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ANAPHYLAXIS AND ANTI-ANAPHYLAXIS

A. BESREDKA

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ANAPHYLAXIS AND ANTI-ANAPHYLAXIS

AND THEIR

EXPERIMENTAL FOUNDATIONS

BY

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WITH A PREFACE BY

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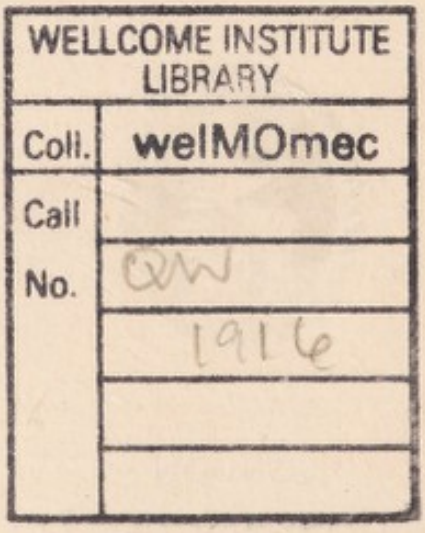

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PREFACE TO THE ENGLISH EDITION

THE translation of a work on an intricate subject such as anaphylaxis is not without its special difficulties. It is not sufficient merely to produce an accurate rendering of the original text; a clear expression of the author's meaning is even more important. Some of the technical terms, for example, have no satisfactory English equivalents. I have therefore endeavoured throughout to keep the sense of the subject, rather than the literal text, intact. Apart from this no alterations or additions to the original have been made. References have all been verified in order to avoid as far as possible any errors in transcribing, and an index has been prepared.

In the concluding chapter I have endeavoured to bring together the results of recent work on this complicated subject, and for this I am solely responsible. Original communications to the journals on anaphylaxis appear almost every month, and at the present time interchange of foreign publications is not always easy; I have therefore to ask the indulgence of the reader for any errors of omission which may have been made.

S. ROODHOUSE GLOYNE.

LONDON.

PREFACE

It is rarely that the significance of a discovery is immediately understood, especially if this discovery is unexpected. Such has been the case with anaphylaxis. In 1902 Charles Richet and Portier discovered that a dog that, twelve days previously, had received a harmless dose of actino-congestin, became so sensitive to the effect of this substance that it succumbed in a few minutes to a fresh injection of an amount far less than the dose lethal to another dog. In spite of the fact that the authors laid stress on the very peculiar characteristics of the phenomenon they had just discovered, and that they had marked its novelty by giving it a special name, there were many who only saw in it a striking example of sensitisation in respect of a poison. Charles Richet and Portier, however, viewed the matter in quite a different light, and doubts as to the exceptional nature of the phenomenon became impossible after Arthus, Otto, and later Rosenau and Anderson had found that a substance as harmless in appearance as horse serum was able to set up fatal anaphylactic symptoms. It was soon recognised that anaphylaxis could be produced by the majority of albuminoid substances, whether of animal or vegetable origin. The subject of anaphylaxis henceforth assumed an importance of which at first it had not been suspected to be capable. Its quasi-mysterious character so much stimulated the enthusiasm of workers that memoirs innumerable

were published relating to it. Though this plethora of publications has opened up some interesting facts to us, it has nevertheless introduced some confusion into the subject. It is common to see the term "anaphylaxis" applied to symptoms that can lay no claim to it. It is necessary before everything else to set this mass of research in proper order; this is the task Dr. Besredka has accomplished in the present work which I have the pleasure of introducing to biologists. Dr. Besredka is in possession of a special qualification for the fulfilment of this task, since we are indebted to him for some of the most valuable advances in our knowledge of anaphylaxis.

The author takes anaphylaxis in the guinea-pig as typical, because anaphylactic phenomena are reproduced in this animal with a regularity and precision met with in no other subject. The state of anaphylaxis is brought about after the animal has received a preliminary injection, and at a definite point of time after the reception of this injection. Preliminary injection and consecutive incubation are the conditions necessary for anaphylaxis. As the result of a second or "exciting" injection, a condition is produced which, owing to the violent symptoms of sudden onset, has rightly been compared to shock. Once acquired, the anaphylactic state persists for a long while, probably during the whole life of the animal. Moreover, anaphylaxis is specific—that is to say, the substance which has served for sensitisation is alone capable of setting up the anaphylactic shock. The dose which causes the death of the animal when it is injected at one time, whether intravenously or by way of the nervous centres, is shown to be harmless if it is introduced by fractional and intermittent injections. The animal which has received it in this fashion becomes like a new being; it is desensitised, and, without suffering any harm,

tolerates a large quantity of the substance which, previously, would have killed it in the smallest doses. Whilst sensitisation is only acquired after an interval, desensitisation is, so to speak, instantaneous, and constitutes anti-anaphylaxis, which is as specific as anaphylaxis itself.

Having specified these characteristics of anaphylaxis, Dr. Besredka goes on to discard all those experiments in which preliminary preparation and specificity are lacking, as they have nothing to do with true anaphylaxis. Indeed, the fact of a guinea-pig dying speedily following the injection of some substance intravenously, after having exhibited convulsive movements and arrest of respiration, does not authorise us to cite the case as one of anaphylaxis. The almost sudden death of guinea-pigs which are injected intravenously with serum shaken up with kaolin or brought into contact with agar, does not constitute a death due to anaphylaxis. It is due to circulatory trouble, caused, possibly, by the coagulation of blood in the small vessels, but in these cases we do not find the characteristics of anaphylaxis. In order to avoid the mishaps that follow intravenous injection, Dr. Besredka prefers to use intrathecal injection. This test has led him to eliminate from the signs of anaphylaxis the anaphylotoxins of Friedberger, alarming in their effects if introduced intravenously, and harmless when they are made to penetrate the nervous centres.

Having thus very wisely narrowed the limits of the domain of anaphylaxis, Dr. Besredka arrives at the more interesting point of his subject—namely, that of ascertaining the mechanism of the phenomena of anaphylaxis.

Everyone admits that after the preparative injection and during the incubation period a fresh substance is formed in the organism—namely, a specific

antibody. The sensitisation of the animal is due to the presence of the latter. Indeed, it is sufficient to introduce a little blood from a rabbit sensitised to horse serum subcutaneously into another guinea-pig to witness the latter becoming sensitive in its turn in the space of a few hours. The foreign blood introduced into the animal as a fully prepared antibody confers upon it a state of passive anaphylaxis. The sudden symptoms which follow the exciting injection are caused by the affinity of the antigen for the antibody.

So far there is entire agreement between scientists, but divergences begin to manifest themselves when one is called upon to explain how the reaction of the antigen and the antibody set up the anaphylactic shock. In the opinion of one school the product of this reaction is a very potent poison and the anaphylactic symptoms depend upon an intoxication. This is the opinion of Charles Richet, and it is shared by the majority of authors.

Dr. Besredka maintains that there is no anaphylactic poison. A harmless complex results from the union of antigen with antibody. The antibody becomes attached to certain nerve-cells, and the antigen combines with it and suddenly penetrates the nerve-cells. This produces the disturbance which finds its expression in the anaphylactic shock. If the cells are rendered anæsthetic they do not react, and this explains the harmlessness of the second injection in the sensitised guinea-pig when it is anæsthetised with ether. In the same way there will be no anaphylactic shock if the introduction of the antigen be graduated so that the combination with antibody is brought about slowly and not hurriedly.

This conception of anaphylaxis is a simple one; it takes account of known facts, and it has led Dr. Besredka to carry out the practice of graduated injec-

tions which has been of so great service. Such are the serious claims for its adoption.

The résumé which I have just given of Dr. Besredka's book is out of all comparison with the value of the book itself. I must apologise to the reader for having kept him so long upon the threshold. In discoursing on the pleasure I have had in reading them, I have delayed his enjoyment of the perusal of these pages in which a very interesting but at the same time very complicated subject is expounded in an attractive manner.

DR. ROUX.

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ANAPHYLAXIS AND ANTI-ANAPHYLAXIS

CHAPTER I

INTRODUCTION

General Characters of the Phenomena of Anaphylaxis.

What is anaphylaxis? Had this question been put to the most highly qualified bacteriologists only ten years ago, in nine cases out of ten no reply would have been forthcoming. At that time the few persons who had heard of Richet's experiments on actino-congestin were decidedly of the opinion that it was a matter of pure physiology which would scarcely interest the bacteriologist and still less the clinician.

Since then this subject has made such strides that to-day if one does not wish to pass for a clinician of the "old school" a knowledge, at least upon its broad lines, of all that has to do with anaphylaxis is indispensable, and especially of the means that should be taken to avoid it. Just as often happens in similar cases, we go from one extreme to the other. To-day we see anaphylaxis in everything, and should a biological phenomenon that is a little out of the ordinary type make its appearance, we immediately regard it as related to anaphylaxis.

The echo of this has reached the ears of persons with no claim to be regarded as professional. Do we not hear of mothers of families bringing the worst

charges against serum therapy, and raising the bogey of mishaps resulting from anaphylaxis whenever an injection of serum is to be made ?

Anaphylaxis has become quite the fashion. There is no doubt that in the realm of general pathology there are few subjects invested with so captivating an interest. As we are still aiding in its evolution, there necessarily remain some obscure points about it which excite the imagination of scientists. Moreover, the interest attaching to the problem is not entirely speculative. On certain sides it touches our most vital problems—namely, serum therapy and even alimentation.

What constitutes the most striking trait in anaphylaxis is its quasi-paradoxical character. This is perplexing to one who has been brought up in the traditions of immunity, and at one time made us speak of anaphylaxis as reversed immunity.

