorbed in the way just pointed out.

Action of the Poison on the Pulse and Respiration.

We have already seen that in slow poisoning the respirations become more and more laboured until convulsions set in and they quickly cease, while the heart continues to beat for a short time. For the accurate study of the exact effects on the respiratory and circulatory systems, proper recording apparatus is necessary, but as these were not available, I had to content myself with a record of the rate of the pulse and respiration after the intravascular injection of a large and rapidly fatal dose of the poison into rabbits under the influence of chloroform, with the following results. A dose of 1 milligramme per kilo. weight, or at least twenty times a minimal lethal dose was used, and death resulted in from six to eight minutes, taking the time up to

the cessation of the heart's beat. The effect on the respiration was simply a uniformly steady slowing down until convulsions set in, when the breathing finally ceased at once. For example, in a rabbit which had received a dose of 1 milligramme per kilo. directly into the carotid artery (the artery being clamped immediately afterwards to prevent hæmorrhage), the respirations were 60 per minute immediately before the injection of the poison. During the four minutes immediately following the injection, the number of respirations were as follows:—first minute, 56; second minute, 51; third minute, 42, and the fourth minute 33. In the first quarter of the 5th minute they were 8, at which point convulsions set in and the breathing stopped. The respirations were written down every quarter of a minute, and the figures for the separate quarters show an equally steady diminution of the number of respirations as the minute periods just given. In the same experiment the pulse showed the following changes. Before the injection it was 105 per minute. During the second half of the first minute after the injection it was 47 (that of the first half minute was lost), during the second minute it was 106, showing no alteration up to this time. During the third minute it fell to 99, and during the fourth it further fell to 48, that for its first half having been 32, and for the second half 16. During this steady fall in the pulse rate, its volume and force became increased. During the fourth minute, as already mentioned, convulsions set in, and the pulse was lost for about a minute, only the first and third quarters of the fifth minute having been recorded as 8 and 11 beats respectively. During the last three quarters of the sixth minute the beats were 15, 15 and 17 respectively, being now very feeble instead of unusually full, as before the cessation of respiration and onset of convulsions. During the first and second quarters of the seventh minute, the beats were 26 and 20 respectively, at which point the heart finally ceased to beat, that is, three and a half minutes after the cessation of the breathing. Very similar results have been obtained in another experiment, in which the same dose was injected into the jugular vein, a steady fall in the respirations first occurring, and they ceased with the onset of convulsions, while an equally steady fall in the pulse rate occurred later than that of the respirations, accompanied with an increased volume of the artery, the tension rapidly falling when the respiratory convulsions set in, but the pulse at the same time became more rapid again until it finally declined once more and then ceased. These experiments appear to show that the primary effect of the poison is a paralysing action on the respiratory centre, and that the cardiac failure is secondary to that of the respirations. The exact explanation of the slowing of the pulse with increased volume of the artery, I am not prepared to say without the aid of pressure tracings, which I have not yet been able to take.

The Affinity of the Nervous System for the Poison.

We have seen that the poison of the Enhydrina is much more potent than even that of Cobra, and it appears to be somewhere intermediate in virulence between Cobra and tetanus toxins. Further, we know that the repeated injection of gradually increasing doses of the latter two poisons into susceptible animals leads to the formation of an antitoxin in the system. This marked similarity of the nerve poisons of the Colubrine Snakes and tetanus toxin leads one to inquire whether these snake venoms do not exert their noxious influences in the same way that tetanus toxin does, namely, by being taken up from the circulation and fixed in the nerve cells until a sufficient dose has been absorbed to paralyse the nervous matter. We know from the experiments of Wassermann that small amounts of tetanus toxin can be thus fixed by fresh nerve matter in a test-tube, and so rendered inert when subsequently injected into a susceptible animal. It seemed to be worth while to repeat these experiments with the poison of the Enhydrina, and although I have not had time to carry out a sufficiently exhaustive series of experiments to settle this point, yet the following data appear to me to have some value as being highly suggestive of the mode of action of these nerve-paralysing snake venoms.

