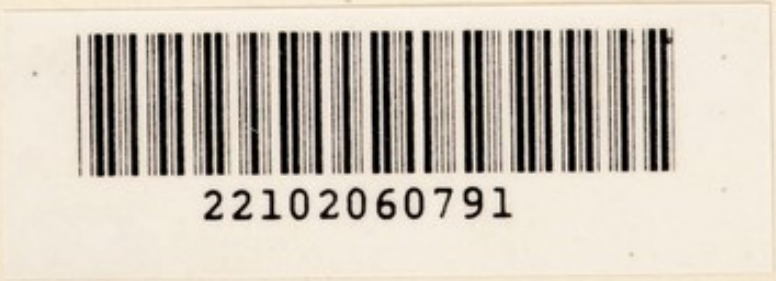
Phylaxis / by G. Billard ; translated by G. Gainsborough.



PHYLAXIS

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PHYLAXIS

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EDITORIAL NOTE

In spite of closer bonds between British and French men of science, their exchange of medical and biological thought is far from being so full and free as could be wished. That arises, in the first place, from the modern lack, in science, of a common medium for expression.

One purpose of the Anglo-French Library of Medical and Biological Science is to remedy this lack, in some measure, by the publication, in England and in France, of monographs, written by authors of distinction, on subjects that they have made their own. Each volume written and printed in English will be at once translated into French and published in Paris: each volume written in French will be at once translated into English.

Phylaxis stands in the foremost rank of those new conceptions which, since the beginning of the twentieth century, have opened up so many new horizons to Medicine. We owe it to the late Dr G. Billard, professor of physiology in the Medical School of Clermont-Ferrand.

The great provincial medical schools of France do not always obtain their just meed, at home or abroad. Their achievements are too willingly passed over by the many, and are remembered but by the few. We have, therefore, the greater pleasure in presenting this noteworthy volume

which reflects so much credit upon contemporary French thought.

Unfortunately, Billard died before his work was done. This volume is, therefore, incomplete. We miss the connecting threads which would have gathered up and brought together the separate chapters: we miss, too, the clear-cut summaries into which the author would have crystallised his findings, the better to bring them to our notice.

Nevertheless, the main thought which Billard so often expounded to his intimates stands out clearly from the few chapters which we now give, and we see how he was led, from a whole series of observations, to an hypothesis which he set out to verify by appeal to fresh facts.

With infinite patience he demonstrated the production of phylaxis: against venom by sparteine: against certain poisons by certain natural waters: against certain neurotoxins by formol, by chloroform and by gardenal; and by one neurotoxin against another. He brought divers facts in experience together by supposing one common, ultimate mechanism. Rejecting the classical humoral explanations, he looked towards a modification of the sensitive element itself: the nerve cell. In a word, he found that the phylactic agent, *dyeing* the nerve cell, renders it impermeable by the toxic agent. Such is the whole theory of PHYLAXIS as conceived by Billard.

But, that this hypothesis may be justified, it must, in the first place, be shown that the phylactic and toxic agents seize upon the same cell-constituents. As a matter of fact, it does appear as if their chemical affinities are

identical. Emulsions of brain substance, and lipoid extracts of brain and spinal cord do " fix " sparteine, venom, diphtheria toxins and the like, and so, when we find that, *in vitro*, such lipoids, once impregnated by any one of these substances, can then " fix " no other, we say that we have produced phylaxis.

It was Pierre Dodel, one of Billard's pupils and his closest collaborator, who actually completed his master's demonstration by thus producing phylaxis. He showed that such a cell as an isolated red blood corpuscle can be " stained " by a non-toxic dose of certain substances in such fashion that it cannot be adversely affected by such a second dose of the same substance as would, normally, be toxic. Thus the ease with which the red blood corpuscle can be studied has enabled us to set up, as it were, working models of what doubtless does happen, in the whole organism, in respect of such complex elements as the nerve cells.[1]

So the materialised hypothesis comes to rest upon the bases of experiment ; so fall the seemingly paradoxical facts into normal alignment : so does Billard return to Overton's theory of cellular impermeability : if, indeed, he had ever for a moment departed from it !

" No substance "—we would say, no *toxic* substance—" can permeate a cell unless it is soluble in the lipoid constituents of that cell. But, if these lipoids are first of all saturated by a preparatory phylactic agent, they become incapable of receiving the toxic agent."

[1] P. Dodel. La Phylaxie cellulaire. *Données expérimentales relatives aux phénomènes de phylaxie*, p. 45 et seq.

In these two sentences we have the upshot of these admirable researches, of which the bare narration is so fascinating.

But there is one other point of cardinal importance, of which we find no mention in the unfinished manuscript of the Clermont physiologist. This is his purely personal notion of the link between phylaxis and anaphylaxis, and it is one which provokes certain theoretical and practical considerations of the highest import. Only Billard could have given us an authoritative exposition of his conception of the link between these two sets of phenomena, but the writings that he has left perhaps enable us to outline it in essentials.

In 1920, then, Billard formally enunciated his own theory of anaphylaxis[1] in which he suggests that, if the lipoidal equilibrium of the cell normally controls the cell exchanges, it is only by favour of a rupture of this equilibrium that what would then be a truly colloido-clasic entry of foreign substances into the cell can be effected. But how can such a rupture of equilibrium be effected ?

We must remember that, in the case of anaphylaxis in respect of an albumin, the albumin is really a complex lipo-proteid, of which the lipoid part only is retained by the organism after the preparatory injection, because it only is soluble in the lipid constituents of the cell.

Since then, for the sensitised animal the lipid constituents of the cell are, in part homogeneous, in part heterogeneous, we have to recognise a new constitution, a

[1] *Journ. méd. français*, Dec., 1920.

new formula, of which the dangerous nature only becomes apparent when, a *second* injection of albumin being given, the heterogeneous lipids, by virtue of their natural affinities, allow the foreign protein to traverse the cell membrane.

Now, according to Billard, the divers antianaphylactic procedures in vogue tend to dislodge the heterogeneous lipids by the intermediacy of soluble soaps which entangle and withdraw them. This is how Billard conceived what he loved to call the " scouring of the organism " to be effected, by natural mineral waters in particular.

But all this is much more than mere hypothesis, glibly set out, and Billard had already begun, and partly achieved, the experimental vindication of his conceptions.

Two sets of experiments were devised. In the first, animals were sensitised by injections of lipoids extracted from foreign serums. When provocative injections of the " whole " foreign serums were given, anaphylactic shock invariably resulted.

In the second set of experiments, devised as counter-proof, the preparatory injections were made with the " whole " serums, and the provocative injections with the lipoids only of these serums. Under these conditions *no* anaphylactic shock occurred.

We can now easily grasp the nature of the link between phylaxis and anaphylaxis. PHYLAXIS results from the artificial impermeability of the cell to a toxin: ANAPHYLAXIS is the consequence of increased permeability to such toxins, whether they be crystalloids, or foreign proteins. Such impermeability is brought about by

saturation of the cellular lipids: such increased permeability by an abnormal receptivity of these lipids.

Those who affect surprise that natural mineral waters should be, some phylactic, others anaphylactic, are sufficiently answered by reference to Billard's work upon the part played by *soaps* in the phylaxis due to such waters, and to his notion of the like parts played by soaps and lipoids in both phylaxis and antianaphylaxis. "The waters which 'soap' badly are phylactic: those which 'soap' well are antianaphylactic."

Billard was possessed of an extraordinarily fertile and inventive imagination. His friends willingly acknowledged that at least one new and original idea sprang daily from his brain. His discourse was wonderfully lucid, and he expressed himself abundantly with a warmth and a conviction that carried all before them. But, beneath this seeming turbulence of ebullition, he concealed remarkable tenacity in the pursuit of those researches that lay ever nearest his heart. So it was that he came to embody his darling vision of PHYLAXIS with the living flesh of his innumerable experiments, and in this guise we are happy to present it to the medical profession.

F. G. CROOKSHANK. RENÉ CRUCHET.

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INTRODUCTION

THE ORIGIN OF THE CONCEPTION OF "PHYLAXIS"

A.—The Empirical Basis of Phylaxis

"In Nature's wonderful edifice all things are bound together in sequence and therefore any sufficiently analytical and complete study of even the smallest phenomenon can lead to the recognition of universal laws." (Armand Gautier, *Mécanisme de la variation des races et des espèces.*, *Rev. gén. Sciences*, 1901, p. 1,046.)

While I was studying an empirical treatment for viper bites I conceived the idea of phylaxis. Although I did not imagine that I had achieved a knowledge of the laws of the universe, I tried to discover some harmonious relationships in the facts I had observed; and if these relationships do not present the whole scheme as it appears to me, it is because I have been unable to express the sequence of the thoughts which have passed through my mind during the years in which I have considered the significance of my data.

Even before the War I was interested in the action of remedies which had been empirically recognised as anti-venomous. "Amongst the popular methods of treatment of snake bites there are some which are so ridiculous as not to deserve mention if it was not essential to analyse

and explain these notions for the end we have in view." (*La Défense contre l'ophidisme*, Pascal Weiss et Cie., São-Paulo, 1914, p. 221.)

I do not entirely share this opinion for " amongst the weeds that must be destroyed to make room for the new sowing " there may be some weeds, as in fact there are, which are worth while isolating and cultivating.

Claude Bernard, in the beginning of his book *Méthode Expérimentale*, has written " compelled by necessity, Medical Science, in its progress through the centuries, has led to innumerable experimental endeavours and has gained therefrom much useful knowledge".

Here I would ask you to imagine the giant figure of an old shepherd, silhouetted against the sky, and clothed in his ancient woollen cloak. He stands on the slope of one of the mountains of the Puy-de-Dôme. One day I saw him, in sculptural pose, his chin resting upon his hands which grasped a long rough staff, while he gazed in contemplation of the far-off horizon. Ever since, I have thought of him as the embodiment of the time-honoured empiricism of our peasants and of our doctors even, for indeed our shepherd was the healer of animals and sometimes of men.

Peasants, worthy of the name, are generally keen observers, who tenaciously retain in their minds a number of biological facts of much interest, for which they seek to find some explanation. Often, in their ignorance, they assume that some occult or cosmic influences are at work, and are laughed at by more pretentious folk who think they know better.

In Spring, despite the earth being snow-covered, the shepherds of the Auvergne, after a few fine sunny days, often bring out their flocks in order to avoid the ill-effects of too long a stay under cover. The sheep browse greedily on the green shoots of the broom which peep out above the snowy carpet ; those which eat too much, in spite of their guardian, become slow in their movements. The shepherds say they are benumbed. Poachers also are aware of the fact that deer, when satiated with the flower of the broom, are very easily caught in the chase—" they have not yet stretched their limbs " say the country folk, whereas we realise that the animals are suffering from sparteine poisoning. However my shepherd also tells me that the sheep and the deer, during this time, are apparently immune to viper's venom.

My laboratory is near the range of the Dômes and I wondered whether it would be possible to confirm in the laboratory the shepherd's knowledge.

It is easy to put a guinea-pig in a state of sparteinism, without killing it, by injecting 5 mg. of sparteine per 100 g. of body weight. If the dose given is more than 7 mg. per 100 g. body weight the animal dies in a paralysis similar to that produced by curare. If we then take a guinea-pig in a state of sparteinism and inject it with more than five times the minimum lethal dose of venom (say 0.5 mg. per 100 g. of body weight) we find that the animal is quite immune to such poisoning. I have seen some guinea-pigs survive even ten times the fatal dosage. Furthermore, if venom and sparteine, previously mixed in

a syringe, are injected together the animal will present no symptoms.

By our experiments we have established the fact that sparteine inactivates venom and have thus confirmed the observation of our shepherd. He however can go no further ; he merely observes and remembers his observations ; he has not the curiosity to reproduce or confirm the facts—in a word he does not practise experimental science, which is perhaps just as well. As Cuvier said "The observer listens to Nature, the experimenter enquires further and compels her to unveil".

I have also heard that Dr Combaud of Sancerre, has successfully used sparteine sulphate in the treatment of snake bites. This experiment, without doubt, must have been suggested by some observations similar to those which my shepherd had described to me. However I have only very rarely been able to save guinea-pigs by injecting sparteine after poisoning by venom and moreover I have never succeeded in curing an animal even by using almost lethal doses of sparteine, if the amount of venom injected is in the slightest excess of the minimal lethal dose. Clearly the curative properties of sparteine are very limited.