Indeed, how should we otherwise regard this strange fact that the person who reacts to a first injection reacts to the second much more strongly than to the first ? On the contrary, has not the practice of vaccination accustomed us to see injections borne with all the greater tolerance the more frequently they are made ?

Take, for instance, the case of a person who at some time in his life has been injected subcutaneously with therapeutic serum for curative or preventive purposes. A month, a year, or several years, elapse. That person has completely forgotten that he was injected. One day a fresh injection of serum is considered necessary. It is made; and the needle is hardly withdrawn from the vein or the spinal cavity before a sequence of symptoms occurs—not always happily—that creates a tragic impression. We believe we are witnessing an acute toxæmia ending in death in a few moments.

Has the serum injected accidentally turned out to be toxic? No, for when it has been injected into other individuals who have never previously received any inoculation, no ill-effects, either local or general, have resulted.

What, then, is the cause? It is anaphylaxis.

Instances of this nature are observed with mathematical regularity in the guinea-pig. If we merely inject a guinea-pig, subcutaneously or otherwise, with a minimal dose of horse serum, say about 0·01 c.c., the animal from the moment of this operation becomes "marked" for the rest of its days. If after the lapse of some time—a fortnight, six months, or a year—we give this same guinea-pig an intravenous, intraspinal, or subdural injection of 0·1 c.c. of the same serum—that is, a dose which is absolutely harmless to any ordinary guinea-pig—symptoms of extreme gravity immediately make their appearance The animal is seized with convulsions which increase in severity; signs of paralysis immediately appear, followed in a few minutes by cessation of respiration and terminating in death.

What invests these anaphylactic phenomena with so mysterious a character is the fact that they are set up by substances that are entirely anodyne in nature. This upsets our ideas as to the harmfulness or the reverse of the substance. For instance, we inject an animal subcutaneously with an extremely weak, almost infinitesimal dose of milk or egg-albumen. Six months or a year later the same guinea-pig is inoculated with a dose of milk or egg-albumen that would not cause a normal guinea-pig to move a muscle. Barely two to three minutes have elapsed from the time of operation before the animal becomes overwhelmed. What is so astonishing is the fact that non-toxic substances assume so formidable toxicity in animals that have already been injected

on a single occasion. What again is perplexing to the last degree is that substances such as egg-albumen, milk, and serum, which may be employed in almost unlimited quantities on ordinary occasions, become deadly in doses that are almost infinitesimal during periods of anaphylaxis.

Such is the riddle which numerous scientists have sought to explain. What is the source of this toxicity which nothing justifies and which nevertheless makes its appearance with such suddenness and violence at the time of reinjection of the same substance ?

Clearly this toxicity can only originate in some change that takes place in the body of the animal itself under the influence of the first injection.

We shall return to this point in the course of this monograph, more especially in the chapter devoted to Theories relating to Anaphylaxis.

CHAPTER II

FIRST STUDIES ON ANAPHYLAXIS

Experiments of Richet and Portier on dogs with actino-congestin in 1902—Experiments carried out by Arthus with serum on rabbits in 1903—Observations on the effect produced by repeated injections of serum in man by v. Pirquet and Schick in 1903—Experiments carried out by Otto, and Rosenau and Anderson, on guinea-pigs in 1906—First papers on the subject of anaphylaxis and anti-anaphylaxis by Besredka and Steinhardt in 1907.

ALTHOUGH attention had already been drawn to hypersensitiveness in the more general acceptation of the term by Koch, Hayem, Behring, Brieger, Knorr, Flexner, and J. and P. Courmont, Rist, and others (even by Magendie as early as 1839), it is to Charles Richet that we owe not only the felicitously chosen term of "anaphylaxis," but also the creation of the subject itself. It was through a series of researches carried out by him, first on the serum of snake poison, but more especially later on the toxins of Actiniæ, that this new idea gained admission to the domain of biology. It was Richet who showed that it was not a question of one isolated fact, but of a phenomenon with a wide significance.

Whilst studying the poisonous action of Actiniæ, Richet and Portier succeeded in extracting a particular poison the toxic dose of which could be determined with precision. This they called congestin, on account of its possessing the property of setting up in inoculated animals an intense congestion of all the viscera—stomach, liver, kidneys, and intestine.

Richet and Portier determined the dose of actinocongestin which could be injected into the veins of a dog without killing it; if they exceeded this dose they killed the dog, though not immediately. The animal did not exhibit anything that gave rise to uneasiness immediately after the injection, and it was only after some time that there appeared symptoms which became progressively aggravated during the course of two days and terminated fatally generally about the third day.

But events took a different course in a dog which had previously received a weak dose of congestin; it was sufficient to inject it with a dose equal to one-twentieth of the first dose in order to produce, after only a few seconds, the sudden onset of serious symptoms such as violent attacks of vomiting, dyspnœa, paraplegia, etc.

"The characteristic experiment," says Richet,[1] "which revealed the phenomenon to me in all its undoubted clearness was carried out on a dog called Neptune. He was an exceptionally vigorous and healthy dog. He had first of all received 0·1 c.c. of glycerinated fluid (glycerinated extract of the tentacles of Actiniæ) without any ill-effects resulting. Twenty-two days afterwards, when he was in excellent health, I injected him with the same dose of 0·1 c.c. He then immediately became exceedingly ill—that is to say, some seconds after the termination of the injection; his breathing became distressed and he panted; he could scarcely drag himself along; he lay on his side, was seized with diarrhœa and the vomiting of blood. Sensation became abolished, and he died in thirty-five minutes."

In other words, the dose of congestin which had no effect on the normal dog rendered the sensitised dog extremely ill and killed him.

[1] Ch. Richet, "L'anaphylaxie," p. 3; ed. F. Alcan.

Nothing gives a better idea of the intensity of the phenomenon, according to Richet, than the difference between these two cases. In the case of the normal dog there was no vomiting even with a dose of 0·08 gr.; in that of the sensitised dog vomiting occurred with a dose of 0·001 gr. The anaphylactic state therefore renders the animal eighty times more sensitive in the particular instance of poisoning by congestin.

The first work of Portier and of Richet dates from 1902.

In the following year (1903) Arthus published an account of his researches on the hypersensitiveness of rabbits in the presence of horse serum.[1] Upon renewing the injections at set intervals, he noted that the serum was first of all reabsorbed without any difficulty. But when the fourth injection was made the serum set up a local infiltration. This infiltration, mild to commence with, became indurated after the fifth injection, and would assume a gangrenous appearance after the later injections. The same local phenomena were observed when the first injection was made intraperitoneally and the subsequent ones hypodermically.

Moreover, Arthus discovered that it was possible to produce serious disturbances and even death in rabbits which had received several doses of serum subcutaneously, by afterwards injecting them with the serum intravenously.

It was found possible to produce analogous phenomena by means of treatment with milk. These phenomena were exhibited in a strictly specific fashion—that is to say, the animals treated at first with milk only appeared sensitive to a further injection of milk and not to an injection of serum. As was stated by Arthus, "the rabbit sensitised by and for serum is not so for milk, and *vice versa*."

[1] *Comptes rend. Soc. de Biol.*, 1903, lv., p. 817.

The observations recorded by v. Pirquet and Schick relating to the hypersensitiveness of the human subject in the presence of serum were published in the same year (1903), and were mainly clinical. In a short note preceding their account these authors sought to bring into relief the fact that the body, when treated with certain substances—serum in particular—acquired the power of reacting more rapidly to a reinjection of the same substance.

Having had under observation a large number of children treated with sera—antidiphtheritic and anti-scarlatinal—these authors described under the name of "serum sickness" a variety of symptoms following this treatment. They remarked that in children who had received this serum for the first time serum complications appeared after seven to twelve days from the time of incubation, never before six days. On the other hand, in children who were injected with serum for the second time the incubation period was distinctly shortened; the serum sickness might even make its appearance immediately after the injection, or at the latest some hours afterwards, and that with quite weak doses of serum (1 c.c.).

Therefore the first dose sensitised the child: after a second injection serum complications appeared with greater rapidity and regularity; more than this, they appeared as a sequel to a weaker dose of serum than was injected on the first occasion.

The study of serum anaphylaxis only entered upon its fruitful stage from the experimental point of view when the guinea-pig was adopted as the most suitable animal for the experiments.

In American laboratories for serum therapy this curious fact had long ago been observed—namely, that guinea-pigs which had been employed for the testing of antidiphtheritic serum exhibited as a

result a remarkable sensitiveness to horse serum. In the course of his visit to America P. Ehrlich heard this from Theobald Smith, and upon his return to Frankfort he entrusted the study of the phenomenon to his co-worker Otto.

Rosenau and Anderson also took up this research independently of Otto. In 1906, two accounts dealing with the same subject made their appearance almost simultaneously: that of Otto was published in Leuthold's "Gedenkschrift,"[1] that of Rosenau and Anderson in the collection of monographs issued by the Laboratory of Hygiene, Washington.[2]

The publication of these two papers marked an important advance in the experimental study of anaphylaxis. Fresh works saw the light and succeeded one another with surprising rapidity.

The phenomenon which Otto labelled with the name of Theobald Smith exhibits the following features: For purposes of testing the antisera a guinea-pig receives a mixture of diphtheria toxin and antitoxin; if it is injected some time afterwards with several cubic centimetres of normal horse serum intraperitoneally or subcutaneously it may manifest grave and even lethal symptoms.