The experiments were carried out in the following manner. A weak solution of the venom, such as is used when giving minimal lethal doses, was placed in a small sterilised test-tube, and a given quantity of fresh brain matter from a pigeon was added to it, and the whole kept at blood temperature for a given time. Another solution of the same strength was kept at the same temperature for an equal period of time without the addition of any brain matter, for the purpose of injecting control animals, which were always used. Double and quadruple minimal lethal doses were used, and the brain matter was broken up so as to mix it with the poison as intimately as possible, and subsequently injected without filtering, so that most of the brain matter in a fine emulsion was injected with the poison. It was found that pigeons injected with these emulsions always lived longer than the control one, while they sometimes recovered from double, and in one instance from quadruple, minimal lethal doses of the poison after being mixed for from half an hour to eighteen hours with a small quantity (from 3 to 20 centigrammes) of fresh brain matter. The most marked effects were obtained by the use of the hemispheres of the cerebrum, the instances of complete recovery from lethal doses having occurred in these instances. The cerebellum had a less marked effect, only considerable prolongation of life having occurred, while in one experiment with the medulla and pons no very marked effect was observed. The grey matter, then, appears to have most effect in fixing the poison, as is also the case with tetanus toxin. These experi-

ments, then, point to the action of the toxins of the Enhydrina being very similar in nature to that produced by the tetanus bacillus. A few experiments were also done with Cobra poison in the same way, using the cerebrum only, but here the results were not so marked as in the case of the Enhydrina poison, only a retardation of the onset of symptoms and of death having been observed.

Antitoxins.

Lastly, we have to deal with the question of the possibility of obtaining an antitoxin against the poison of the Hydrophidæ. It has now been abundantly proved that Calmette's antivenin is not a specific against all kinds of snake venom, as he claimed, although in large doses (40 c.c. according to Lamb) it is undoubtedly of great value against the poison of the Cobra. The very marked similarity of the symptoms of poisoning by the Hydrophidæ with that produced by Cobra, lead one to hope that the antitoxin, which is efficient against the latter, would also be of value against Sea-snake venom. This has been put to the test by adding minimal and slightly supraminimal lethal doses of the poison of the Enhydrina to one half c.c. of fresh Calmette's antivenin (which had only reached Calcutta a very short time before it was used), and after allowing the mixture to stand at blood heat for half an hour, injecting the whole subcutaneously. White rats were used in the experiments, and the amount of antivenin in proportion to the amount of poison was relatively enormous as compared with the dose recommended in the treatment of men bitten by venomous snakes. Yet the animals uniformly died in just about the same time as the controls, so that it is evident that Calmette's serum is of no use against the poison of the Hydrophidæ.

On the other hand, the similarity in the action of this poison to the Cobra and tetanus toxins leads one to expect that an antidote could be prepared against it in a similar way to those of the latter poisons. It is only during three months that I have been able to experiment on this point, fowls being used. It soon appeared that the doses had to be very slowly increased, or fatalities occurred, and in the limited time these experiments lasted, I was only able to immunise one fowl against the minimal lethal dose of this poison, and a slightly larger dose proved fatal with the usual symptoms. My intention was to immunise a series of animals against the Enhydrina poison, and then to test them with small doses of poisons from the other Hydrophidæ, as owing to the large variety of this class of snakes, no antidote would be of any practical value unless it was equally potent against all the genera and species, or at least against the ones most commonly met with. This important and interesting question must await further investigation.

One experiment, which was carried out in order to test if the serum

or bile of the Enhydrina had any antidotal properties, deserves mention in this connection. Three puppies of the same litter were used, all very much of the same size. Each received an equal quantity of Enhydrina poison, but in the first this was mixed with a four minims of the serum of the Enhydrina; in the second it was mixed with four minims of the bile of the same snake; and the third received the poison solution only as a control. The mixtures were injected ten minutes after being made. The result was that all three animals died in a little over an hour, the control surviving slightly longer than the others. It appears, then, that neither the serum nor the bile of this snake has any antidotal properties against the poison, and can not, therefore, be utilised in the treatment of their bites. Further research will be necessary to determine if a practically efficient antidote can be prepared, which I hope to undertake when sufficient venom for the purpose can be obtained.

This concludes the most important experiments so far carried out by me with the poison of the Hydrophidæ. They have necessarily been strictly limited by the very small amount of poison which I have yet been able to obtain, and by the equipment of the laboratory at my disposal, for the use of which I am indebted to the kind permission of the Committee of the Zoological Gardens of Calcutta. I am also indebted to the Bengal Government for a grant towards the expense of this investigation.