From this account it follows that sparteine inactivates venom under certain experimental conditions. Is this inactivation due to a neutralisation of venom by sparteine by some kind of physico-chemical reaction between the two poisons, or on the other hand does sparteine effect some alteration in the sensitivity of the guinea-pig's organism to venom ? This second hypothesis would

suggest that the protection against venom is the result of modification, produced by sparteine, in the organic and humoral states of the animal. I hold to this second hypothesis which incorporates the idea of protection or "Phylaxis" against an intoxication.

I regard the case of sparteine as typical of Phylaxis, and it is an example to which I am much attached, because it has led me to the discovery and interpretation of other cases. Here I will describe two further examples, which though of different appearance, nevertheless belong, in my opinion, to the same group. From these three cases I hope that the idea of phylaxis will become apparent and that I will then be enabled to define it clearly.

B.—The Case of the Mineral Waters

It is common knowledge that mineral salts and water soluble crystalloids dissolve in water at very different rates, and that chemists and physicists have studied this property and measured the so-called rates of diffusion.

I have often noted that with various salt solutions, whether simple or complex, whether isotonic or not with blood and tissue fluids, there are great differences in the rate of evolution of osmotic phenomena. This fact so impressed me, that I thought I might try dissolving some sparteine in solutions of different electrolytes to see if one could increase the rapidity of its absorption into the nervous system. In this way I hoped it might be possible to obtain a rapidity of absorption greater than that of venom and so be able to protect the nervous system by sparteine even after the administration of venom.

Consequently, as Claude Bernard said, " I devised some experiments in order to see " and have attempted " to fish in troubled waters ". I had at hand a remarkable series of different electrolytic solutions in the mineral waters of the Auvergne and, without looking further afield, used them in my experiments.

The following results were obtained :

The sparteine sulphate (Poulenc) that I used often killed a guinea-pig when given in a dose of 7 mg. per 100 g. of animal and with certainty when a bigger dose was employed. I injected sparteine in doses of 10 mg. of sparteine per 100 g. of animal, having previously mixed it, in a syringe with 5 c.c. of mineral water.

Such mixtures made *in vitro* with

Physiological saline	caused death in		40	minutes.
Châtelguyon (Gübler)	,,	,,	39	,,
Royat (Saint-Mart)	,,	,,	35	,,
Saint-Nectaire (Parc)	,,	,,	33	,,

However, when the waters of La Bourboule (Choussy) or Mont-Dore (Madeleine) were used, the mixture not only failed to kill but the animals showed no signs whatever of poisoning.

These experiments have led to conclusions which were not and could not have been foreseen, and these results have been so confirmed by further series of experiments, in which it was shown that the water of Mont-Dore was rather more effective than that of La Bourboule, that I consider these results as adequately established.

Being unable to understand the mode of action of the mineral waters on sparteine I stated, at that time, that

the former had some " power against the toxic action " of sparteine—some kind of " anagotoxic action".

These demonstrations of the inactivation of sparteine by mixing it with mineral water *in vitro* could not be explained by phylaxis but rather as a result of a physico-chemical reaction between the elements concerned. On the other hand, I have noted that guinea pigs which had been treated by intraperitoneal injections of water from Choussy (La Bourboule) survived for eight to fifteen days, or even longer, after a dose of tetanus toxin that would kill control animals in three or four days. Here, unquestionably, one can and ought to invoke the notion of phylaxis, though there is no doubt that the action of the mineral water is very different from that of sparteine. In what follows I shall attempt to point out the connexion between these two modes of action.

C.—The Case of Certain Neurotoxins

In the Auvergne, where I live, it is relatively easy to obtain quantities of the venom of *Vipera aspis*, sufficient for laboratory research. Thanks to this privilege, I was able at first to investigate the natural immunity of certain animals against venom (*cf:* *C. R. Soc. Biol.* Paris, 1910). About the same time I showed that the juice of pig's liver, which had been autolysed in an atmosphere of chloroform vapour, could inactivate various poisons and toxins after mixture *in vitro*. Later I will attempt to explain the mode of action of this autolysis liquor though at the time of publication of my papers I had only a vague idea of this. At the moment however, I would present

certain facts which I then observed, viz., that venom, diphtheria and tetanus toxins, strychnine and cocaine, after mixture in a syringe with this autolysis liquor, can be injected in lethal doses into a guinea-pig without producing any signs whatever of poisoning. During these investigations I was led to inject a lethal dose of viper's venom into a guinea-pig which had received, four days earlier, a mixture of tetanus toxin and autolysis liquor. This guinea-pig survived the administration of the venom. After having assured myself that the autolysis liquor could not produce an effect of this degree, the test was repeated on a dozen animals with various doses of toxin and venom and always with the same results, confirming my first experiment. These results are recorded in a thesis which was presented by my pupil Maublanc in 1911 before the Faculty of Medicine of Paris under the presidency of Professor Roger. I had asked Maublanc to expound these in his discussion on the mechanism of the "Natural immunity of certain animals against the venom of the viper".

In July, 1926, I presented to the Société de Biologie a communication on *Non-specific immunisation by certain neurotoxins against each other*. In this note I recalled the facts that Emile Roux had discovered that the virus of rabies immunises against venom, that Marie Phisalix had noted that the mucous venom of the toad immunises against rabies and that I myself had demonstrated the following:

(1) A guinea-pig, immunised against tetanus toxin, survives a dose of diphtheria toxin which kills a control animal in twenty-four hours.

(2) A guinea-pig, immunised against tetanus toxin, survives a dose of viper's venom four times the minimum lethal dose for the controls.

(3) A guinea-pig, immunised against diphtheria toxin, survives three times the minimum lethal dose of viper's venom.

Thus the neurotoxins can be classified in the descending sequence : tetanus toxin, diphtheria toxin, neurotoxin of venom, and I have shewn that this sequence can only be used in one direction, for venom will not produce immunity against diphtheria toxin, and diphtheria toxin does not immunise against tetanus toxin.

I have described my observations more fully in the *J. Physiol. Path. gén.*, submitted for publication 15th of November, 1926, and appearing in Volume XXV, March, 1927. However an article appeared in the *Paris Médical* of the 4th of December, 1926, by Ramon et Zoeller on *The Theoretical and Practical Applications of Tetanus Anatoxin.* In this, the authors noted (p. 461) that their results in the immunisation by tetanus toxin against diphtheria toxin were not in agreement with those I had published. I read this article on the 15th of December and my descending sequence of neurotoxins seemed to be disrupted. On the one hand, I knew that guinea-pigs resisted diphtheria toxin after treatment by tetanus toxin, but on the other hand, one could not doubt the good faith of Ramon and Zoeller. The problem demanded a solution and in its further study I was led to the idea of " Phylaxis ".

CHAPTER ONE

THE IDEA OF PHYLAXIS

In the *J. Physiol. Path. gén.* (1928, Vol. XXV, No. 1, March) I published an article on "Phylaxis" which commences as follows:

"The title of this article, though so simple, may appear pretentious and even courageous, in that it might recall the more important work of Charles Richet on *Anaphylaxis*; inasmuch also as I have been able to make observations which show that phylaxis and anaphylaxis are in some ways related to each other. It is sometimes very difficult to prove oneself worthy to carry an illustrious family name, and consequently I have a hard task to perform in order that phylaxis should not appear to be merely a poor relation of anaphylaxis".

As a result of the observation of a large number of facts concerning the protection of the guinea pig by the use of some poisons and toxins against other poisons and toxins, I have had almost inevitably to adopt this term because the word immunity, which I had previously used, led to much confusion.

I have earlier mentioned the results obtained by Ramon and Zoeller in their attempts at immunisation by tetanus toxin against diphtheria toxin, results which were negative and in disagreement with those I had myself

published. I hope to show that this disagreement, between the facts observed by Ramon and Zoeller and those of my own, is more apparent than real, and that it exists solely in the use of the term "immunisation" which I had employed in an unfortunate manner (I dare not say regrettable because it was on this account, that after a discussion with G. Ramon and in agreement with him, that I adopted a new term—phylaxis).

The kernel of the problem is really as to whether I was correct, in my study of the sequence of the neurotoxins, in stating that my guinea pigs had been *immunised* against diphtheria toxin by tetanus toxin. In Larousse's popular dictionary immunity is thus defined: (Latin: *Immunitas* from *Immun*, exempt) Exemption from taxes, duties, burdens, and further on "The property of a living organism to exist in safety against a definite disease; a first attack of an infectious disease often confers an immunity of more or less long duration". It follows that, according to the strict sense of the definition given by Larousse, I have not committed a gross error and the public would absolve me without question. However, in this connection, neither I nor my readers are to be classed as the general public. To the medical mind, in fact, the idea of immunity conveys the idea of specific antibodies; so that if tetanus toxin had protected my guinea pigs as the result of antibody formation in the organism then the experiments of Ramon and Zoeller could not have yielded negative results, because Ramon found that antibodies were produced when anatoxin was used. "Which shows how much care it is essential to

take at the commencement of any study, in using terms which are necessary for the exposition of one's observations, and particularly when using expressions which have already a strictly defined scientific usage." (Maurice Arthus in *Praxis. Revue Suisse de Médecine*, 5th and 12th April, 1927.)

Nevertheless there can be no doubt about the facts, which have been recorded in my papers, and a series of them will be detailed in this book. I may have explained them badly, but the facts are there, and can be reproduced. It follows that there must exist a method of protecting organisms against neurotoxins which is different from immunisation by production of antibodies.

By December, 1926, I had made a series of communications to the Société de Biologie on the inactivation of some neurotoxins by sparteine sulphate, scopolamine, and also by certain mineral waters. I had at that time put forward a working hypothesis, which had already been used by other biologists in considering the action of poisons on the nervous system, viz., that such poisons impregnate this tissue as with a dye. As regards sparteine, I have stated that once the nervous system was impregnated by it, or as it were stained, other neurotropic toxins could not attach themselves in the place already occupied. When I read Ramon and Zoeller's paper on the 15th of December, 1926, I considered the matter deeply and arrived at the following conclusions: Ramon and Zoeller had used tetanus anatoxin in order to immunise their guinea-pigs. This material is however very different from mine since anatoxin is a complex

formolised toxin of which we have no precise physico-chemical knowledge, and I do not prejudice the case in referring to it simply as a complex. I knew that anatomists had, for a long time, preferred the use of formalin to any other fixing agent for the purpose of hardening the brain. Consequently I have wondered if formalin has a particular affinity for the nervous system, and if, when the complex formolised toxin is injected into the organism, it perhaps splits in such a way that the formalin impregnates the nervous system *in vivo* and so hinders the fixation of the neurotoxin, just as with sparteine. This of course is all hypothesis and perhaps could be put to the proof.

On December the 16th I injected four guinea-pigs as follows :

A.—With a mixture, in the syringe, of uroformine (Gobey) in a dose of 30 mg. per 100 g. of body weight and of sparteine sulphate in a dose of 10 mg. per 100 g. body weight. Death followed in 45 minutes.

B.—With uroformine in the same dose, but given 15 minutes before the sparteine was injected. The animal survived without symptoms.

C.—With uroformine 15 minutes after injection of sparteine sulphate. Death followed in 20 minutes.

D.—Control with sparteine only. Death followed in 45 minutes.

I have repeated this series of tests and always obtained

analogous results. So it follows that a guinea-pig, of the type used, after preliminary treatment with uroformine, survives a lethal dose of sparteine, and my working hypothesis seems to be well supported by this observation.

Now hexamine (hexamethylene-tetramine), discovered by Bardet and Trillat, is decomposed in the animal organism with liberation of formaldehyde (formalin). Consequently, if the impregnation of the nervous system with formol can protect an animal against neurotoxins, would it not be possible to protect a guinea-pig in the same way against sparteine by using anatoxin (complex formolised toxin) ?

I injected four more guinea-pigs as follows :

A.—With sparteine, 10 mg. per 100 g. body weight and tetanus anatoxin, 1 c.c. per 300 g. of body weight (mixed *in vitro*). The animal survived without symptoms.

B.—Tetanus anatoxin first, followed 15 minutes later by sparteine. Death followed in 43 minutes.

C.—Sparteine first, and 15 minutes later the anatoxin. Death followed in 26 minutes.

D.—Controls with sparteine. Death occurred in 46 minutes.

In this experiment it cannot be doubted that guinea-pig A has been protected by the formalin, because the tetanus toxin moiety of the complex had not had time to act. Why, in the second series, did we not get identical results with the first experiment, and why is it that

guinea-pig A has been protected here while in the first series it is guinea-pig B which survived ? There must be here some difference in the speed of liberation of formalin from the complex in which it is fixed, the manner in which it is fixed being different in uroformin and in anatoxin. These facts seem to me to provide further support for my hypothesis of the protection of the nervous system against a toxin by impregnation with formalin. The following test also leads to this conclusion ; five guinea-pigs were used :

1.—Injected at 9.45 a.m. with a lethal dose of diphtheria toxin. The animal survived for 25 hours 30 minutes.