In Otto's experiments guinea-pigs were used in which the first injection dated back from five to twelve weeks. When they were injected subcutaneously, after this interval, with 6 c.c. of normal horse serum they were observed to exhibit signs of distress and pain; respiration was quickened, and dyspnœa followed; the heart's action grew weak, and the temperature fell to below the normal. Half an hour later half of the guinea-pigs injected were dead; the remainder gradually recovered.

[1] "Das Theobald Smithsche Phaenomen der Serum-Überempfindlichkeit," Berlin, 1906, i., 153-172.

[2] "A Study of the Cause of Sudden Death following the Injection of Horse Serum," Bulletin No. 29, April, 1906.

Not one of the control animals that had received at the time of the first injection the same dose of horse serum (6 c.c.) became ill. Moreover, it was found possible to inject them with a much larger dose without giving rise to any ill-effects.

In view of these findings Otto sought to inquire whether the phenomenon that he had observed was peculiar to horse serum, or whether it was manifested in the presence of other kinds of serum.

Experience soon shewed him that in the presence of rabbit serum, goat serum, or ox serum, the guinea-pigs that had been inoculated with antidiphtheritic serum—that is to say, horse serum—behaved like fresh guinea-pigs. In other words, those guinea-pigs which had been originally injected with the toxin and horse serum were only sensitised to horse serum; and, on the other hand, remained indifferent to reinjection of serum when that serum was derived from an animal other than the horse.

The question now remained, What was this substance which in the mixture as originally injected communicated this state of hypersensitiveness to the guinea-pig; was it the diphtheria toxin or the antitoxin (*i.e.*, the horse serum)?

With this purpose in view, a certain number of the guinea-pigs were injected with diphtheria toxin; four to twelve weeks later they were injected with horse serum. The guinea-pigs thus treated did not exhibit any noteworthy reaction; therefore the toxin had nothing to do with the appearance of the phenomenon.

This fact having been established, another series of guinea-pigs was injected with variable doses of antidiphtheritic (horse) serum only, not mixed with toxin; four to twelve weeks later the guinea-pigs were tested with 6 c.c. of horse serum by subcutaneous injection. None of the guinea-pigs died. A certain number of

them exhibited the characteristic symptoms, whilst others did not shew any indisposition.

To summarise these experiments, Otto concluded that the toxin alone did not play any important part, save to favour the production of hypersensitiveness; it was, however, clearly seen that the horse serum was the essential cause of the phenomenon. Otto, moreover, ascertained the fact that the phenomenon only originated in those guinea-pigs which had first been injected with a weak dose of the serum; and that in guinea-pigs prepared by a mixture of the serum and the diphtheria toxin death occurred in 50 per cent. of the cases.

While fully admitting that he was in ignorance of the mechanism of the phenomenon he described, Otto pointed out the analogy between the phenomenon and the symptoms sometimes observed in man following the injection of serum.

About the same time two American scientists, Rosenau and Anderson, published a very well authenticated study entitled "On the Causes of Sudden Death following the Injection of Horse Serum."

To avoid repetition it is enough for us to say that on broad lines their researches entirely accord with those of Otto. They succeeded, moreover, in rendering the guinea-pigs immune to anaphylactic symptoms by injecting them with repeated massive doses of the serum. We shall return to this point later.

These authors almost succeeded in solving certain other problems relating to anaphylaxis. Thus they established the fact that between the time of sensitisation of the guinea-pig and the time when the animal is ripe for reaction to a second injection there should elapse an incubation period of ten days. They saw that in order to sensitise the guinea-pig a minimum dose of serum (one-millionth of a cubic centimetre) was sufficient, and that once the state of

hypersensitiveness was established it was capable of lasting for months.

Rosenau and Anderson stated that they succeeded in sensitising the guinea-pigs to horse serum by giving them horse-flesh or serum to eat.

Like Otto, they also obtained a clear idea of the specificity of the anaphylactic reaction.

With the object of depriving the serum of its toxicity at the time of the second injection, they attempted to treat it with different reagents either of a physical or chemical nature, but without success. Only heating the serum to 100° C. had any effect on this toxicity; we shall return to this point in detail later on.

As regards the internal mechanism of anaphylaxis, these scientists have sought to discover it in the production of an antibody. According to their views, at the time of the second injection the toxic substance of the serum enters into combination with the antibody in question, and gives rise to the symptoms with which we are now familiar.

Like Otto, they, too, have sought to establish a connexion between the anaphylaxis of guinea-pigs and the symptoms occurring in man.

The facts established by Otto, Rosenau and Anderson may therefore be summed up as follows: (1) The injection of a weak dose of horse serum (0·000001 to 0·004 c.c.) sets up a state of hypersensitiveness or anaphylaxis in the guinea-pig; (2) the addition of diphtheria toxin, without being indispensable, renders this state more pronounced; (3) an interval of at least ten to twelve days is necessary between the "sensitising" injection (weak dose of serum) and the "toxic" injection (5 c.c. of serum) made intraperitoneally; (4) when the interval between the two injections is shorter, and the toxic injection is made before the expiration of the interval of ten to twelve

days, the guinea-pig reacts but very little or not at all. In this latter case it remains for some time unaffected by every fresh injection of serum, even if made after the expiration of twelve days. In other words, after having received by the peritoneal route a massive dose of serum before the establishment of the anaphylactic state, the animal remains immune for some time.

Such was the exact state of our knowledge at the time of the appearance of our first two published memoirs on anaphylaxis and anti-anaphylaxis, written in collaboration with E. Steinhardt.[1]

In order to obtain a clue to the solution of the facts we have just summarised—facts which at the time appeared both without parallel and incomprehensible, because they were altogether outside the province of known phenomena—we put the question to ourselves from the outset as to whether the sensitised guinea-pig, though apparently in the enjoyment of excellent health, had not in reality some latent lesion of the nervous system. Perhaps, we reflected, a second injection, made ten to twelve days after the first, might call into activity such nervous lesion, and this would have the effect of developing those grave complications which result in death.

This hypothesis shews to what point we had advanced into the unknown; indeed, we were in such a state of confusion that progress was impossible save by feeling our way.

Starting from the hypothesis we have just stated, we decided to make the second injection of serum, not intraperitoneally or subcutaneously as our predecessors had done, but directly into the brain. By thus getting into direct contact with the sensitive cell

[1] "De l'anaphylaxie et de l'anti-anaphylaxie, vis-à-vis du sérum de cheval," *Annales de l'Institut Pasteur*, 1907, xxi., p. 117; "Du mécanisme de l'anti-anaphylaxie," *ibid.*, 1907, xxi., p. 384.

we anticipated that anaphylactic symptoms would be set up in a more rapid manner, with weaker doses and with greater constancy than was the case when the injections were made intraperitoneally or subcutaneously.

This is, in fact, what took place: Upon introducing beneath the dura mater of a sensitised guinea-pig 0·25 c.c. of horse serum and even less, we produced in one or two minutes the same symptoms as those which followed the injection of 5 c.c. into the peritoneum. Moreover, while the intraperitoneal injections (5 c.c.) led to death in about 25 per cent. of cases, those made into the brain yielded a mortality of almost 100 per cent. From time to time the guinea-pigs injected by the subdural route did escape death, but they never failed to exhibit a group of the most characteristic symptoms.

It need scarcely be added that we took care to make sure that the fresh guinea-pigs, or those prepared with substances other than serum, tolerated with impunity an intracerebral injection of 0·25 c.c. horse serum. In the same way we made controls with guinea-pigs sensitised to horse serum, and straightway reinjected intracerebrally with 0·25 c.c. of an inert fluid such as beef bouillon or physiological saline solution.

Just as in the case of the intraperitoneal test, we have never noticed anaphylactic symptoms when the interval of ten to twelve days between the sensitising injection and the test injection into the brain has not been adhered to.

This intracerebral test not only exhibited a theoretical interest in exploring the nervous origin of anaphylactic symptoms, but it also shewed this practical advantage, that it henceforth rendered possible researches on anti-anaphylactic immunity. Indeed, before these were undertaken it put us in

possession of a sure means of killing sensitised guinea-pigs. Now, taking into consideration the regularity with which the prepared guinea-pigs succumbed to the cerebral test, we were enabled to make certain that those which resisted this test owed their survival not to mere chance, but in reality to anti-anaphylactic immunity artificially induced. This assurance, which neither intraperitoneal nor, still less, subcutaneous injection was able to afford us—the majority of the guinea-pigs showing themselves to be naturally resistant to injection by these routes—was guaranteed us by the intracerebral method.

From the time of publication of our first monograph in 1907 the study of anti-anaphylaxis has been our primary object. It was also our principal aim in the memoirs that followed in the period covered by the years 1907 to 1912, when we devoted ourselves to the study of various methods of producing anti-anaphylaxis and the mechanism of its production. But, for the sake of clearness of exposition we shall now abandon chronological sequence, and in order to facilitate an understanding of phenomena, so numerous and so varied as those of anaphylaxis, we shall deal with these phenomena in combination with the three main properties, the sensitising, the toxic, and the anti-anaphylactic.

CHAPTER III

THE SENSITISING OR PREPARATIVE INJECTION

Sensitisation to serum by subcutaneous inoculation. Sensitisation by way of the digestive tract.—Sensibiligen and its properties. Attempts to attack its resistance by various agents.—Sensitisation to milk. Sensibiligen of milk and its properties. Oral and rectal sensitisation.—Sensitisation to egg-albumen. Optimum doses. Duration of the anaphylactic State. Heated and non-heated sensibiligens.—Passive anaphylaxis.