2.—Injected at 9.47 a.m. with the same dose of diphtheria toxin mixed *in vitro* with 1 c.c. of tetanus anatoxin. Survived without symptoms.

3.—Injected at 9.49 a.m. with diphtheria toxin and 15 minutes later with 1 c.c. of tetanus anatoxin. Death in 25 hours 45 minutes.

4.—Injected with tetanus anatoxin at 10 a.m. and 15 minutes later with diphtheria toxin. Death in 25 hours 25 minutes.

5.—Injected, in the peritoneal cavity, with tetanus anatoxin and immediately after with diphtheria toxin subcutaneously in the back. Death followed in 30 hours 45 minutes.

In these tests one cannot admit that the tetanus toxin played any part in the protection of guinea-pig number 2, and must assume it to be due to the formalin. As to

number 5, which survived about five hours longer than the control animal, there is clearly some protection produced and the action is definitely *in vivo*. I am therefore brought again to my hypothesis of the impregnation of the nervous system by formalin (*in vivo*). This would explain why Ramon is able to inject formidable doses of toxin in association with formalin without the nervous system being affected. It would also explain how it is that, though the anatoxin is harmless, it yet is able to keep its antigenic power, for the toxin, liberated from its association with formalin, can circulate in the organism, which reacts by producing the anti-toxins.

If one admits this hypothesis of the mode of action of anatoxins, it is possible to understand why Ramon and Zoeller could not obtain the least protection from it against diphtheria toxin, and particularly so after a prolonged attempt at preventive immunisation. Here, the nervous system, which was protected for a time by formalin, after this body has been eliminated, is no longer protected at the time of the injection of diphtheria toxin. Furthermore, one can assume that the nervous system at this time is quite free from any impregnation with neurotoxin of tetanus and consequently it is quite easily accessible to the diphtheria toxin.

I will deal with this point in further detail when I set out the phenomena of phylaxis by neurotoxins, but it is now clear that the disagreement between the results of Ramon and Zoeller and the results which I had published is more apparent than real. So it is, that after these observations, which I have just presented, I began to use

the term phylaxis with the idea of a process which is not specific like immunity and which does not involve the notion of specific antibodies.

The word " phylaxis " (Greek, *ψυλασσειν*, *protection*) is not, so far as I know, used in medicine, even though the terms " prophylaxis " and " anaphylaxis " are employed. It is not therefore, properly speaking, a new word, but its value will be determined only by the sense in which it is used. Guided by the hypotheses which I have just put forward, I am endeavouring to formulate this meaning. As Pasteur has said : " In the laboratory, hypotheses are always kept moving ". The most attractive hypotheses are often the most dangerous if strictly established facts do not confirm their value. So it is fortunate that the facts, which I am going to bring forward, are in favour of my conception of " Phylaxis ".

CHAPTER TWO

PHYLAXIS BY SPARTEINE

I HAVE already mentioned how it was that, after a conversation with a shepherd who used broom in an empirical manner as protection against viper bites, I began to study the effects of sparteine sulphate on the venom of the vipers of the Auvergne.

A. Viand Grand-Marais had already noted the value of the heads of the broom in some recipes for use against poisonous bites (*Journal de Médecine de l'Ouest*, Nantes, 1879, 2nd series). I have also reported that M. Perrin of Nancy, in a study of sparteine, has written that Dr Combaud of Sancerre had utilised sparteine successfully against viper bites.

The sulphate of sparteine (Poulenc) that I have used in my investigations nearly always kills a guinea-pig in a dose of 7 mg. per 100 g. of body weight and death occurs in between forty to fifty minutes after intraperitoneal injection. Those animals which survived for more than one hour, then recover quite rapidly, and half an hour later become normal. Thus cure is rapid.

The first symptoms generally appear about fifteen minutes after injection, when the animal lies flat on its belly and is shortly seized by tremors which occur in waves, at first well separated, but which become more and

more close, till there occurs a convulsion which begins at the anterior end of the animal and is propagated violently to the posterior extremity. At this moment the animal attempts to get up but its fore legs give way, and its head falls again, with the nose against the ground. The hind legs are not yet paralysed and, during some minutes, are subject to violent contractions which propel the animal in a jerky and inco-ordinated manner, its fore-part paralysed and its head scraping the ground. There soon appear marked asphyxial movements, during which the animal occasionally emits one or two loud cries ; the body then falls quite flaccid, small asphyxial movements of the head continue but the heart beats can still be detected by thoracic palpation. Soon the sphincters relax, the hair stands on end, and the animal dies.

These phenomena do not persist, in all, for more than thirty minutes from the onset of the first signs of intoxication. It cannot be doubted that there occurs a progressive curarisation of the animal ; but with curare the spread of the paralysis begins with the hind legs, whereas with sparteine the forelegs are first affected. This particular difference without doubt, deserves further study but I have not, up to the present time, worked on this subject. However it is certain that sparteine acts in a similar manner to curare, and the following facts show this clearly. When sparteine is injected into a guinea-pig in a dose of 5 mg. per 100 g. body weight, no signs of intoxication appear ; if however this dose is repeated every second day, there appear some slight symptoms of sparteinism on the fourth or fifth

injection, that is to say about the eighth or the tenth day. This observation demonstrates the cumulative action of the drug and the slowness of its elimination. These minor symptoms, which are relatively transitory, constitute what I have called the state of sparteinism.

A.—Phylaxis against Viper's Venom by Sparteine

A guinea-pig in a state of sparteinism is very resistent to viper's venom. During my investigations I have treated several hundred guinea-pigs with sparteine and have observed that after a protecting dose of 5 to 6 mg. of sparteine per 100 g. of body weight, the animals resist more than five minimum lethal doses, this being 0.1 mg. per 100 g. body weight. Sometimes the animals survive ten times this minimal lethal dose, when it has been administered between half to one hour after the sparteine injection. On the other hand if one injects venom and sparteine mixed in a syringe, the animals show no symptoms. Here one might consider it a case of neutralisation *in vitro*, but I cannot admit it.

The rapidity of diffusion of venom injected into the tissues is very great and the following experiment of Calmette demonstrates this: a lethal dose of venom is injected into the tip of the tail of a rat and this organ is then amputated within two to three minutes. Nevertheless the animal dies from the effect of the venom. The speed of diffusion of sparteine is certainly much greater than that of venom, and the rapidity with which a guinea-pig dies from a minimum lethal dose of sparteine (death within forty-five to fifty minutes) is sufficient

proof of this, for with a minimum lethal dose of venom the animal remains alive for twenty-four hours. When sparteine and venom, mixed in a syringe, are injected there then ensues a race for the central nervous system, in which the sparteine arrives first, fixes itself there, and thus prevents any subsequent attachment of the venom. The phylaxis which occurs is actually *in vivo*.

Further on, in a study of cellular phylaxis I will show more conclusively that this explanation is not purely hypothetical.

B.—Phylaxis against certain Neurotoxins by Sparteine

(*Diphtheria toxin and tetanus toxin*).

R. Dujarric de La Rivière, M. Tissier, and A. Lafaille, in a communication to the Société de Biologie (séance du 23 juillet, 1927) on *The effects of diphtheria and tetanus toxin on animals treated by certain poisons*, state: "We have seen, in agreement with what Billard has shown for snake venom, that sparteine protects, to a certain degree, against diphtheria or tetanus intoxication". The following, however, appears in *J. Physiol. Path. gén.*, March, 1927, xxv, 53 under my signature:

> "(1) *Inactivation of diphtheria toxin by sparteine sulphate.*
>
> Guinea pig no. 71 received an injection of diphtheria toxin, which killed a control in twenty-four hours. This toxin was mixed in a syringe with a dose of sparteine sulphate corresponding to 5 mg. per 100 g. of body weight. The animal survived.

We have repeated the test a number of times and always with the same results.

(2) *Attempts at inactivation of tetanus toxin by sparteine sulphate.*

We have completely failed in our experiments. The tetanus toxin is a toxin with an incubation period; there is "a quiescent period" before its action as Maurice Nicolle has stated. Perhaps by producing a prolonged state of sparteinism I might be able to inactivate the tetanus toxin."

Moreover in the *C. R. Soc. Biol. Paris*, 27th February, 1927, there appeared my communication on *Non-specific phylaxis and specific immunity* wherein I stated:

"I have observed a series of facts which appear to me to bring into definite relationship non-specific phylaxis with the specific anaphylaxis of Richet. I have in fact discovered a phenomenon of incubation in the development of phylaxis by sparteine and certain mineral waters, analogous to the incubation period in the evolution of states of anaphylaxis, but with opposed final results.

This is what I observed with sparteine.

Four guinea pigs received a preparatory injection of a sub-lethal dose of sparteine (5 mg. per 100 g. body weight). The test injection was made with a dose of tetanus toxin, lethal for controls in ninety-two hours.

(1) Tetanus toxin injected 24 hours after the sparteine. Death in 101 hours.

(2) Tetanus toxin injected 3 days after the sparteine. Death in 130 hours.

(3) Tetanus toxin injected 9 days after the sparteine. Survived without symptoms.

(4) Tetanus toxin injected 13 days after the sparteine. Survived without symptoms.

This phylaxis by sparteine, after a single injection, has appeared to exist (to a greater or less degree), for from 15 to 21 days."

From the dates of my publications it will be seen that I had definitely demonstrated before R. Dujarric de La Rivière, M. Tissier, and A. Lafaille, " that sparteine protects, to a certain degree, against diphtheria and tetanus toxin ". As these authors were not aware of my results, it is even more interesting to observe that their researches have confirmed the occurrence of phylaxis by sparteine against neurotoxins. I will return later to the interpretation which they give to their own results, but I would add here that sparteine does not only protect against diphtheria toxin when mixed with it *in vitro*, as I have said earlier, but also by preventive inoculation.

C.—The Mode of Action of Phylaxis by Sparteine

In the well documented book by G. Ogier and E. Kohn-Abrès (*Chimie toxicologique*, Doin., 1924, Vol. 2, p. 291) we read " the action of the usual reagents on sparteine has very little interest : it does not possess any characteristic specific reactions ".

It follows that the chemical study of the mode of action of sparteine has failed and it becomes necessary to

use bio-physical methods which, fortunately, yield precise and valuable results. Thanks to the work of Lapicque and his school on chronaxis, we know that certain poisons produce a dissociation between the chronaxis of muscles and that of the nerves. Such poisons induce curarisation. Mlle Jeanne Weill has shown that " sparteine is certainly a poison which leads to curarisation : it creates heterochronism by increasing the chronaxis of the muscles ". (*J. Physiol. Path. gén.*, 1913, p. 792.)

On the other hand, M. Arthus, by a rigorous experimental procedure, has demonstrated that the venoms also kill in the same way as the poisons which resemble curare.

These results are eminently suggestive : two poisons, with similar properties, are allowed to attach themselves to the nervous system, but instead of producing an additive effect, when one of them (sparteine) has impregnated the nervous system the other (venom) cannot then attach itself thereto, and its poisonous effects do not ensue. When both are injected at the same time there probably occurs what I have previously described as a race for the central nervous system ; the sparteine is the first to arrive and so prevents the fixation here of the venom. Doubtless this is only the same hypothesis, but nevertheless one is led inevitably to its formulation. The question arises whether it is the same with other neurotoxins such as those of tetanus and diphtheria.

Here I would quote from Mme Marie Phisalix (*Animaux Venimeux et Venins*, Masson, 1922, ii, 593) a summary on the neurotropism of the toxins which seems

to me to be very adequate : " The neurotoxins of the venoms possess a specific affinity for nervous tissue, whence their name. This fact was brought to light by Flexner and Noguchi in a study of the venom of ancistrodon : the toxicity of this venom disappears more or less completely when it is mixed with the cerebral substance of animals which are sensitive to it, and the liquid, which can be separated from the mixture by centrifugalisation, has lost the greater part of its toxicity. Other tissues have not the same capacity. Myers, in some similar work, did not obtain such positive results but Calmette observed fixation of the neurotoxin of cobra venom by an emulsion of cerebral substance. In many descriptions there have been presented the antihæmolytic properties of cholesterol, its anti-venomous action against viper's venom (Ch. Phisalix), the discovery by Ramon of its anti-saponin action and consequently its antiaphiotoxic action, and these have thrown a new light on the fixation of neurotoxin by nervous tissue, which had been observed by Flexner and Noguchi. If the aphiotoxin of Faust is neutralised by cholesterol, the phenomenon of its fixation could be due simply to the cholesterol in the brain emulsion : the fixation of tetanus toxin by this emulsion, discovered by Wassermann and Takaki, is today definitely attributed to cholesterol or cerebrin or protagon etc. In the same way Noguchi shewed that tetanolysin is neutralised by cholesterol, and Landsteiner that tetanus toxin is inactivated by protagon. Somewhat earlier experiments by Fraser demonstrated the anti-venomous action of bile against cobra venom, and those of Ch.

Phisalix, as regards bile against viper venom, which action he attributed to cholesterol and to the bile salts.

Are all the neurotoxins identical? This is a question, interesting not only from the theoretical point of view, but also from the practical aspect of the anti-venoms."

In what follows, however, the author reports a whole series of observations which prove that the neurotoxins are not identical, but nevertheless it is of great interest and a support to the hypothesis, which I have set out, that Mme Phisalix has been led to pose this question.

As regards the diphtheria toxin our ideas about its neurotropism are less definite than in the case of tetanus. Further on I will deal with this very important point in greater detail and will merely mention here that Laroche and Grigaut have observed that cholesterol does not fix diphtheria toxin but that lecithin fixes and activates the poison.

[Laroche and Grigaut have distinguished between the capacities of fixation and of neutralisation; diphtheria toxin, for example, though attracted by the "lipoid" is at the same time activated.—*Ed.*]

In further investigations with viper's venom and with sparteine, Dodel and I have shown that these poisons can be perfectly fixed *in vitro* by emulsion of nervous tissue, or by suspension of lipoids, which have been extracted from nerve tissue.

In considering the whole of these investigations, as clearly set out, it seems quite definite that the neurotropic poisons become attached at first to the lipoids, and it also appears that each neurotoxin may have a special

affinity for one only of the constituents of the group of lipoids.

"Overton (1900-1901) invented the term 'lipoid' when he evolved a theory concerning the mechanism of the permeability of the enveloping membrane of cells. The substances, which are classed together under this name, includes principally the lecithins, the sterols, protagon and cerebrin. From the chemical point of view it is a very heterogeneous family, but Overton only regarded this classification from a purely physical aspect. He labelled those substances as lipoids which, in a general manner, had the property of being solvents for the same group of bodies (the anæsthetics for example) and which behaved in a manner somewhat resembling the fats." (E. Lambling, *Précis de Biochimie*, Masson, 1919, p. 69.)

We might be entitled to say that, just as the lipoids are characterised by their property of being solvents for anæsthetics, so are they also, in the same way, characterised by their property of being solvents for neurotoxins. These latter, one might say, are only distinguished by the effects which they produce, but to produce such an effect the first condition necessary, to enable them to reach the nervous structures, is that they should be soluble in the lipoids which cover the more important elements of the nervous system. From another standpoint, we know that during a surgical operation it is difficult to obtain good anæsthesia if the anæsthetic is changed. This brings us back again to our hypothesis by which we believe that when a neurotoxin is fixed in the nervous system (or more definitely on the lipoids of this system) another toxin is

then unable to attach itself to these lipoids. They are, as it were, stained by the first neurotoxin.

R. Dujarric de La Rivière, M. Tissier, A. Lafaille, in a communication to the Société de Biologie, 27th of July, 1927, say in relation to tetanus toxin : " For this toxin, just as with the others, there does not appear to be any absolute neutralisation of the toxin in the organism, but there is created only a temporary condition of resistance which disappears as the poison is eliminated ". I do not believe that the toxin could be neutralised by sparteine any more than that it could be neutralised by formalin in Ramon's anatoxin, for in this latter case, animals treated with this could not produce antitoxins. I still think that the toxin remains intact and I agree with these authors when they say that sparteine " creates a temporary condition of resistance of the organism ", but I can be more precise by saying that it is the impregnation of the lipoids of the nervous system that produces phylaxis.

In case I have not yet demonstrated the rôle of the lipoids in the phenomena of phylaxis as clearly as I should have wished, I will add many further reasons, both theoretical and experimental, in support of my hypothesis. In the following chapter on phylaxis by mineral waters, I think I have succeeded in explaining the internal mechanism of phylaxis and in getting an understanding, from the maximum amount of evidence, of the rôle of the lipoids (soaps and lipoids) in these phenomena.

CHAPTER THREE

PHYLAXIS BY CERTAIN MINERAL WATERS AGAINST CERTAIN NEUROTOXINS

In my introduction I have discussed how it was that I discovered that certain mineral waters inactivated certain of the poisons of the nervous system. I will reproduce here the details of my experiments.

I injected a series of guinea pigs with sparteine sulphate, in a dose of 10 mg. per 100 g. of body weight, mixed in a syringe with 5 c.c. of different mineral waters from springs in the Auvergne.

The mixture *in vitro* with

Physiological saline killed the animal in 40 minutes.

Châtelguyon (Gübler)	,,	,,	39	,,
Royat (Saint-Mart)	,,	,,	35	,,
Saint-Nectaire (Parc)	,,	,,	33	,,

The mixture with La Bourboule (Choussy) and Mont-Dore (Madeleine) not only did not kill, but animals so treated showed no signs whatever of poisoning.

From these experiments two types of results are to be distinguished :

(1) Some electrolytic solutions, when used as vehicles for certain nerve poisons, favour the diffusion of the poison and the consequent intoxication. Thus, when using physiological saline, death only occurs after forty

minutes, but when using Saint-Nectaire water death ensues in thirty-three minutes—a difference of seven minutes, which is a significant figure when compared with the forty minutes usually noted for the survival time. Reckoning from these figures, we can say that, by comparison with physiological saline, the Saint-Nectaire water, when used as a solvent, increased the toxicity of sparteine by $\frac{7}{40} = \frac{1}{5{\cdot}7}$, that is to say, by almost one-fifth.

This fact alone is well worth noting, because it shows that remarkable aggravation of some intoxications and affections might occur, as a result of mineral water cures, as I will show later.

(2) There is no doubt that in the experiments, which have just been reported, I have obtained results which were both unforeseen and unforseeable, viz. the inactivation of sparteine by the water of Mont-Dore and of La Bourboule.

I have thus been lucky in " fishing in troubled waters " and in making experiments " in order to see ", as Bernard said.

I have been able to reproduce these tests, in series, with the same water and the same poison and always with the same results. I therefore consider this fact as being well established and analogous to the inactivation of venom by sparteine sulphate *in vitro*. One can easily understand how these results then led me to investigate the inactivating property of the mineral waters of my neighbourhood for those neurotoxins, the action of sparteine on which, I had already determined.

Inactivation *in vitro* of some Neurotoxins by certain Mineral Waters

After many investigations, on a number of animals, by the method previously used for sparteine, I have noted that, by mixture *in vitro*, it is possible to inactivate :

Venom by Châtelguyon (Yvonne, Gübler, Deval) ;
Tetanus toxin by La Bourboule (Choussy) ;
Diphtheria toxin by Saint-Nectaire (Rocher, Saint-Cérain) ;
Phalline by Mont-Dore (Chanteur).

I have included phalline, although it is not usually considered to be a neurotoxin, because I have concluded from the facts that I have observed, that " We have today the firm conviction that there probably does not exist a poison of organic origin for which one cannot find a mineral water which will inactivate it ". (*C. R. Soc. Biol. Paris*, July, 1926.) My opinion has not changed since that date. Nevertheless one foresees what an enormous amount of labour such a research might entail, although stimulated by the end in view and the abundant harvest to be reaped. For my part I have been fortunate in finding, in the springs of the five stations of the Auvergne, waters which possess unsuspected properties of inactivating the poisons and neurotoxins which I had by me. This good chance however necessitated the investigation of the problem of the mechanism of this inactivation.

It is possible to conceive the inactivation of neurotoxins *in vitro* in the very simple way, which first comes

to mind, namely that it consists of a phenomenon of adsorption or of precipitation. "Different toxins, as with the diastases, have the property of adhering to precipitates which are produced in the liquids in which they are in solution (or apparently in solution). Thus, diphtheria toxin is precipitated along with calcium sulphate ; this salt or alumina precipitates in part tetanus toxin, and animal charcoal and the colloidal hydrates of chromium, iron and zinc have also the same property ; the neurotoxin of the venom of the cobra is precipitated with various fatty bodies. These are all phenomena of adsorption." (E. Gley, *Physiologie*, Masson, p. 102.) This interpretation might be permissible in so far as it concerns the inactivation *in vitro* during the time that the mineral water and the poisons remain in contact in the syringe. It is however impossible to look upon the inactivating action of the mineral waters in any such simple and commonplace manner, for this action not only occurs *in vitro* but also *in vivo*.

Inactivation *in vivo* of some Neurotoxins by certain Mineral Waters

In my researches on inactivation *in vivo* I have been guided partly by the results I have obtained with sparteine, but here again I had to make a number of experiments "in order to see" and to continue "to fish in troubled waters".

There is actually one factor in the foreground which must be considered in the phenomenon of poisoning, that is, the time factor. Many tests that I have attempted

in vain, and which have given results which at first appeared to me as negative, would have been more useful if I had known the incubation period. I would cite an example from the study of tetanus toxin. In *J. Physiol. Path. gén.*, vol. xxv, p. 53, under the title *Attempts at inactivation of tetanus toxin by sparteine sulphate*, I have written : " We have completely failed in our experiments. The toxin of tetanus is a toxin with an incubation period—there is ' a quiescent period ' before the action, as Maurice Nicolle has stated. Perhaps by producing a prolonged state of sparteinism it might be possible to inactivate the tetanus toxin ". This was actually written in October and November, 1926, and though it was not published till March, 1927, it left my hands on the 15th of November 1926. Thus it was that a communication appeared in the *C.R. Soc. Biol. Paris*, 26th February, 1927, in which I presented the inactivation of tetanus toxin by sparteine *in vivo* as a type of phylaxis. It is essential that the sparteine should be injected eight days before the tetanus toxin in order that this latter should produce no effects. So that to obtain good results with sparteine there must be a period of incubation, just as there is such a period before the symptoms of tetanus become manifest.

We will see shortly that analogous facts can be observed with the mineral waters. With these I have proceeded by the following method :

1.—Preventive injection of mineral waters before that of toxins.

2.—Injection of waters after that of toxins.

1.—*Injection of mineral waters before the toxins*

I have obtained definite inactivation of viper's venom by preventive injections of sparteine, even when these last only preceded the injection of venom by a quarter of an hour. With mineral waters, however, when injected a quarter, a half, one or two hours before the toxins, I have been unable to obtain inactivation of the toxic effect. From what I have just said about sparteine and tetanus toxin one can guess that, for mineral waters to be effective, it is not only a matter of hours but of days. Consequently a number of animals were sacrificed and numerous failures encountered before I succeeded in producing phylaxis in my animals.

The time factor is a tyrant, against which our impatience is of no avail, yet nevertheless in its more or less slow rhythm it holds the secret of vital manifestations. I was too impatient at first and wished to go too quickly, in fact as quickly as the diffusion of sparteine and venom, with the result that I paid for my haste by prolonged researches and a long wait. I will not set out the results I noted at this period because they contain in essence the whole problem of phylaxis, and they will be discussed more leisurely in what follows later.

2.—*Injection of mineral waters after the toxins*

Here again I have injected mineral waters after the toxins in periods, measurable in hours, and contrary to what I observed with preventive injections, using similar time periods, I noted that my animals could be saved.

I have seen guinea-pigs survive after truly lethal doses of tetanus toxin and diphtheria toxin by injecting, after a six hour delay, the water of Choussy (La Bourboule) against tetanus toxin, and the water of Rocher (Saint-Nectaire) against diphtheria toxin. Clearly we have here to deal with inactivation *in vivo*. I would add that the results just mentioned only occur with a period of about six hours for the best results, and these are less good when the time is under five or over eight hours. Here again, we observe a phenomenon in which the time factor plays a part and in a singularly precise manner. Is this inactivation, at a definite time after the intoxication, of a curative nature or of a phylactic nature ? What is certain, however, is that these facts do demonstrate that the mineral waters act not only *in vitro* but also *in vivo*. Having been mostly interested in the study of the problem of phylaxis I have not yet followed up the study of this inactivation after intoxication, but I do not hesitate in separating it from phylaxis.