FROM a study of the preceding pages it will be seen that in the phenomena of anaphylaxis the same substance—serum, milk, or egg-albumen—may be exhibited under the guise of three different properties. According to the circumstances of the experiment, this substance may be sometimes sensitising, sometimes toxic, and, on the contrary, may sometimes act as a vaccine.

We shall study these three functions separately, always keeping before us the example of serum which is the substance of choice from the point of view of anaphylaxis.

Sensitisation with Serum.—We know already that in order to sensitise the guinea-pig it is sufficient to inject into it a minimum dose of serum (0·01 c.c.) either subcutaneously or intraperitoneally. In the majority of instances it is needless to repeat the injection: one is sufficient. If it be repeated, we can only sensitise the animal further if the doses of serum are very weak.

We can also sensitise with strong doses of serum —with doses as strong as we like (*e.g.*, 5 to 10 c.c.);

only in this case the condition of anaphylaxis appears much more slowly than with weak doses. As a general rule anaphylaxis takes a longer time for its production in proportion to the strength of the initial dose of the serum. For instance, if, in order to sensitise it, we inject a guiena-pig with 5 c.c. of serum, we must not be surprised if we fail to see the onset of the anaphylactic state till after the lapse of several months; whereas with the initial dose of 0·01 c.c. the ordinary interval hardly exceeds ten to twelve days.

Therefore, in order to sensitise rapidly and satisfactorily, it is to our interest to employ weak doses. As to strong doses, they, too, certainly sensitise, but only after a long time. Our impression is that, in order to become capable of sensitising, the serum needs to be eliminated to a great extent, either just as it is, or after having undergone a transformation within the animal. It does not become truly active—that is to say, sensitising—till the very moment that it becomes very much diluted in the body. This is one of the most curious peculiarities of anaphylaxis, and, we may add, one that has remained almost unsolved to the present day.

If weak doses of serum are those that should be chosen by preference, they must not, however, exceed certain limits. It is true that, according to Rosenau and Anderson, even a millionth of a cubic centimetre of serum is sufficient for sensitisation. We would venture to remark, however, that in our experience when the dose has been less than one-thousandth of a cubic centimetre the results have been very uncertain.

Rosenau and Anderson state that animals may be sensitised by the intestinal route. By feeding guinea-pigs with horse-flesh or giving them serum to drink they have succeeded in bringing about hypersensi-

tisation to horse serum. In order to succeed better in this, according to these authors the animals should be thus fed over a long period of time, a single ingestion not being sufficient. Personally, we have not succeeded in sensitising guinea-pigs under these conditions. *A priori* the thing seems possible, and perhaps this is the explanation of those frequent and particularly violent serum symptoms which Russian practitioners have pointed out as occurring in Tartars, because, as is well known, the diet of this race often consists of horse-flesh.[1]

Let us call to mind that in the report which he presented to the International Medical Congress held in London in 1913 Charles Richet stated that "experimental alimentary anaphylaxis is difficult to bring about under conditions of healthy digestion, since it is a question of toxalbumins or nutritive albumins, either because the digestive juices actively intervene in transforming these albumins and rendering them innocuous, or because the individual is immunised against them—at all events, because he passes a minimal quantity of unchanged albumin."

Without coming to any conclusion as to the nature of sensitisation, we fully appreciate the fact that an antibody is produced during the process of sensitisation. We propose to term this antibody *sensibiligen*.

The sensibiligen of serum, when the latter is suitably diluted—it should always be diluted when a rapid sensitising effect is sought—is resistant to high temperatures. Thus a serum which is diluted one in a hundred is resistant to temperatures which much exceed that of coagulation. Serum may be heated

[1] Rist and Richet, junior (quoted by Charles Richet in "Anaphylaxie," p. 77), have noticed that patients feeding on raw horse-flesh react more rapidly than do normal subjects to a subcutaneous (antituberculous) injection of horse serum.

to 100° C. and even to 120° C. without effecting a disappearance of the sensitising power.

The result of experiments which we have carried out along these lines shews that the sensitising property of serum is of a heat-resisting character.[1] This fact was at first disputed by some authors. In fact, since our experiments were undertaken several papers have been published in which different conclusions are drawn. Rosenau and Anderson,[2] for instance, have stated that sensibiligen entirely disappears from serum heated to 100° C. Doerr and Russ[3] affirm that it is impossible to sensitise with serum even when heated to 80° C. On the other hand, Kraus and Volk[4] succeeded in sensitising with serum heated to 90° C. Arthus[5] has likewise confirmed our results by showing that rabbits can be sensitised with serum heated to 100° C.

It was not without interest, therefore, that we examined the reason for these contradictory statements; especially as it was of importance to dispel all idea of a possible error on the part of the experimenters.

It did not take long to convince us that there was, in fact, no question of any error having been made. If the authors had been unable to agree, it was because they were working with sera of different dilutions. In fact, it follows from our experiments that the sensitising property (just as is the case, as we shall see later, with the vaccine and the toxin) is dependent on the physical state of the serum. The more the serum is diluted the less is it coagulated by heat, and the less is its sensitising property affected. In

[1] *Comptes rend. Soc. de Biol.*, 1907, lxiii., p. 294.
[2] *Journal of Medical Research*, 1908, xix., p. 37.
[3] *Zeitschrift f. Immunitätsforschung*, I. Orig., 1909, p. 109.
[4] *Ibid.*, 1909, p. 731.
[5] *Arch. Internat. de Physiol.*, 1909, vii., p. 471.

other words, the more the serum is diluted the more thermostable does the sensibiligen appear to be.

If Rosenau and Anderson, and Doerr and Russ as well, had carried out their sensitisation experiments under the conditions we have indicated, they would have seen that sensibiligen is in reality thermostable; that is to say, that sera adequately diluted, although heated to 100° C. for twenty minutes, preserve their sensitising power in its integrity.

If we have dwelt upon these experiments in some little detail, it is because they shew how far the physical state of the substance is important in anaphylaxis; we shall, moreover, see other examples of it in the course of this account.

Certain authors have sought to isolate in the pure or almost pure state the sensibiligen contained in the serum. For example, Gay and Adler[1] believed that it was possible to obtain it by fractional precipitation of the serum with ammonium sulphate. In the opinion of these workers sensibiligen would be found in the pure state in the euglobulin. This latter substance would also be capable of sensitising the animal in the space of four to five days, instead of taking eight to twelve days. The euglobulin, in its quality of pure sensibiligen, would be deprived of its vaccine and toxic properties peculiar to the whole serum, as we shall see farther on.

Numerous attempts have been made to deprive serum of its sensitising function. In order to effect this experimenters have tried the action of various chemical products such as formaldehyde, hydrochloric acid, etc., upon sera. Without going into detail on this point, we may say at once that every one of these experiments has missed its aim; either the reagents employed failed to destroy anything at all, or else they made such profound alterations in

[1] *Journal of Medical Research*, xiii., 1908, p. 433.

the albuminoid substances that not only did the sensibiligen disappear, but the serum itself also lost its biological characters.

The anaphylactic state may also be produced with proteins other than those contained in blood-serum. The animal may be sensitised with milk, egg-albumen, organic extracts, toxalbumins, bacteria, etc.

For the present we shall only mention milk and egg-albumen, which exhibit many features in common with sera from the point of view of anaphylaxis. The other substances will form the subject of a special chapter, the more so as their sensitising functions have been and are still disputed, because they are not so definite in their results as milk, egg-albumen, or serum.

Sensitisation with Milk.[1]—Upon injecting unboiled (raw) and boiled cow's milk into guinea-pigs subcutaneously, we have noticed that sensitisation by this route has disadvantages which are due to the slow and unequal absorption of the milk. The intraperitoneal route is better suited—so long, however, as unboiled milk is not used. The latter often causes the animals to become emaciated, and in time may set up a condition of cachexia. Milk heated to 100° C. for twenty minutes is best for sensitisation. We inject 1 c.c. intraperitoneally, and for this purpose select by preference guinea-pigs weighing 300 to 400 grammes. Starting from the sixteenth day—or, with greater certainty still, from the twentieth day—the guinea-pigs, in the majority of cases, acquire a hypersensitiveness to a fresh injection of milk which is quite remarkable.

The substance in the milk which produces anaphylaxis in the guinea-pig, or the sensibiligen of the milk, to make use of a term now sanctioned by usage, is thermostable. It withstands a temperature

[1] *Annales de l'Institut Pasteur*, 1909, xxiii., p. 166.

of 100° and even of 120° C. Very often we have sensitised guinea-pigs with milk that has been sterilised in the autoclave at 120° C. for a quarter of an hour; the result has been quite as good as with milk heated to 100° C. or with unboiled milk.

When heated to higher temperatures—130° to 140° C.—the milk sensitises less and less satisfactorily. We have noted that its sensitising power advances, from the point of view of resistance to the temperature, *pari passu* with its toxic power. As we shall see later, the two functions, the sensitising and the toxic, are resistant to 120° C.; beyond that temperature they decline, and disappear completely when the temperature approaches 135° to 140° C.

Let us observe by the way that this parallelism is not absolute: it does not hold good for sera. The sensibiligen of the latter is thermostable; their toxic power is, on the contrary, thermolabile. Already diminished at 70° C., this power, in the case of sera, declines progressively with the rise of temperature. At 100° C. the toxic power no longer exists, whilst the serum sensitises fully.

We have repeatedly attempted to sensitise guinea-pigs with milk by way of the mouth or rectum. We have introduced into the mouths of a large number of guinea-pigs from 3 c.c. to 7 c.c. of unboiled milk; then after varying intervals (16, 18, 23, 30, 44 days) we have tested them by intracerebral injections (0·25 c.c.). Never, in the course of our experiments, have we noticed the slightest symptom of anaphylaxis.

In the same way the guinea-pigs which we attempted to sensitise *per rectum* always proved to be resistant to the intracerebral injection of milk. In order to favour the absorption of milk *per rectum* we first administered to our guinea-pigs a glycerin enema, after which we introduced into the rectum from 1 to 20 c.c. of milk. About a month afterwards we

submitted our animals to the most severe test (0·25 c.c. of milk introduced subdurally): not one of them exhibited the slightest hypersensitiveness upon re-injection.

Therefore, the introduction of milk into the mouth or into the rectum of guinea-pigs has proved useless. We have not succeeded in sensitising them; at least, we have not succeeded under the conditions of experiment indicated.

Sensitisation with Egg-Albumen.[1]—In order to bring about hypersensitiveness in guinea-pigs with egg-albumen we injected them subcutaneously at the outset of our researches with 0·5 c.c. of egg-albumen in its own volume of physiological solution.

The anaphylactic condition made its appearance in this case from the sixteenth day, or more properly from the twentieth day. In our later experiments we saw that the guinea-pigs could be rendered anaphylactic with far weaker doses: for instance, we rendered the guinea-pigs hypersensitive with 0·01 c.c. of egg-albumen, with this additional advantage, that the anaphylactic state was established at the end of a brief interval—on an average, twelve days.

It was interesting to discover whether the sensibiligen of egg-albumen was resistant to raised temperatures; in other words, whether egg-albumen heated to 100° C. retained the property of sensitising animals.

Experiments were carried out along the same lines in the case of egg-albumen as in the case of serum and of milk. The result shewed that although it was diminished by heating, the sensitising property of egg-albumen none the less persists. It is therefore thermostable.

It might be asked whether an animal sensitised with egg-albumen remains in its anaphylactic state

[1] *Annales de l'Institut Pasteur*, xxiii., January, 1909.

for any length of time. Having had occasion to keep guinea-pigs thus treated for over a year, we have been able to make certain that the anaphylactic state after injection with egg-albumen persists quite as well as with serum injection. We have been led to believe that this state when once acquired only disappears with the death of the animal.

Let us note by the way this curious feature in specificity with egg-albumen. Guinea-pigs sensitised with egg-albumen heated to 100° C. appear extremely sensitive to reinjection of this substance. Now, as we shall see later, heated egg-albumen has no action on guinea-pigs sensitised under normal conditions—that is to say, on guinea-pigs that have been sensitised with the raw substance. It therefore appears that from the point of view of its biochemical constitution there exists between heated and non-heated egg-albumen a difference as profound as that between proteins derived from different species of animals. That is an extremely curious fact which deserves the attention of chemists. For details of a biological nature we refer the reader to a paper which we devoted to the study of anaphylaxis produced by egg-albumen.

Passive Anaphylaxis.—So far, for the purposes of sensitisation, whether with serum, milk, or egg-albumen, we have seen that it is sufficient to inject a small quantity of one of these substances in any part of the animal's body. We then wait eight to ten days, experience having shewn us that this is the minimum interval to be allowed for the anaphylactic state to make its appearance.

But there is another means of creating the state of hypersensibility, a method just as certain and extremely rapid. By that we mean *passive anaphylaxis.* Just in the same way as with passive immunity, this anaphylaxis is conferred on the fresh animal by means of antibodies manufactured by another animal actively sensitised.

Von Pirquet and Schick had already expressed the opinion that horse serum when injected would give rise in the body of the animal to a reaction product which is a kind of antibody ("anti-Körperartiges Reaktionsprodukt").[1] These authors thus explained the incubation period, which is observed in individuals injected with the serum, and which lasts eight to twelve days, and never less than six days.

In the opinion of Rosenau and Anderson also, the anaphylactic state was due not to the substance injected, but to the reaction which this substance set up in the animal, a reaction which is made possible by the formation of antibody.

From the date of our first experiments dealing with anaphylaxis we have been very positive about this: "Under the influence of a very weak dose of normal serum injected subcutaneously," we wrote in collaboration with Steinhardt, "the guinea-pig elaborates a new substance—namely, a *sensibilisin*.[2]

In the course of his studies on Arthus's phenomenon in the rabbit Nicolle[3] was successful in obtaining a clear view of the presence of sensibilisin; in fact, he was enabled to produce Arthus's phenomenon in fresh rabbits by injecting them with serum derived from prepared rabbits.

Working on the same lines, Charles Richet[4] carried out the following pretty experiment: A dog was twice injected with crepitin, one month being allowed to elapse between the injections. Six weeks after the second injection the dog was venesected: it need hardly be said that its serum was innocuous. To this serum Richet added some crepitin in an innocuous dose: the mixture was forthwith injected into a fresh dog. The animal was immediately seized with acute ana-

[1] *Loc. cit.* [2] *Annales de l'Institut Pasteur*, 1907, xxi., p. 384.
[3] *Ibid.*, p. 136.
[4] *Comptes rend. Soc. de Biol.*, 1909, lxvi., p. 1055.

phylactic symptoms such as attacks of vomiting, diarrhœa, collapse, micturition, dyspnœa, abolition of the reflexes, etc.

Evidently the serum of the dog that had been first injected contained the anaphylactic antibody which we have termed sensibilisin, to which Charles Richet gave the name of toxogenin.

Passive anaphylaxis possesses the same characteristics and the same specificity as active, with this difference—namely, that it is produced at the outset, that is to say, instantaneously.

Owing to the researches of Doerr[1] and his collaborators, the technique of passive anaphylactisation is at the present date considerably simplified.

A rabbit is injected twice or three times with several days' intervals with serum, milk, or some other substance against which it is desired to obtain sensibilisin. These injections are made indifferently either subcutaneously, intraperitoneally, or intravenously. Six days after the last injection the rabbit is venesected and its serum collected. In the majority of cases it is sufficient to inject 1 c.c. of it into a fresh guinea-pig, in order that it may become hypersensitive at the first onset to the substance which had been injected into the rabbit that supplied the serum.

Passive anaphylaxis has been produced with bacteria by Kraus and Amiradzibi,[2] Briot,[3] and Dopter, Briot and Dujardin-Beaumetz;[4] with pancreatic juice by Nicolle and Pozersky;[5] with tuberculin, antipyrin, and iodoform by Bruck.[6] We shall return to this subject in the chapter dealing with bacterial and therapeutic anaphylaxis.

[1] *Zeitschr. f. Immunitätsf.* I. Orig., 1909, iii., pp. 181 and 706.
[2] *Ibid.*, I. Orig., 1910, iv., p. 607.
[3] *Comptes rend. Soc. de Biol.*, 1910, lxix., pp. 10 and 126.
[4] *Comptes rend. Soc. de Biol.*, 1910, lxix., p. 14.
[5] *Ibid.*, 1910, lxviii., p. 1113.
[6] *Berl. klin. Wochenschr.*, 1910, xlvii., p. 192.

CHAPTER IV

THE TOXIC OR EXCITING INJECTION

Sensibilisin and the conditions which govern its appearance. Different methods of carrying out the test injection; their severity. Intracerebral test; its advantages.—Toxicity of sera; its variations; its dosage.—Toxicity of milk; influence of heat on the toxicity.—Specificity of the toxic effect.—Toxicity of egg-albumen; its variations according to place of injection.—Toxicity of heated egg-albumen; its specificity.—Symptoms of anaphylactic intoxication in the guinea-pig, rabbit, dog, ox, horse, man.

Now that we know how animals are sensitised, it remains for us to investigate what takes place in the organism after sensitisation has produced its effect. At what point of time do the animals pass into the anaphylactic state? How is one to make sure of this new state? How is the toxic effect of the second injection to be estimated? Lastly, how can the toxic effect of the latter be moderated?

We have already seen that sensitisation only becomes operative when a fixed interval of time elapses between it and the second injection. This interval, which is from eight to twelve days, is indispensable for the production of a new body which we have termed sensibilisin and which is none other than the antibody of sensibiligen discussed in the preceding chapter.

Experiments shew, indeed, that the corollary of every injection of sensibiligen is the appearance in the serum of the animal of a special antibody which is the material substratum of anaphylaxis. It is the persistence of this antibody, of this sensibilisin, which

is the cause of the duration of the hypersensitiveness. It is due to this antibody that the animal, while still seemingly quite normal, reacts in the violent manner we are familiar with as soon as the sensibiligen is reintroduced—that is to say, as soon as we proceed to make what we have termed the *test injection*, or *toxic injection*.

What takes place in the animal at the point of time when the sensibiligen encounters the sensibilisin? To this question we cannot give an exact reply. We shall revert to it in detail when we discuss the theories of anaphylaxis. What is of present importance to know is the fact that the anaphylactic antibody, or sensibilism, does exist. With regard to passive anaphylaxis we have seen that nothing is easier than to demonstrate it experimentally. Let us recollect that not long ago observers as acute as Gay and Southard denied its existence.[1] In order to explain the anaphylactic state, these workers formulated an imaginary substance contained in the serum: this substance, or "anaphylactin," was held to persist in the body after all the other constituent parts had been eliminated from it, and this it was which would directly sensitise the animal, in the same way as a toxin or a stain selectively fixes on particular cells. This conception, which, at the present day, possesses only an historical interest, shews into what a state of confusion even enlightened experimenters have been led by anaphylaxis.