Phylaxis by Châtelguyon Waters against Venom

We know that the venom of the Auvergne viper contains a whole series of elements which play different rôles in the intoxication and which can be classified under two heads : the neurotoxins (which resemble curare in their mode of killing), and the hæmorrhagins (which produce local necrotic inflammation, hæmolysis, etc.). Thus it is conceivable that a mineral water might protect against the neurotoxins without protecting against the hæmorrhagins, or vice versa. According to the way in

which the animals are treated, it is possible to obtain, using Châtelguyon water, almost complete inactivation as regards the hæmorrhagin and complete as regards the neurotoxin, or on the other hand inactivation of the neurotoxin only, which latter is clearly the most dangerous constituent, even though its effects are less painful than those of hæmorrhagin.

When a guinea-pig receives an intraperitoneal injection of a mixture of venom and Châtelguyon water, *in vitro*, the animals shows very little subsequent reaction, it is uncommon for it to cry out like the controls and shortly it commences to feed just as before the injection. If the injection is made subcutaneously the local inflammatory reaction is attenuated, and scarcely to be compared with the controls. I have already stated that Châtelguyon inactivates venom and I wish to stress particularly that *in vitro* it inactivates these two noxious active principles.

The inactivation of the hæmorrhagin *in vitro* appears to me to belong to the same group of phenomena of precipitation and adsorption as I have quoted earlier from E. Gley, for one sees immediately that the animal is not protected against hæmorrhagin by treatment *in vivo*.

I must point out as well that, when experimenting with Châtelguyon water, it is very essential to use water that is absolutely clear. Bottled Châtelguyon water rapidly throws a deposit, which increases with time, but I do not know if this is due to liberation of carbon dioxide, or to interaction between the mineral water and the glass of the bottle, or to any other reasons. Whatever the case may be, if the bottle is in any way shaken before use and

the very fine deposit clouds the liquid ever so slightly, then instead of protecting the animal such water mixed with venom destroys it in a few minutes. I have thought it important to mention this for the benefit of those who like myself cannot work at the springs but only with waters which have been bottled, perhaps for a long time. I believe that the majority of the waters of Châtelguyon can produce phylaxis against venom. My own researches have been made *in vitro* with water from the following springs, Deval, Gübler, Yvonne, Louise, all of which inactivate venom quite well. For my investigations *in vivo* I have used Gübler and Yvonne because of the clarity of the bottled waters at my disposal.

Warned by my previous failures, when I have given a preventive injection (which I could truly term sensitising) I have given the venom (provocative injection) after a delay, not of a few hours, but only after some days. I have treated eight guinea-pigs with intraperitoneal injections of 5 c.cs. of water from the source Yvonne, and eight others, in the same way, with water from the source Gübler II.

Researches on the Mechanism of the Phylactic Action of the Mineral Waters

The interpretation of the mode of action of the mineral waters against the neurotoxins has necessitated the consideration of different hypotheses according as to whether the action is *in vitro* or *in vivo*.

Action "in vitro"

It is manifest that the simplest hypothesis that can

be adopted to explain the mode of action *in vitro* is that of precipitation and adsorption, as I have previously explained. However, though this hypothesis can be admitted in so far as we are concerned with the hæmorrhagin of venom (which behaves in the same way as the proteolytic ferments) it is useless as an explanation of the action as regards the neurotoxin of venom and the other neurotoxins I have studied, because the phylactic action of the water is manifested above all *in vivo*.

Action "in vivo"

As regards the inactivating action *in vivo*, which truly arises from phylaxis, it seems to me that two hypotheses should be considered.

First hypothesis: *Certain free ions of the complex electrolyte, constituted by the thermal waters, while circulating in the blood and body fluids, might become attached to the toxins and so inactivate them.*

It can be seen that this hypothesis has certain points in common with the one that I have put forward as regards inactivation *in vitro*. The effects of the thermal waters would be produced by the direct action of the ions on the toxin, a sort of neutralisation as it were. This hypothesis however deserves further examination.

The toxins belong to the group of proteins which are characterised by the grouping of simple elements, carbon, nitrogen and hydrogen, united by oxygen. All the protein substances always contain mineral matter (Ca., Mg., P., S., etc.). "Pure albumen, freed from mineral matter, is only obtained at the cost of profound changes

in its state, shown for example by the loss of its solubility properties (it becomes soluble in water), and does not correspond to the substance in its natural condition, since proteins are always intimately united with mineral moieties. Thus it was thought that potassium, sodium, calcium, etc., just as in the case of iron in hæmoglobin, are not only an integral part of the different albuminoid molecules, but that they play a definite rôle, on which the special function of the different molecules depends : a substance combined with an alkaline metal or with a metal of the alkaline earths does not behave in the same way as the substance combined with chlorine or phosphoric acid." (E. Gley, *loc. cit.*, p. 30.)

On the other hand, we know what important parts are played by the minutest quantities, as for example, silver in Raulin's solution, manganese in relation to the oxydases (G. Bertrand), zinc in the case of snake's venom (Delezenne), cobalt and nickel in insulin (G. Bertrand and M. Macheboeuf). Doubtless these elements have a particular activating effect on which the special functions of oxydases, venom and insulin are dependent ; but is it possible to imagine that each neurotoxin can undergo a modification of its molecular constitution which inactivates it as a result of the fixation of a particular ion contained in the mineral waters from definite sources ? M. C. Delezenne, after demonstrating the stimulating action of calcium salts on trypsin has said : " The neutral salts of the alkaline metals, on the other hand, oppose the activation by calcium ". (*Livre jubilaire* de Ch. Richet, 1916.)

In spite of the attractiveness of this hypothesis I have been unable to accept it, at least in so far as phylaxis *in vivo* is being considered, because this conception implies a direct action on the toxin, a sort of neutralisation of the toxin, and one must remember, in connection with the phylactic action of sparteine, that the toxin persists intact since the animal is able to produce specific antibodies, which enable it to resist further lethal doses of toxin. I have noted the same facts in phylaxis by mineral waters and I have come to the conclusion that these waters modify the state of receptivity of the animal and so render it refractory, for a time, to the action of the toxin. The investigation of this refractory state has led me to suggest a second hypothesis which is as follows :

Second hypothesis : *Certain free ions of the complex electrolyte, constituted by certain thermal waters, become attached to the nervous system and render it refractory to impregnation by toxins.*

We know that neurotoxins owe their name to their specific affinity for the nervous system, as with the neurotoxins of the venoms, tetanus toxin, diphtheria toxin and many other poisons, the neurotropic properties of which, determine what part of the central nervous system or peripheral nervous system is selected.

There is however a common property of the neurotoxins which has been well demonstrated and that is their solubility in the lipoids of the nervous system. As regards the phylactic action of sparteine I have insisted at some length on the particular affinity of the neurotoxins for the lipoids, so here I will only outline some suggestions

concerning the constitution of the lipoid complexes, which have led me to study firstly the rôle played in phylaxis by some of the elements of this complex.

Considerations on certain Functions of the Lipoids and their Rôle in the Phenomena of Phylaxis

The first hypothesis concerning phylaxis by mineral waters, which I have set out, I have abandoned, but the second one, which I have just stated, I find to be more acceptable and the results of investigations, which I am going to discuss, justify this preference, or at least I hope so.

I have mentioned above how Overton, concerned solely in a study of the rates of diffusion across cellular membranes, had been led to classify as lipoids those substances, which had as common link one physical property only—their solubility in the anæsthetics. From the chemical point of view this group is completely heterogeneous for, to cite the two principal elements of the lipoid complexes, it classes together cholesterol, which is a monovalent alcohol, with the lecithins, which are phosphorised fats. Nevertheless many authors have considered that this grouping together of many substances, unrelated from the chemical point of view, under the term lipoids could be defended from the point of view of their functional rôle or physiological activity.

I do not wish to present a long summary of the very numerous works which touch on this question, but from all these investigations I have retained certain facts which support my thesis.

Amongst the large number of workers who, following the initial experiments of Wassermann and Takaki, have investigated which elements of the nervous system are fixed on by the neurotoxins, the majority, if not almost all, have concluded that the neurotoxins become attached to the lipoids. Only Marie and Tiffeneau and their pupils Laroche and Grigaut believe that the neurotoxins are fixed by the proteins of the central nervous system. (*Ann. Inst. Pasteur*, 1911.) From the publications of these authors it would appear that they worked with emulsions of the well purified lipoids—cholesterol, cerebrosides, phosphatides and protagon. There is no doubt that they used purified products emulsified in either distilled water or physiological saline; this technique, though no information is given on the subject in their article (Guy Laroche and Grigaut, *loc. cit.*), is otherwise the same as that which has been employed by the great majority of investigators and is the method of Wassermann and Takaki which Laroche and Grigaut describe as follows:

"These authors have stated, that when white mice are injected with one or more lethal doses of tetanus toxin, the effect of the toxin could be reduced considerably, or even abolished, if the toxin was previously mixed with an emulsion of brain substance."

On the same subject they note that Landsteiner and Botteri "place the emulsified or finely powdered substance in contact with a more or less dilute solution of the toxin at the ordinary temperature". Landsteiner and Botteri (*Uber Verbindungen von Tetanustoxin mit lipoïden*,

Centr. für Bakt., 1906, vi, 582) have in this way studied the adsorbant action of lecithin, cholesterol and protagon for neurotoxin.

Though I do not wish to criticise at length this method of making emulsions for the fixation of neurotoxins by adsorption, or to discuss the importance of the degree of subdivision of the substance which has been powdered, I must insist that there is a considerable difference between an emulsion of cerebral substance, as made by Wassermann and Takaki, on the one hand and an emulsion of pure cholesterol on the other.

Cholesterol is insoluble in water but, inasmuch as it is a hydrophobe colloid, it will form with water, in the course of time suspensoid colloidal solutions. (Höber).

We know from the work of Mayer and Schaeffer that there is a constant ratio between the lipoids and the soaps contained in all the organs and tissues (*Lipocytic ratio* of Mayer and Schaeffer). Further it is clear that soap favours solution of lipoids in the body tissues and fluids ; and also, besides the classical examples of lipoids (Overton), a cerebral emulsion as prepared by Wassermann and Takaki would contain soaps, which many authors classify amongst the lipoids, from consideration of their physiological rôle (Bang, Iscovesco). From this one sees how much difference there is between emulsions of cerebral tissue and emulsions of purified and powdered lipoids. It also seems that there is a difference, no less important, between emulsions of cerebral substance and living nervous tissue.

We know that " a colloidal substance can exist under

two different forms, either as a sol or as a gel, the former resembles a solution, the latter a jelly. Almost all the inorganic colloids are sols, though silica forms a gel, and almost all the organic colloids are gels, though the lipoids can form sols." (F. d'Hérelle, *Les Défenses de l'organisme*, E. Flammarion, 1923.)

In a living animal the lipoids appear in the form of a solution, while on the other hand in a dead animal they are not only in the form of a gel but are in an even more advanced stage, that of coagulation. This fact is a matter of common knowledge, and butchers, after having skinned their animals immediately after death, wait while the fat coagulates before quartering them. There is thus a considerable difference between emulsions of cerebral substance and living cerebral substance from the point of view of their constitutional state, even if one only considers their condition of physical equilibrium.

It is due to the soaps that lipoids exist in the state of a sol in the living animal. " In fact, solutions of cholesterol in aqueous solutions of bile salts and of soaps (E. Gérard), or in sterilised bile will give a precipitate of cholesterol if the bacillus coli or the bacillus of Eberth is allowed to grow therein. This precipitation can be explained by the bacterial destruction of the bile salts and the soaps, which constituents of the bile hold cholesterol in solution." (E. Lambling, *Précis de Biochimie*, Masson, 1925, p. 188.) If one considers these facts, it is obvious that the soaps play a part of utmost importance in maintaining the equilibrium of the lipoids in the body fluids and tissues. Thus we are led to ask ourselves what part they play in

the fixation of neurotoxins, and further, what is their rôle in phylaxis produced by mineral waters.

We have seen that, according to d'Hérelle, the lipoids exist in our organism in the form of sols. " What is the difference between a sol and a gel ? If one takes a sol containing in a given volume a sufficient number of micellæ and adds thereto a quantity of a salt, insufficient to produce immediate coagulation, one can then observe, if the solution is left at rest, the change en masse into the form of a gel. If this gel is then shaken, it breaks up into small isolated flakes. The gel is therefore in the state of a colloid, the equilibrium of which is very near coagulation. At the moment it changes to a gel, Brownian movement ceases to be observable." (d'Hérelle, *loc. cit.*)

I have quoted d'Hérelle here for many reasons :

(1) Because he is short, simple and clear, and because I cannot find that he is in serious contradiction anywhere with the authors who have particularly studied the lipoids, Mayer, Schaeffer, Terroine.

(2) Because in his book on " *Les Defenses de l'Organisme* " d'Hérelle does not anywhere consider the lipoids in this connection, and consequently he does not make any suggestion concerning their possible rôle in the phenomena of defence.