How much time is needed for the formation of sensibilisin—that is to say, for the state of anaphylaxis to arise? As we have already remarked, it all depends on the dose of sensibiligen; when the dose is weak the sensitisation is accomplished in eight days; when the dose of sensibiligen is stronger it needs a

[1] *Journal of Medical Research*, 1908, xviii., p. 407; *ibid.*, 1908, xix., pp. 1, 5, and 17.

much longer interval—weeks, and sometimes months. One thing is certain: whatever be the sensitising dose, it is hardly possible to obtain an anaphylactic condition in less than eight days. This is the interval, according to our view, which is necessary for the production of antibody in general, whether this be against cells or against bacteria or against toxins.

There are many ways of carrying out the test injection in a sensitised animal: one may do it intravenously, intracerebrally, intraperitoneally, or even subcutaneously.

The intravenous route is the most effective of all; it also allows of our establishing the fact, quicker than the other routes, that the animal is in an anaphylactic condition—sometimes at the end of seven days. We should not, however, place too great confidence in the intravenous test, especially in the case of fluids containing particulate matter, cells, or bacteria. One may, indeed, by its use set up embolism and help in bringing about phenomena that entirely simulate anaphylaxis though in reality the particular phenomenon present may be one of quite a different nature. The intravenous test is sometimes attended by another disadvantage; when substances that alter the coagulability of the blood are employed, such as peptone or organic extracts, the intravenous route may tend to confusion and to an erroneous interpretation of facts. We shall return to this subject later.

After the intravenous route, the intracerebral route is the most effective; this is the one we have adopted from the beginning of our researches on anaphylaxis. Without its possessing so great a sensitiveness as the intravenous route, it has the advantage of not exhibiting any of its drawbacks. Indeed, there is a whole series of substances which when injected intravenously give rise to toxic symptoms which resemble

anaphylactic shock, while in reality those symptoms are due to coagulation and subsequent embolism. Now, these accidents are never to be feared when the subdural route is employed. If this latter is a trifle less effective than the venous route it is more satisfactory than the intraperitoneal route, and incomparably more so than the subcutaneous route.

The following concrete example will supply a satisfactory estimate of these differences in sensitiveness. Guinea-pigs sensitised to serum were inoculated with a test injection after the necessary interval had elapsed: they exhibited grave or lethal anaphylactic symptoms with 0·05 to 0·1 c.c. serum introduced intravenously. To produce the same effect subdurally 0·07 to 0·125 c.c. is needed. With intraperitoneal injection the guinea-pigs only reacted in half the cases, and even then a dose of 5 to 6 c.c. was necessary. The same dose injected subcutaneously rarely gave rise to serious or lethal symptoms.

As we have pointed out, our preference from the outset has all been for the subdural test. We have discovered that in the case of the guinea-pig one cannot inject more than 0·25 c.c. of the fluid into one hemisphere without setting up symptoms due to compression; this is the dose, therefore, that we have been in the habit of employing in all our experiments.

Toxicity of Sera.—In the course of our experiments we have noted that the sensitiveness of guinea-pigs to serum is far from being uniform, and that sera of various origins set up more or less severe reactions. In the case of the same serum, however, the intensity of the reaction is in direct ratio to the dose injected.

This finding, which nowadays appears so natural, was not so simple at the time of its discovery. This suggested to us the idea of graduating the dosage of therapeutic and normal sera.[1] The test for this

[1] *Annales de l'Institut Pasteur*, 1909, xxiii., January.

dosage was clear: it must be a guinea-pig sensitised to the serum.

It will be understood that while we—and others with us—always limited ourselves to the subdural injection of the same dose (0·25 c.c.) of the serum, it was impossible to estimate the difference between one serum and another. When injected in this quantity all the sera, irrespective of their origin, nature, or age, invariably kill the guinea-pig in a few minutes. If the animal survives—and this does sometimes happen—this dose never fails at the very least to set up characteristic symptoms of a very grave nature.

By progressively diminishing the doses of the serum we were enabled to demonstrate the fact that all sera were not toxic to the same extent, but that there were very marked individual differences between them. In that way we hit upon the idea of determining in the case of each serum its coefficient of toxicity.

In order to form a general idea of the toxicity of sera we had samples of them imported from Russia, Germany, England, Switzerland, and America, and after having established the titre of toxicity for each one of them, we arrived at the conclusion that there are sometimes very great differences between one serum and another. These differences depend largely on the age of the serum, but in addition there exists an individual factor which depends altogether on the breed of the horses, on their food-supply, and perhaps on the manner in which the serum is collected.

We can demonstrate the fact that the addition of preservative fluids and antiseptics (carbolic acid, trikresol, chloroform) in use in most countries fails to exercise any influence on the toxicity of sera.

Amongst the causes of toxicity, that due to the age of the serum deserves the most attention. As our experiments have indicated, the age of the serum no

longer intervenes as a factor when six weeks or two months have elapsed since the venesection. After that individual toxicity alone has to be reckoned with. We have established it as a fact that in the great majority of cases the lethal dose for the sensitised guinea-pig is from 0·084 to 0·125 c.c. of serum. We have, however, seen sera destroying guinea-pigs, in intracerebral or intravenous injections, when the dose has been 0·0625 c.c.; and, moreover, other sera—not many, it is true—which proved to be exceedingly toxic in doses as small as 0·015 to 0·032 c.c., and which set up anaphylactic symptoms in still weaker doses (*e.g.*, 0·0063 c.c.).

The causes of the marked toxicity of certain sera, independent of their age, still elude us. The fact of its occurrence is useful to know; and we shall be on our guard against it.

On the other hand, the toxicity which is due to the age of the serum lends itself to more accurate analysis. This toxicity has been studied in horses belonging to the Pasteur Institute, all living under the same conditions, receiving the same kind of food, and being treated in the same way.

In spite of this equality of conditions, we found from time to time instances of sera exhibiting a somewhat unequal toxicity. If the sera be examined at various intervals of time after venesection the findings are as follows: At the outset the toxicity of all the sera is almost equal; after this it decreases at a fairly regular rate. The serum is very toxic on the first day—that is, the day of the venesection (lethal dose =0·032 c.c.), but it rapidly loses its toxicity during the next ten days. As the eleventh day approaches, the toxicity is almost diminished by half (lethal dose=0·063 c.c.). It continues very slowly to decrease during the next thirty or forty days, and when injected in the dose which previously has been

fatal (0·063 c.c.), the serum gives rise only to anaphylactic symptoms—grave, it is true, but not necessarily fatal. After the two months' interval the toxicity of the serum is indefinitely maintained at the same level (lethal dose=0·125 c.c.). All the sera which we examined, and which were more than two months old, exhibited the same degree of toxicity (lethal dose=0·125 c.c.). It never entirely disappeared. We have had occasion to examine a bottle of antidiphtheritic serum about twenty years old; as was indeed to be expected, it was only necessary to inject a sensitised guinea-pig intracerebrally with 0·25 c.c. to produce the anaphylactic syndrome in the animal, followed by death in less than five minutes.

Without our being authorised to make the statement that a serum which is very toxic when injected into a sensitised guinea-pig is equally so in the case of man, it is clear that it is absolutely in our interest to forbid the use of toxic sera in treating the human subject.

In only using serum which is at least two months old we eliminate at any rate one factor in the causation of the toxicity. As to the other factors, if we are unable to modify them by means of procedures which will be indicated farther on, we can at least verify their presence, form an estimate of their harmfulness, and take measures accordingly.

According to the rules drawn up by the Frankfort Institute,[1] every therapeutic serum should satisfy four conditions: (1) it should be limpid; (2) it should not contain any bacteria; (3) it should not contain more than 0·5 per cent. carbolic acid; (4) it should be devoid of free toxin, tetanus toxin especially. In our opinion it is well to add a fifth desideratum which

[1] Otto, "Die staatliche Prüfung der Heilsera," Arb. a. d. k. Inst. f. Exper. therap. zu Frank. a. M. Jena, 1906, Heft ii., pp. 1-86.

may be thus formulated: any serum prepared for human use, if found capable of giving rise to grave anaphylactic symptoms in a sensitised guinea-pig in doses of from 0·05 to 0·6 c.c., should be discarded.

Quantitative estimation of the toxicity by the intracranial route is very simple. It can be done instantaneously, and entails no expense. The guinea-pigs that have been used for the dosage of diphtheria antitoxin are quite suitable for this purpose.

We may note, in passing, that high temperatures distinctly diminish the toxicity of sera; we shall deal with this question in detail in the following chapter.

To sum up: quantitative experiments have shown that there exists a whole range of sera that are more or less toxic. The variations in toxicity are connected with the age of the sera and with factors that are yet unknown. The sera are hypertoxic on the day of venesection, but they lose their toxicity by degrees; this lowering of toxicity, which is rapid to commence with, slackens down from the tenth day onwards. After two months, the toxicity due to the age of the sera becomes negligible. Every serum which sets up grave anaphylactic symptoms in the dose of 0·05 to 0·063 c.c., and *a fortiori* below that dose, should be considered toxic for man.

Toxicity of Milk.[1]—If we have insisted at so great a length on the toxicity of serum it is, amongst other reasons, on account of its practical interest. But it is not only sera that are toxic: milk kills the sensitised guinea-pig quite as often. As our experiments have shown us, a quarter of a cubic centimetre of milk injected subdurally destroys the sensitised guinea-pig in a few minutes and sometimes 0·1 c.c. is enough to cause death.

The milk used for injection into the brain should not be unboiled: very often we have seen guinea-

[1] *Annales de l'Institut Pasteur*, xxiii., January, 1909.

pigs die the day after the operation independently of their anaphylactic state as a result of local infection. In all anaphylaxis experiments we therefore recommend the employment of milk heated to 100° C. This temperature does not perceptibly diminish the toxicity of the milk.

It should be recollected that the reverse is the case with sera; they lose all their toxic action at a temperature of 100° C. even when they are not coagulated.

Milk when heated to 100° and even to 120° C. for a quarter of an hour kills a hypersensitive guinea-pig in a dose of 0·25 c.c., and even 0·1 c.c. The persistence of this toxicity is clearly due to the fact that the milk remains perfectly fluid even at high temperatures. Above 120° C. the milk, while apparently remaining quite fluid, loses its toxicity so much that when heated to 130° C. for fifteen minutes, although slightly toxic (*i.e.*, producing cough, hurried respiration, tendency to prostration), it no longer kills when injected in a dose of 0·25 c.c. When the milk is heated to 135° to 140° C. it becomes gelatinous, and in that state of semi-coagulation it is not toxic at all.

It is just as well to add that in the normal guinea-pig—that is to say, in one not previously sensitised—the intracerebral injection of milk never gives rise to the slightest trouble.

Experiments have shown us that the toxicity of milk is specific without, however, its being as strictly so as in the case of serum. Thus we verified the fact that guinea-pigs sensitised with cow's milk did not react to the intracerebral injection (0·25 c.c.) of human milk; on the other hand, they reacted readily to goat's milk, and succumbed to the effect of the injection in a few minutes. The specificity of lactic anaphylaxis is therefore not absolute; it is of less degree than serum anaphylaxis.

We may add that cow's milk does not exhibit any toxicity in the case of guinea-pigs that have been sensitised with cow's serum.

Toxicity of Egg-Albumen.[1]—In order to demonstrate this, two routes were open to us—the intravenous and the intracerebral. In both instances it was sufficient to make the test injection with a minimal dose of egg-albumen in order immediately to set up the anaphylactic syndrome similar in every respect to serum or lactic anaphylaxis.

Grave anaphylactic symptoms are rarely observed when the injection is made intraperitoneally, and we have never seen them produced by subcutaneous injection.

These reactions following the second injection differ according to the method of inoculation of the egg-albumen, and evidently depend upon the consistency of this substance, and consequently upon its more or less rapid absorption. Thus, the absorption is very rapid when the injection is made into the general circulation. It will be understood that it is slightly less rapid when the egg-albumen is injected subdurally. The absorption takes a distinctly longer time in the peritoneal cavity, and even longer still in the case of subcutaneous injection: hence the same egg-albumen decreases proportionately in toxicity according as the injection is made intravenously, subdurally, intraperitoneally, or subcutaneously.

In order to give an idea of the toxicity of egg-albumen in the sensitised guinea-pig when the injection is made intravenously, we may say that a dose of 0·002 c.c., and sometimes of 0·001 c.c., is at once fatal, whilst in the normal guinea-pig the injection of 1 or 2 c.c. of pure egg-albumen (that is, a dose 500 or 1,000 times greater) does not give rise to the slightest trouble.

[1] *Annales de l'Institut Pasteur*, xxv., p. 392, 1911.

The toxicity of egg-albumen disappears after it has been heated even without coagulation. Its behaviour in this respect is the same as that of blood-sera; whilst the dose of 0·002 c.c. of egg-albumen is sufficient to determine fatal anaphylactic shock, a dose of 0·1 c.c. or even of 0·3 c.c. of this same solution, heated to 100° C., is tolerated by the sensitised animal without any ill-effects.

Does an animal sensitised with the white of the hen's egg react anaphylactically if the test injection is made with the white of egg of another species of bird—say, for example, the pigeon or the turtle-dove? In other words, is the toxic function of the egg-albumen specific?

The result of our experiments goes to shew that the toxicity is specific for a given species, but that this specificity is not absolute. Thus, a guinea-pig sensitised with the white of the hen's egg tolerates as much as 0·1 c.c. of the white of the pigeon's or turtle-dove's egg, but if the dose of this latter be raised ever so little (0·5 c.c.), the most characteristic anaphylactic symptoms are immediately produced. The egg-albumen of alien species therefore only becomes toxic as the doses are raised; egg anaphylaxis then possesses a relative specificity.

In speaking of the action of heat on the toxicity of egg-albumen we have said that the latter is thermolabile—that is to say, that the *heated* solution appears to be deprived of toxicity. We should remark, however, that this loss of toxicity is only quite relative. It is in evidence when it is administered to guinea-pigs that have been sensitised with *raw* egg-albumen, but if the test be carried out in animals that have been sensitised with the *heated* egg-albumen, one is quite astonished to discover—and we have repeated this experiment a number of times—that intravenous injection made with *heated* egg-albumen ranks amongst

the most deadly. The atoxicity of heated egg-albumen is therefore only apparent. In other words, heated egg-albumen and raw egg-albumen behave when compared with one another just as if they were albumens of different species of animals.

Whatever be the substance employed, whether one is dealing with serum, milk, or egg-albumen, the symptoms which the animals exhibit are almost the same in each case. The symptoms follow with more or less rapidity, according to the dose injected and to the route chosen for the injection (*i.e.*, in proportion to the rapidity of the absorption of the albumen), but on general lines their characteristics are always the same.

From the point of view of symptomatology, however, certain peculiarities should be noted which vary with the particular species of animal. Thus the anaphylactic symptoms set up by the same substance are not always the same in guinea-pigs and in dogs; the ox and the horse react a little differently; whilst serum disease in man differs in certain points from that observed in animals.

The classical picture of anaphylaxis is that which is shown us by the guinea-pig, and which is commonly designated to-day in accordance with our proposition under the name of *anaphylactic shock*. Scarcely is the test injection terminated than the animal begins to struggle; it scratches its muzzle as if wanting to remove a foreign body. Its struggles become more and more marked; it commences to turn round and round and then to turn somersaults which become more and more frequent and violent. Then these convulsive attacks begin to take place at greater intervals, and the animal having become weakened from the output of so much energy, lies on its side. The vesical and anal sphincters become relaxed; there is involuntary discharge of urine and fæces. The

respiratory movements, dyspnœic at first, become slower and slower, and finally become paralysed, and at the end of an interval which varies from three to seven minutes, the animal dies from asphyxia. The internal mechanism of this asphyxia has not yet been satisfactorily explained. At autopsies hæmorrhagic congestion has been found in the stomach, in the intestine, in the lungs, and in the heart. No lesion has been discovered, even microscopically, in the region of the nerve centres.

In the rabbit the clinical symptoms are almost the same as in the guinea-pig; with this difference, however, that the phase of excitation is often of much shorter duration and the paralytic phase much longer. Anaphylactic shock is particularly well marked when reinjection is carried out intravenously.

We have already described above (p. 7) the local anaphylaxis which is observed so constantly in the rabbit and so rarely in the guinea-pig, following repeated *subcutaneous* injections of serum or of milk (Arthus).

As our co-worker Grineff[1] has noted, the symptoms of local anaphylaxis are the same when the rabbit is injected subcutaneously with a solution of egg-albumen instead of serum or milk.

Anaphylaxis was first studied in the dog (Charles Richet). When slight, the symptoms noted are pruritus, acceleration of respiration and of the heart's action, a lowering of arterial pressure, and diarrhœa. When severe, the first symptom to appear—and the one which is constant, and overshadows all others—is vomiting. This is only absent in cases of anaphylaxis of peculiarly sudden and violent onset. Paralytic symptoms are then present. The dog staggers as though intoxicated; and its hind quarters become paralysed. The eyes assume a wild expression and

[1] *Comptes rend. Soc. de Biol.*, lxxii., p. 974, 1912.

the animal falls down exhausted. Urine and blood-stained liquid fæces are passed involuntarily. Respiration becomes more and more difficult and death takes place after some hours; or if the symptoms take a favourable course, recovery may follow in about half an hour.

According to Charles Richet,[1] the cause of death in the dog is asphyxia set up by pulmonary congestion; to this asphyxia is superadded marked catarrh of the intestines. The pulmonary circulation becomes more and more congested, the blood-pressure is lowered, and the central nervous system consequently ischæmic. Death ensues from failure of circulation. In the ox and in the horse, serum anaphylaxis is manifested (Alexandrescu, A. Ciuca[2]), in slight cases by œdema of the muzzle, of the nasal mucous membrane, and of the vulva; by attacks of colic; by mammary cyanosis and a diminution in the secretion of milk.

In serious cases dyspnœa, pulmonary œdema, vertigo, excessive salivary and buccal secretion occur, whilst there is also loss of consciousness for a period of some three-quarters of an hour. In fulminating cases death may supervene in from five to six minutes.

In the horse sometimes extreme nervous excitement and urticaria are present, at other times œdema of the head and neck.

In man the condition known as serum sickness (v. Pirquet and Schick) is nowadays well recognised, so that a detailed description of it is unnecessary. Generally speaking, eight days after the injection of the serum an irritating eruption makes its appearance together with pains in the joints and slight rise of temperature. In certain cases the sequelæ of serum injection assume a more serious character.

[1] "L'Anaphylaxie," p. 44.

[2] *Comptes rend. Soc. de Biol.*, lxviii., p. 685, 1910.

The lymphatic glands become enlarged, there is some œdema, and the temperature rises to 140° F. The general condition of the patient is such that there can be no doubt as to the production of a general toxæmia.

Symptoms of serum sickness are produced sometimes—though not always—with quite a peculiar intensity in persons who have already been injected with serum either recently or at some distant date. In these patients the symptoms may appear very rapidly after the injection. Even a minute or two after inoculation one may witness true anaphylactic shock such as is seen in the guinea-pig or rabbit, with asphyxia and a condition resembling status lymphaticus (to say nothing of other symptoms already described) which are displayed with an intensity that is peculiarly impressive. Cases of sudden death following reinjection have been recorded. It must be added, however, that some of these cases are not to be trusted, the part played by the serum not having been properly established.