(3) Because, in his brief exposition, he indicates the action of the mineral salts and their electrolytes in the formation of gels, and because, simply presented therein, I have found the reason which has led me to think that the electrolytes of the mineral waters play a similar part in the phenomena of phylaxis by these waters.

However, after all the considerations which I have just discussed, one realises at once that only the experimental method can yield a decision for or against my suggestion. My investigations, at first, have been carried out on the fixation of neurotoxins by solutions of soaps.

Fixation of neurotoxins by soaps

I have limited myself in these researches to the palmitates, oleates and stearates of soda, firstly because they are the commonest in our body tissues and fluids, and also because these soaps are usually present in the fatty part of the constitution of the lecithins which, we know, are phosphorised lipoids. In this way I preserved a binding link between the lipoids and the soaps.

If I make a mixture *in vitro* of tetanus toxin and a solution of sodium palmitate, and several days later inject this mixture into an animal, I find that this animal does not present any of the symptoms of tetanus, and I can say that the toxin has been inactivated by the soap. H. Vincent has already observed and published this fact, and further he has shown that sodium palmitate can fix considerable doses of toxins to such a point, that these can later be injected without injuring the animal.

We know that Vincent has given the name *cryptotoxins* to those toxins hidden away, as it were, by the palmitates. In effect, the toxin and its neurotoxic properties are no longer apparent. These phenomena of inactivation of toxins by adsorption *in vitro* are very different from the phenomena of phylaxis *in vivo* that I communicated to the Société de Biologie in January, 1927 and described in

adequate detail in the *J. Physiol. Path. gén.*, March, 1928.

Although I have not used such doses of toxins as were employed by Vincent, and my investigations had not the same end in view, I would remark here that, of the soaps which I have tested, the palmitates fix the neurotoxins to the greatest extent, and also that the oleates are capable of some degree of fixation, while the stearates are inactive.

I do not wish however to lose sight of the fact that my researches were intended for the elucidation of the problem of the inactivation of toxins by mineral waters and of phylaxis by these waters. In defining the selective affinity of certain neurotoxins for certain soaps I have not departed from my plan, for I can now study the action of phylaxis producing waters on the soaps and see if the properties of the former are modified.

The action of the mineral waters on the soaps

I have not been able to understand why hydrotimetric studies of the mineral waters have never been made, although these are compulsory in the case of all potable waters. Do not mineral waters belong to the category of potable waters ? We know that Carnot and Villaret rightly consider these as medicinal waters, but even then, is that sufficient reason for hydrotimetric studies not having been made ? We cannot foresee whether some suggestions, useful in the interpretation of their effects, might not well arise from such a study.

I have had the curiosity to investigate to what extent

certain mineral waters can produce variation in the surface tension of soap solution. To that end, I have made a series of mixtures using a solution of ordinary household soap by taking 1 c.c. of 0.5 per cent. solution of this soap in distilled water and 19 c.cs. of mineral water from different springs in our neighbourhood. By the use of stalagmometric methods, employing the drop counting pipette of Duclaux and a densimeter, and working at 16° C., I have encountered differences of surface tension from 56.9 dynes to 9.75 dynes and a whole series of intermediate values, according to the origin of the water I had used for the experiments (twenty-three springs), and these results I will describe shortly. They possess only relative value, since they were obtained with water which had been bottled for various periods and using any soap, yet nevertheless one consideration appears to follow—that of these waters, some soap well, others badly—some disperse well the colloidal particles of soap, others jellify them, and others precipitate them. Though these facts are only results of varying degree it may be possible to find something of value.

In what follows I have limited myself to the stalagmometric study of the phylaxis producing waters that I have already mentioned, that is to say, Choussy, Yvonne, Rocher and Madeleine. I have made these investigations with the collaboration of Laville who has incorporated them in his thesis for doctorate. We first determined the surface tensions of 0.1 per cent. solutions of the soaps in distilled water. These, reckoned in dynes at 18° C., were as follows :

Oleate	34.2
Palmitate	53.9
Stearate	12.6

The surface tension of the mineral waters was estimated after the following treatment. Equal volumes of each mineral water, viz. 15 c.c., were treated with 0.5 c.c. of a 2 per cent. solution of sodium oleate in distilled water, 0.5 c.c. of 2 per cent. sodium palmitate, and 1.5 c.c. of 2 per cent. solution of sodium stearate (the explanation of this difference of the quantities of soap solution utilised will be given later). Reckoned in dynes the surface tensions were as follows :

	Choussy.	Yvonne.	Rocher.	Madeleine.
Oleate ..	47.84	72.09	60.11	61.55
Palmitate..	56.60	72.09	67.83	68.39
Stearate ..	66.21	72.09	69.10	69.68

We should mention that the surface tension of the pure mineral waters are approximately 75.2 dynes per cm., the variations only affecting the decimal place.

From these figures certain facts appear ; firstly, that the waters of Choussy are those which soap best and those of Yvonne soap least well ; secondly, that the oleate and palmitate in the same quantities give with Yvonne water the same surface tension, but that to get this same value with stearate it was necessary to use three times the quantity as of the other soaps, viz., 1.5 c.c. instead of 0.5 c.c.

These results are certainly very interesting and I will consider them later, but the end we pursue at the moment is not only to study these modifications of surface tension

but to determine the actual points of precipitation of the different soaps by the different waters. We have had to use stalagmometry because, as we will see in a moment, it is so extremely helpful in the determination of these precipitation points. In order to determine these, we put up a series of tubes, each containing 15 c.c. of mineral water, and added increasing volumes of relatively concentrated soap solutions (2 per cent.). After shaking in order thoroughly to mix the liquids, they are left for twenty-four hours. The tubes are then examined: in some, containing the smallest amount of soap, this is entirely precipitated; then one finds tubes the contents of which appear as a colloidal gel. The critical point lies between the last tube where precipitation has occurred and the first which appears to contain a gel. A series is then put up, with greater degree of accuracy, between these two points, and this series is controlled by stalagmometry. Here one takes the last tube which contains a gel. Using stalagmometric methods it is very easy to discover traces of non-precipitated soap, and thus determine the critical point at which precipitation occurs. Our results are as follows:

	Mineral water 15 c.c.			
	Choussy.	Yvonne.	Rocher.	Madeleine.
Sodium oleate 2 per cent.	0.05 c.c.	5.5 c.c.	0.20 c.c.	0.06 c.c.
Sodium palmitate, 2 per cent.	0.05 c.c.	5.5 c.c.	0.30 c.c.	0.12 c.c.
Sodium stearate, 2 per cent.	0.50 c.c.	7.5 c.c.	1.25 c.c.	0.30 c.c.

These results have been further controlled by means of the ultramicroscope by observing the extinction of the Brownian movements at the moment of precipitation.

Why have we so meticulously determined the critical point of precipitation ? We have already observed, and we know from Vincent's work, how powerfully some soaps can fix certain neurotoxins, so that *if we wish to estimate the phylactic properties of the water after precipitation with soap, it is essential that no soap should remain in solution, for otherwise it would falsify our results.*

By proceeding in this manner we have been able to demonstrate that by the precipitation of certain soaps by mineral waters capable of producing phylaxis, these waters lose their power against the neurotoxins.

After a number of tests in series we have observed that :

(1) Waters treated by *sodium palmitate* up to the critical point of precipitation of the soap completely lose their phylactic properties. We have verified this fact with, Mont-Dore (Madeleine) for sparteine, La Bourboule (Choussy) for tetanus toxin, Saint-Nectaire (Rocher) for diphtheria toxin. Experiments, that we have repeated several times, with Châtelguyon and venom have given us less precise results, but I will return to this point later.

(2) Waters treated with *sodium oleate* have not completely lost their phylactic power. Animals treated with such water die later than the controls, while using waters treated with the palmitate animals die as quickly and sometimes more quickly than the controls.

(3) Waters treated by *sodium stearate* do not lose their phylactic properties and animals become resistent to poisons in the same way as when using untreated waters.

Here then we have clear and definite facts concerning sparteine, diphtheria toxin, and tetanus toxin.

The results obtained with venom and Châtelguyon water have been different and less definite. Sometimes I have found that the oleate treated waters mixed with venom protect better than the palmitate, other times the palmitate treated waters have been more effective than the oleate, in other words the phylactic properties of these waters have been destroyed sometimes by oleate, sometimes by palmitate. In fact, I have not obtained such precise results as with the other poisons. This lack of precision might perhaps be due to insufficient time of contact but mostly, I think, to the fact that the venom of the Auvergne viper contains, besides the neurotoxins, hæmorrhagins or cytolysins, which render the problem more complex. I will try later to determine these unknown factors. However it may be, the following conclusions can be drawn from the results I have just presented:

(1) *The palmitates, precipitated by the ions of the electrolytes of those mineral waters, which can produce phylaxis, bring down in their floccules all the ions which produce phylaxis.*

(2) *The oleates, precipitated by the electrolytes of these same waters, bring down only a part of the ions which produce phylaxis.*

(3) *The precipitated stearates leave intact in the mineral waters the ions which produce phylaxis.*

(4) *The electrolytes brought down with the floccules of soap are different according to the soap that has been used.*

(5) The electrolytes fixed on the soaps form different precipitates according to the mineral water used, that is to say, one obtains different insoluble soaps from each mineral water.

I have been able to verify this fact in the following way: Some palmitate precipitated by Châtelguyon water, which is not phylactic for sparteine, is recovered on filter, washed with distilled water and physiological saline, and then ground up in a mortar with some sulphate of sparteine in an amount certainly lethal for a guinea-pig (8 mg. per 100 g. of body weight). After treatment with this palmitate the animal has shown some signs of sparteinism but has survived. I can therefore say that this palmitate had not been layered by Châtelguyon with such ions as were suitable in order that it should no longer fix sparteine.

[This experiment assumes a knowledge of some work published by Laville (*Thèse de Pharmacie*, Toulouse, 1929, on "*The Modification of the Phylactic properties of soap by mineral waters and reciprocally of the mineral waters by the soaps*," p. 45). Laville stated: "We have tried to discover if a soap flocculated by a mineral water could still fix the neurotoxin corresponding to the mineral water used. We experimented with sparteine, as it was more easily handled than other neurotoxins, and because it has no inoculation time. The water of Mont-Dore (Madeleine), which produces phylaxis in a guinea pig against sparteine, has been brought to the flocculation point in relation to three soaps. The mixture was then filtered, and the precipitate resting on the filter was then emulsified in physiological saline. To the three emulsions so obtained, a lethal dose of sparteine (10 mg. per 100 g. of animal) was added and after a period of contact of two hours the mixtures were injected into guinea pigs. These all died in the same time—thirty-five minutes: thus the three soaps after flocculation were no longer able to fix sparteine." In the experiment described here by Billard, the precipitate Châtelguyon-palmitate does not "protect" the soap but still leaves it with the capacity of fixing sparteine because it has not been "layered" with the specific ions; on the other hand the precipitate Mont-Dore-palmitate, "layered" with specific ions, can no longer hold sparteine.—*Ed.*]

We see from these facts how the study of the new soaps, formed by different mineral waters, will allow us to obtain more definite knowledge concerning the therapeutic properties of the thermal waters, and still more so when we understand better the rôle of the soaps in the defence of the organism and in their capacity as regulators of intracellular exchanges, because for these substances the lipoid barriers of Overton offer no resistance.

(6) This last test, together with those I have set out above, shows definitely that organisms are put in a state of phylaxis by ions which are specific for each toxin.

All these considerations and facts, which I have brought forward, have led me to visualise the whole of these phenomena in the following way :

(1) The lipoid colloids exist in the state of a sol in some sort of soapy phase. The latter being a colloidal solution of soaps, of which the micellæ have an electro-negative charge, as also have the lipoid micellæ, therefore promotes a dispersion of the latter and thus produces a sol.

(2) The penetration of toxins or poisons up to the lipoids of Overton necessitates firstly that they be dissolved or fixed by the soap micellæ, these being considered as a special type of intermediary bodies.

(3) In the body fluids this soapy phase will also carry the common electrolytes, which primarily maintain the osmotic pressure of the cells.

(4) The dissociated ions of these electrolytes or of any foreign electrolytes can promote, in the case of

electro-negative ions, molecular dispersion; or on the contrary, in the case of mobilisation of electropositive ions, can provoke gel formation or even local or general precipitation. It is easy to understand what perturbations could accompany such phenomena even when they are localised to one portion of the nervous system, e.g. thermal crises.

(5) The phylaxis producing ions of the mineral waters have a selective action on the palmitates, for which the neurotoxins equally exhibit a selective affinity.

(6) When the physical equilibrium of the palmitates has been upset by the fixation of phylaxis producing ions, toxins can no longer attach themselves to these palmitates.

This provides the explanation of the phylactic action of certain mineral waters.

CHAPTER FOUR

PHYLAXIS BY CHLOROFORM

According to my original plan I ought to continue by a study of phylaxis by neurotoxins immediately after the discussion of the mineral waters, but phylaxis by neurotoxins is difficult to effect, as one will see later, and further the interpretation of the results requires complicated analysis. In order that no confusion should arise in the linking up of the various facts, phylaxis by chloroform and by gardenal will be considered next. This, I hope, will enable us to understand more easily and definitely the phylaxis by neurotoxins.

Phylactic Capacity of Chloroform

Convinced as I am of the all-important rôle of lipoids in the phenomena of phylaxis, I have wondered if the anæsthetics, the tropism of which for the lipoids of different segments of the central nervous system was so clearly brought forward by M. Nicloux, and particularly if chloroform, which helped Overton to establish the group of lipoids, could not modify *in vivo* the lipoid equilibrium sufficiently to render animals either more sensitive or more resistant to neurotoxins.

I have already said that I, like most living biologists, imagine the impregnation of the central nervous system by neurotoxins as similar to staining by a dye. An example will make this clearer. We know that " basic dyes and

their neutral salts, neutral red, methylene blue, toluidine blue, Nile blue, and safranin are vital stains, that is to say, they can penetrate living tissues. Whereas the non-vital stains are insoluble in lipoids the vital stains are all soluble in those bodies ". (E. Lambling, *Précis de Biochimie*, Masson, 1919, p. 136.)

Amongst these liposoluble and therefore vital dyes, there is one, methylene blue, which is especially interesting for our exposition. To this end I will here quote the experiments of Leonard Hill from the summary of E. Gley in his *Physiologie*, page 383. " If we inject this substance into the blood of a living animal the nervous centres remain quite unstained, but if the animal (dog or rabbit), on which we are experimenting, is anæsthetised, the brain takes on a blue colouration." Leonard Hill attempted to explain this fact by the successive phenomena of oxidation and reduction. However that may be, we have here two substances which possess a marked tropism for the lipoids of the central nervous system and of which the action of one appears to assist that of the other. Is it the same for the neurotoxins ?

I have not yet been able to determine sufficiently precisely the effects of methylene blue on the neurotoxins so I must confine myself here to the results I have obtained with chloroform.

Phylaxis by Chloroform against Sparteine

1.—Control guinea-pig, weight 400 g. Injected with sparteine 8 mg. per 100 g. body weight. Death after 21 minutes. (Sparteine Byla.)

2.—Guinea pig, weight 270 g. Anæsthetised for 3 minutes and awakens 6 minutes later. After further 7 minutes sparteine was injected. Death after 36 minutes.

3.—Guinea-pig, weight 290 g. Injection of sparteine followed immediately by anæsthesia. Half an hour later there were symptoms of severe sparteine poisoning which lasted an hour, followed by rapid recovery. Two hours later the animal appeared normal.

4.—Guinea-pig, weight 310 g. Anæsthesia for 3 minutes 15 seconds and sparteine was injected before awakening, which occurred after 8 minutes. The animal survived after having presented a slight paresis of the abdominal wall.

Thus chloroform possesses a true phylactic action against sparteine, but I have noted that is of short duration and is only manifested during anæsthesia, since in a batch of twenty-five guinea pigs, I have only been able to save those which received the injection of sparteine during anæsthesia or those which were anæsthetised immediately after injection.

Phylaxis by Chloroform against Venom

1.—Control guinea pig, weight 370 g. Injected with 0.2 mg. of venom per 100 g. body weight. Death after 14 hours 45 minutes.

2.—Guinea pig, weight 300 g. Anæsthetised for 3 minutes, 12 minutes later injection of 0.2 mg.

of venom per 100 g. body weight. The animal survived without noteworthy symptoms.

3.—Guinea-pig, weight 330 g. Venom injected 10 minutes before anæsthesia. Death after 9 hours.

Here again chloroform shows definite phylactic action against venom, but it is purely preventive. Further, another fact which was previously observed with formalin and sparteine, is to be noted, that attempts at cure are ill-omened and that death occurs more quickly in these cases than with the controls.

Phylaxis by Chloroform against Diphtheria Toxin

1.—Control guinea-pig. Death from diphtheria toxin in 31 hours.

2.—Guinea-pig, weight 400 g. Anæsthetised at 5.25 p.m. Injection of diphtheria toxin at 5.40 p.m. The animal survived.

3.—Guinea-pig, weight 360 g. Injection of diphtheria toxin at 5 p.m., and anæsthetised at 5.15 p.m. Death after 16 hours.

Here again preventive anæsthesia has had a phylactic action, but such anæsthesia has had an unfortunate effect from the curative point of view.

Phylaxis by Chloroform against Tetanus Toxin

My attempts at this have given insufficient results, and this can be explained without doubt by the transient nature of the phylaxis produced by chloroform, and which

cannot arrest the progressive but slow invasion of tetanus toxin.

Attempts at producing an anatoxin with Chloroform

It is impossible to collect snake venom aseptically; further, after it has been dried and one prepares standardised solutions, these cannot be kept for any length of time. If sterilised by heat the qualities of venom may be considerably modified, and also if it is filtered through a candle, this retains part of the venom or its elements by adsorption. I once tried to preserve some by chloroform, and some by ether. I noticed then, that the solution had rapidly lost the greater part of its toxicity in a few hours. Was this venom attenuated by ageing, or by physico-chemical modifications produced by the chloroform, or more simply, by the phylactic action *in vivo* of this last. Biological experiments seem to support the final suggestion.

A.—A guinea-pig is given a dose of sparteine (lethal in 45 minutes). This was mixed in a syringe with 5 c.c. of chloroformed physiological saline. It survived without showing any symptoms of sparteinism.

B.—A guinea-pig is given 0.2 mg. of venom per 100 g. body weight, mixed in the syringe with 5 c.c. of chloroformed physiological saline. It survived without noteworthy symptoms.

C.—A guinea-pig, weight 310 g., receives an injection of 0.2 mg. of venom which had been

mixed with chloroformed physiological saline 15 hours previously. Very little reaction followed the injection, and some hours later the animal appeared normal.

D.—Guinea-pig, weight 340 g. Injected with 0.3 mg. of chloroformed venom (preparation 40 hours old). Survival without symptoms.

E.—Guinea-pig, weight 300 g. Injected with 0.4 mg. of chloroformed venom (preparation 96 hours old). Survival time 10 days, whereas the control died in 6 hours.

F.—Guinea-pig, weight 410 g. Injected with 0.5 mg. of chloroformed venom (preparation 120 hours old). Survival time 7 days.

G.—Guinea-pig, weight 460 g. Injected with 1.0 mg. of chloroformed venom (preparation 120 hours old). Survival time 2 days.

H.—Guinea-pig, weight 310 g. Injected with 0.3 mg. of chloroformed venom (preparation 168 hours old). Survived without symptoms. Five days later, injected with 0.3 mg. of non-chloroformed venom—the animal survived.

I do not presume to have produced a true chloroformed anatoxin, nevertheless this would seem possible.

Phylaxis by Chloroform and Antitoxic Sera

Dufour and Duhamel presented a communication to the Société Médicale des Hôpitaux on the 2nd of April,

1926, on the treatment of tetanus by injection of anti-tetanic serum while the patient was under chloroform anæsthesia. They reported good results, obtained by this method of treatment. Dufour, Vidiez and Casteran further stressed this observation at the Société Médicale des Hôpitaux on July the 1st, 1926. Ravina, at that time, also reported favourable results to the same society. Since then many articles on this subject have appeared in La Presse Médicale. Do these observations bear any relation to the phylactic properties of chloroform which I have just described ? for in my researches, I noted that chloroform, when used as treatment, had an unfavourable, in fact, fatal influence. We know, it is true, that we must not apply rapid conclusions, drawn from results of animal experiments, to human beings. With diphtheria toxin I have made the following observations :

A.—Guinea-pig, weight 250 g. Received a dose of diphtheria toxin which killed it in fifteen hours.

B.—Guinea-pig, weight 230 g. Received a dose of diphtheria toxin and six hours later 0.25 c.c. of antidiphtheritic serum per 100 g. body weight. Death after 30 hours.

C.—Guinea-pig, weight 280 g. Receives a similar dose of the same diphtheria toxin and 6 hours later 0.5 c.c. of antidiphtheritic serum per 100 g. body weight. Death after 35 hours.

D.—Guinea-pig, weight 380 g. Injection of a dose of diphtheritic toxin (lethal in 15 hours) and six hours later injection of 0.5 c.c. of anti-

diphtheritic serum per 100 g. body weight. Anæsthesia immediately after the serum. The animal survived.

Five other guinea-pigs, treated in the same way as D, also survived. It is manifest therefore, that chloroform has had a favourable influence in these cases.

The results are also satisfactory when smaller doses of antitoxic sera are employed. Using 0.2 c.c. of serum per 100 g. body weight, 3 animals had survival periods of 45, 48 and 55 hours respectively.

It appears, consequently, that anæsthesia aids the neutralisation by antitoxin of toxin already fixed to the lipoids.

With tetanus toxin we have obtained similar results when the animals are treated by serum and anæsthesia fifteen hours after the injection of an amount of toxin, lethal in two or three days (series of twenty-five guinea-pigs).

It is generally held, and particularly as regards tetanus toxin, that antitoxin is unable to neutralise toxin already attached to the nervous system. How is it then possible to explain these observations ?

The Interpretation of the Mode of Action of Chloroform

Certain considerations, relevant to the observations we have made, will, I hope, re-attach us to the thread of Ariadne which has guided us to this point. I will quote at first a summary of these from Etienne Burnet,

who discussed a number of toxins, and from this I will select that which concerns tetanus toxin.

"The experiments of Meyer and Ramon, and of Marie and Morax show that tetanus toxin does not pass directly from the circulation to the nervous centres, but that it penetrates into the peripheral nerves through the motor terminations and arrives at the centres by following the course of the nerves. Some dissociation experiments have demonstrated that antitoxin acts by neutralising such toxin as is still circulating but it does not neutralise the part which has been already absorbed by the nerve trunks, these latter being incapable of absorbing antitoxin."

Injected toxin disappears more or less quickly from the blood and from the circulation because it becomes fixed on the cells of the organism, and further it cannot be recovered to any appreciable extent from the excretions. Seventeen hours after the inoculation of a rabbit no free toxin can be found in the blood and organs, and there is never any in the blood at the moment that tetanus appears (about forty-eight hours after intravenous inoculation. A. Marie).

"When tetanus toxin becomes fixed to other cells than nerve cells it is clear that the former are retaining some portion of the toxin and so protect the nerve cells in the manner of a screen."

From another quarter one can read in *La Presse Médicale*, 1928, No. 97, 5th October, an abstract by Robert Clément of an article by R. Le Clerc, *On the liberation of neurotropic toxins by anæsthetics* (*Le Bulletin*

Médicale de Québec, July, 1928, vol. xxix, No. 7). "We realise the affinity of the nervous system for toxins. This affinity is such that the latter can absorb many times the lethal dose of toxin, and further, if cerebral matter is washed with water, the liquid, poured off by decantation, contains no toxin. Anæsthetics and particularly ether, have the property of being able to liberate, in experiments *in vitro*, toxin so fixed. This explains the good results obtained by the use of general anæsthesia in severe cases of tetanus." Do the considerations, which have just been expressed, aid us in the interpretation of the mode of action of chloroform in the experiments which I have made and are described above ?

From these tests, two conclusions appear :

(1) Injection of toxin into an anæsthetised guinea-pig does not give rise to intoxication.

Is this phenomenon of a phylactic nature ? There cannot be the least doubt of this, though the state of phylaxis commences immediately and without any appreciable incubation period, which is explained by the proper nature of the phylactising substance.

If in fact neurotoxins owe their name to their specific affinity for the lipoids of the nervous system, it is also the case that the grouping together of the different lipoids was established by their not less specific affinity for the anæsthetics. It is not therefore surprising that anæsthetics can impregnate the lipoids of the nervous system with a power, greater than that of toxins ; it is easy to understand that the toxins being unable to fix themselves on the nervous system during anæsthesia (the

place of attachment belonging to the first and most powerful occupant) can attach themselves " to other cells than the cells of the nervous system, and so protect the nerve cells in the manner of a screen ".

The toxins are neither transformed nor destroyed, but at the level of the " other cells " they are able to provoke the elaboration of antibodies.

Thus I have been able, with some degree of success, to outline the study of a sort of anatoxin with chloroformed venom.

(2) Antitoxins neutralise toxins *in vivo* better during and after anæsthesia.

We have seen that antitoxins do not neutralise toxins which are fixed to the nervous system but only the circulating toxins. Chloroform, as a result of its powerful affinity for the lipoids of the central nervous system displaces the toxins fixed here, and so returns them to the circulation. It then becomes possible to neutralise the toxins, and this would appear to have been confirmed by the experiments I have set out above, not only for tetanus toxin but also for diphtheria toxin.

These observations justify the treatment of severe cases of diphtheria by anæsthesia and injection of large doses of antitoxin while the anæsthetic has mobilised the toxins.

Chloroform, through its affinity for the lipoids, is a powerful phylactic agent, perhaps even a curative one. May we not see here a further proof of the hypothesis we have put forward to explain phylaxis.

CHAPTER FIVE

PHYLAXIS BY GARDENAL

I COMMENCED to study the action of gardenal as the result of a series of considerations regarding the mode of action of this drug, and also because gardenal serves as a link between phylaxis by mineral waters and phylaxis by chloroform, at least that is the way in which the facts appear to me.

Gardenal was first used by the Germans, under the name of luminal, as a hypnotic and consequently as a neurotropic drug. Therapeutic applications of this drug have, by chance, shown that it also possesses the singular quality of suppressing, more or less completely, epileptic fits.

We know now that gardenal possesses, apart from its hypnotic properties, the remarkable power of promoting the fixation of the calcium ion in the organism. As a result it allows the fixation of a buffer (regulator of the acid-base equilibrium of the body).

We realise, above all since the work of Bigwood, the part played by alkalosis in spasmophilia, tetany, convulsions, etc., and this throws a new light which renders comprehensible the action of gardenal.

W. Koch in 1903, while studying the physico-chemical properties of aqueous emulsions of phosphoaminolipids,

noted the precipitating action of certain salts on these emulsions and he concluded that :

(1) Salts of monovalent metals are without action.

(2) Salts of bivalent metals produce precipitation.

(3) Salts of trivalent metals produce precipitation to the same degree.

(4) Non-electrolytes have no appreciable precipitating power.

These considerations, which have been briefly presented, have led me to suppose that gardenal might be considered as having two forms of activity :

(1) as hypnotic.

(2) as regulator of the acid-base equilibrium by fixing calcium on the soaps or lipoids of the nervous system.

From my point of view, the power of fixing the calcium ion on the lipoids of the nervous system suggests to me a relationship between its own proper activity and that of the mineral waters. Consequently I was not very surprised when I obtained the following results.

Tetanus Toxin

Although the results I obtained with chloroform against tetanus toxin had not seemed to me sufficiently precise for presentation, those I obtained with gardenal are quite definite :

A.—Two guinea-pigs used as controls—one died in 73 hours, the other in 75 hours.

B.—This guinea-pig received at 3 p.m., 22nd August, 1927, a similar injection to that given to the controls, but it was mixed *in vitro* with 0.01 g. gardenal (Poulenc). At 10 a.m., 25th August, the animal ingested 0.01 g. gardenal. Following this the animal became sluggish and stuporose but survived without any signs of tetanus.

C.—Three other guinea pigs, treated in an identical manner, have survived injection of tetanus toxin without any symptoms.

The doses of gardenal were 0.01 g. per 300 g. of animal.

Viper's Venom

With viper's venom we have obtained the following results :

(1) Gardenal, when injected five to six hours before the venom, protects an animal against three times the minimum lethal dose, which is 0.1 mg. per 100 g. body weight.

(2) Gardenal, mixed *in vitro* with venom, protects an animal against doses up to 0.5 mg. per 100 g. body weight and after a period of contact in the syringe of five to ten minutes.

(3) Treatment by gardenal after injection of venom has disastrous effects. I will return to this point later.

Phylaxis by gardenal is therefore undeniable. Is it due to the hypnotic action of the substance or to its

regulator action on the calcium metabolism ? Where the effects are rapid, as in the case of phylaxis against venom, I would willingly consider that there is a hypnotic action comparable to that of chloroform but as regards the effects on tetanus toxin I would rather stress the action of calcium, the fixation of which, particularly on the lipoids of the nervous system, is stimulated by this drug. It is easy to see that this mode of action is related to that of the mineral waters, on which I have particularly laid stress.

The reason why I have described here the phylactic action of gardenal is mainly because I have chosen this drug following some deductions which arose from my working hypothesis. The results which I obtained quite support this hypothesis, and up to now I do not believe that I have been following any false path.

CHAPTER SIX

PHYLAXIS BY NEUROTOXINS AGAINST OTHER NEUROTOXINS

If we glance back at the path we have just traversed in order to find some view point, we soon see that a simple idea has been our guide, and that is, that certain poisons have a selective affinity for the lipoids (here including the soaps) of the nervous system. This selective affinity is more powerful for some poisons than for others, which may also be neurotropic. When one of these poisons become fixed, others of feebler affinity cannot displace it and reach their goal.

Up till now, my working hypothesis is apparently quite in agreement with the observations which have been established, however dissimilar the phylaxis producing substances which have been used. Now we come to a study of more complex phenomena, the interpretation of which will be more difficult. I have already mentioned some cases of phylaxis against a neurotoxin which has not been produced by an ordinary phylactic agent but by the use of another neurotoxin, administered in a sublethal dose.

In a series of communications to the Société de Biologie (1910, 1911), I have shown that the autolysis

juice of pig's liver, obtained in an atmosphere of chloroform vapour, inactivates different poisons and toxins after admixture *in vitro*. During these researches I have been led to performing an injection of a lethal dose of viper's venom into a guinea-pig which, four days previously, had received a lethal dose of tetanus toxin, inactivated by liver juice. This animal resisted perfectly the injection of venom. This test which was repeated with a series of animals with identical results, led me to conclude that tetanus toxin protects a guinea-pig against venom. This protective action *in vivo* cannot be certainly obtained by the liver juice only, according to my observations. Further, though tetanus toxin protects against venom, the inverse does not take place, that is to say, that an animal treated by venom succumbs after a lethal dose of tetanus toxin. I did not follow up this investigation till after I had accurately determined the phylactic effect of sparteine against the neurotoxins.

Is it possible to consider that the protection of a guinea-pig against the effects of venom by a preliminary injection of tetanus toxin is a phenomenon of phylaxis ? I have at first thought this to be so. It is after all easy to suppose that when the nervous system of a guinea-pig is more or less impregnated with a poison, so eminently neurotropic as tetanus toxin, that one should find that the neurotoxin of venom is no longer able to fix itself to the same nervous system. This indeed appears to be the conclusion to be derived from the following typical experiments which concern the protection against venom by tetanus toxin, and against diphtheria toxin by tetanus

toxin. The interpretation of these facts will be discussed after their exposition.

A.—Tetanus Toxin and Venom

Guinea-pig No. 7 on the 20th January received a dose of tetanus toxin (which killed the controls in three days) mixed *in vitro* with 1 c.c. of the autolysis liquor of pig's liver. On the 24th January the animal is injected with 0.4 mg. of venom per 100 g. body weight. It survived without symptoms.

Guinea-pig 192, on the 24th March at 10.30 a.m. received an injection of tetanus toxin in a dose which killed the control animals in ten hours. The same day at 3.30 p.m. it received an injection of 5 c.c. of Choussy water containing 0.01 g. of stovarsol. Later it was given an injection of 0.2 mg. of venom per 100 g. body weight and the animal survived without showing any symptoms.

I could multiply these examples of this *in vivo* inactivation of venom by previous injection of tetanus toxin, because it is relatively easy to effect these tests and to obtain successful results.

B.—Phylaxis against Diphtheria Toxin by Tetanus Toxin

Protection of a guinea-pig against a lethal dose of diphtheria toxin by previous injection of tetanus toxin is somewhat difficult to realise. Out of fifty animals treated I have only been successful in effecting this ten times.

If one admits my hypothesis concerning the staining or impregnation of the nervous system by toxins, in the

same way as with sparteine, it becomes easy to realise that once this system has been dyed, to however small an extent, by tetanus toxin, it can be no longer stainable by diphtheria toxin because the site of fixation belongs to the first occupant, a toxin possessing the greatest selective affinity for the nervous system. Often it happens that the impregnation of the nervous system is insufficient and the diphtheria toxin passes through and kills, or that the dose of tetanus toxin is too strong and it is this that slays the animal. It is therefore difficult to perform these tests but they become easier when the diphtheria toxin is injected nearby the site of injection of the tetanus toxin (intraperitoneal route).

I would repeat that I have only succeeded in obtaining ten positive results out of fifty animals treated, and I will discuss these facts at greater length while I am dealing with the interpretation of these results.

Interpretation of the Results

Although all the observations that I have just produced appear to be quite related to phylaxis, nevertheless I do not consider that this is so. I have been careful in establishing the existence of definite limits between immunity and phylaxis, in the sense that the idea of neutralisation of toxins plays no part in phylaxis, where there can be no question of the action of antibodies. It is not, however, certain that antibodies do not intervene in the preservation of a guinea-pig by one toxin against another toxin.

Here are some observations, put forward by Emile

Roux in a communication to the Congrès de Budapest, where it seems indeed that antibodies play a rôle :

(1) " The serum of a healthy horse mixed with cobra venom in no way prevents the latter from acting, while the serum of an animal immunised against tetanus, if added to venom, renders it innocuous. Such antitetanic serum injected before the venom considerably retards the fatal issue, and even prevents it if given in repeated doses." (*Ann. Inst. Pasteur*, 1894, viii, 726. "*Sur les sérums antitoxiques*".)

(2) " A rabbit, immunised against tetanus, dies if given a dose of venom a little larger than that which kills a new rabbit."

From these observations of E. Roux it would appear that venom is neutralised by tetanus antitoxin and this suggests that antitetanic serum contains a powerful antineurotoxin, which is not purely specific for tetanus toxin. E. Roux states (p. 727) " Assuredly antitetanic serum is much more efficacious against the poison of tetanus than against the venoms"; although he adds, "but these are only questions of degree". Further he writes " I have noted that antitetanic serum was not without action on snake's venom, and I have given Dr Calmette, who studies the serum therapy of venom in my laboratory, this problem for investigation."

However, in Calmette's book, *Les Venins, les Animaux Venimeux et La Sérothérapie Antivenimeuse* (Masson, 1927), there is no reference to researches on the use of antitetanic serum against snake's venom, neither do we find this

serum mentioned in the work of Vital-Brazil, *La Défense contre l'ophidisme* (Pascall Weiss et Cie, São Paulo, 1924).

I have no intention here of discussing the specificity of the antitoxic sera, but from the observations which have just been put forward, it appears definitely that antitetanic serum neutralises the neurotoxin of venom and that it has some antitoxic power against it. This fact does not fit in with the notion of phylaxis as I have considered it since, in the phenomena of phylaxis, the neurotoxins remain intact though they are unable to attach themselves to the nervous system.

E. Roux, in the same communication, reports an impressive series of facts which suggest the existence of many non-specific immunisations :

(1) " Recently, M. Duntschman has noted that the serum of animals immunised against symptomatic anthrax acts on the ' bacillus of acute septicæmia ' " (*loc. cit.*, p. 723).

(2) " The serum of untouched rabbits has no action on venom, that of rabbits vaccinated against rabies is antivenomous to a high degree" (*loc. cit.*, p. 727).

(3) " Antivenomous serum renders rabbits more resistant to rabies, and antirabic serum has also an action on the venoms " (*loc. cit.*).

(4) Finally he states " I could give other examples of the action of a single antitoxin against many poisons."

I would remark here that E. Roux does not, at any time, mention immunisation by one toxin against another toxin (this remark has some importance as we will see

later) but can we deduce from his facts that animals, which produce antitoxins and whose serum inactivates " many poisons " are themselves more or less immunised against these poisons ?

Though there is no question of considering a habitual non-specific rôle of the antibodies, it is nevertheless curious to note that a *preliminary* injection of " antitetanic serum considerably retards the fatal issue " following injection of venom. Does it not seem that the nervous tissue, when impregnated with antitoxin, has not been able to absorb the neurotoxic poison as easily as usual. An antitoxin could thus play a phylactic rôle in the already defined sense of the word. Thus we have endeavoured to demonstrate the non-specificity of the phenomena of phylaxis.

Conceived in this manner, phylaxis by neurotoxins takes a place beside immunity without supplanting it in any way. We wish to show simply the possibility of such interpretations.