Whatever the case may be, granting that serum shock does not necessarily endanger human life, it is none the less a mishap of sufficient gravity for the practitioner to take the most serious account of it.

Every time we make the statement that serum, milk, and egg-albumen are toxic or lethal in such and such a dose it must be understood that we by no means intend to imply that they contain a substance which is actually poisonous. There was a time when this was believed to be the case, as we shall see; but this time has now gone by, and at the present day we know that this toxicity is due to the union of two substances—sensibiligen and sensibilisin—neither of which taken separately is toxic at all.

Why is this union toxic? Is it because these two substances, atoxic separately, by their combination

liberate a third substance which is toxic? Is it because the sudden encounter between the antigen and antibody upsets the equilibrium of the nerve cells and gives rise to symptoms that simulate a true intoxication? The question has not yet been definitely settled, but we shall discuss it at length in the last chapter. At present the mechanism of this toxic action is not an essential point, and it is relatively of secondary importance compared with the need for counteracting this toxicity in animals and, above all, in man.

CHAPTER V

VACCINATING OR ANTI-ANAPHYLACTIC INJECTION

Experiments on the destruction of the toxic substance of sera by chemical and physical agents—Attenuation of the toxicity of sera by heating to 100°, 95°, 89°, 76° C.—Effect of repeated heating at 56° C.—Effect of narcotics (ether, alcohol) on the production of anaphylactic shock in guinea-pigs—Experiments on vaccination against the so-called toxin contained in sera—Rapid vaccination by a single injection against anaphylactic shock — Vaccination by graduated small doses—Vaccination against local anaphylaxis — Vaccination against anaphylactic shock in the course of immunisation of horses with bacteria (gonococcus, meningococcus, diphtheria bacillus, streptococcus, etc.)—The method of Auer and Ascoli for dealing with anaphylactic accidents—Application of the method of graduated small doses in man: choice of method—Anti-anaphylaxis by the digestive tract—Summary of various anti-anaphylactic processes; their respective value—Mechanism of anti-anaphylactic vaccination.

In view of the marked resemblance which exists between anaphylaxis in the guinea-pig and the symptoms which immediately follow serum inoculation in man, we set ourselves to discover, from the beginning of our researches on anti-anaphylaxis (1907), by what means the guinea-pig could be preserved from anaphylactic shock. We hoped thus to reach the solution of the problem which we had most at heart—viz., the checking of serum sickness in man.

The problem before us might *a priori* be attacked on two sides: on one side we could endeavour to attenuate the toxicity of sera for injection; on the other we could endeavour to produce in the animal an immunity against sera.

Starting from the idea that the serum contained a true poison, we set ourselves to attack this poison by the most varied chemical substances—permanganate of potash, alcohol, hydrogen peroxide, chloroform, ferments, alkaloids, salts—but all these attempts to destroy the so-called anaphylactic poison came to nothing.

We were not more successful with atropine, strychnine, morphia, caffeine, calcium chloride, magnesium sulphate, ox-bile, formaldehyde; whilst the same failure confronted us in alternately freezing and thawing the serum. Maintaining the serum at 60° C. for six consecutive hours, after the method of Rosenau and Anderson, had likewise no effect on the toxicity, and a temperature of no less than 100° C. for fifteen minutes was necessary to destroy the toxicity of the serum.

Starting from the conception prevailing at this time on the subject of serum poison, we also, in our turn, strove to attenuate the poison. Let us say at once that all our attempts to alter the toxicity of the serum by means of chemical products, one after another, completely failed; neither Gram's solution, nor precipitation with distilled water, nor extraction with ether, nor prolonged contact (for two days) with animal charcoal, altered the toxicity of the serum for the sensitised guinea-pig.

We decided to try physical agents, and to have recourse afterwards to various histological processes. Our previous experiences shewed that the toxicity of sera varied in different cases, and that some sera may be extremely toxic, whilst others may be much less so. Thus, the French sera used in our examination proved to be so feebly toxic that to produce anaphylactic shock it was necessary to inject not less than 0·1-0·125 c.c. either into the brain or intravenously.

In seeking the cause of this feeble toxicity of our

sera, we asked ourselves if the serum poison was not modified by temperature. We were led to this view still more definitely from the fact that in previous experiments we observed that, when tested a short time after the venesection, the sera were quite as toxic as those of other countries. It appeared from that time very probable that the feeble toxicity of our commercial sera was connected with the raising of the temperature to 55°-56° C. which they underwent before being put on the market. To settle the question clearly, it only remained to make an experimental control; that is to say, it was necessary to choose a serum of fixed toxicity, to submit it to different temperatures, and to follow the modifications of its toxicity in the course of the experiment. The complete disappearance of the toxicity at boiling-point has been already pointed out by Rosenau and Anderson in their first memoir. These scientists have not, however, stated precisely if their serum, when it had become atoxic, was coagulated or not, a detail which is not without importance. In our experiments we have always worked with uncoagulated sera, whatever the temperature to which they were raised. To prevent coagulation we added to 1 part of serum 3 parts of distilled water. The serum thus diluted was kept for twenty minutes at 100° C. When injected into the brain of sensitised guinea-pigs in the maximum dose of 0·25 c.c., the serum thus heated appeared almost harmless. The animals experienced a little discomfort, it is true, immediately after the injection, but they did not shew the slightest symptoms of anaphylaxis.

Another series of sensitised guinea-pigs were injected intracerebrally with the same serum, diluted with 3 volumes of distilled water, but not heated; at the end of two to three minutes all these guinea-pigs succumbed with the most marked anaphylactic

symptoms. Heating the serum to 100° C. without coagulation is sufficient, therefore, to render it completely harmless, even with the maximum dose injected by the intradural route. Indeed, heating the serum, though beneficial, from the point of view of toxicity, is unfortunate from the point of view of therapeutics. But, it might be asked whether in heating sera to a lower temperature it would not be possible perceptibly to reduce the toxic power, and yet preserve the useful—that is to say, the preventive and curative—properties.

This is, indeed, what experience teaches. Three parts of a serum of known toxicity were, after adequate dilution (1 : 4), heated respectively to 76°, 89°, and 95° C. for twenty minutes. From each of the three portions of the serum 0·25 c.c. was taken for injection subdurally into sensitised guinea-pigs. In order to demonstrate the toxicity of this serum before heating, two sensitised guinea-pigs were injected with 0·25 and 0·1 c.c. The first of these guinea-pigs (0·25 c.c.) died in a few moments with characteristic symptoms; the second (0·1 c.c.) was very resistant, and recovered half an hour after. In the case of the guinea-pigs which were injected with heated sera the results were as follows. One of two guinea-pigs which was inoculated with serum heated to 76° C. exhibited rather severe anaphylactic symptoms; the other was scarcely ill at all. Four guinea-pigs which were injected with sera heated to 89° and 95° C. shewed slight, hardly noticeable symptoms. By means of these experiments we have satisfied ourselves that the substance which is the cause of the toxicity of sera is partially destroyed at a temperature below 100° C.

However, to be applicable to therapeutic sera, the heating ought to be effective at temperatures still lower than 76° C. In order to obtain the maximum

diminution of toxicity with the minimum loss of curative power, it is necessary not to exceed 59°-60° C., a temperature at which antibodies generally remain intact.

The following is a short résumé of experiments made on the above lines. We chose a very toxic serum in order to be able to follow the progressive diminution of the toxicity, in proportion to the duration of the heating.

This unheated serum was of such virulence that 0·025 c.c. injected by the subdural route either killed the guinea-pig in a few minutes, or set up very serious anaphylactic symptoms, from which the guinea-pig only recovered by degrees.

After heating to 60° C. for an hour on three successive days, the toxicity of the serum was as follows:

0·25 c.c. Certain death.

0·1 c.c. Symptoms very serious, but not followed by death.

0·05 c.c. No symptoms.

After heating to 60° for an hour on five successive days:

0·25 c.c. Certain death.

0·125 c.c. Symptoms serious, but transitory.

0·0625 c.c. Hardly any symptoms.

After heating to 60° C. for an hour on seven successive days, the results were the same as above.

It will be seen, therefore, that even moderate but prolonged heating is capable of reducing the toxicity of the serum to one fourth or fifth of its virulence.

In the same way the toxicity undergoes an appreciable modification with a temperature of 56° C. It follows, indeed, from our experiments that serum which has been heated on three successive days to 56° C. for an hour, and on the fourth day for two hours, is three times less toxic than the same serum when not heated.

At the Pasteur Institute therapeutic sera are heated to 56° C. four days in succession, for an hour each time. In the first place, raising the temperature in this way prevents the possible risk of contamination, but without doubt this practice also renders the sera considerably less toxic. This explains why in France serum accidents have always been relatively rare, and why in the small number of cases (13 per cent.) in which they occur they are not of such a serious nature as they are in countries where sera are not heated.

We ought, however, to recognise the fact that the heating of serum is only a last resource; it is a palliative—valuable, it is true, but very inadequate in certain cases.

The ideal would be to find a means of not only rendering possible the mitigation of serum accidents, but of avoiding them and preventing them. With that aim in view it would be necessary to be able to act, not on the sera by heating them, but on the animal itself, by rendering it impervious or insensitive to the test injection.

As our experiments have shown, this immunity of the animal to reinjection can easily be realised, and may be of either a transitory or durable nature. Let us examine two cases.

If it be true that serum sickness is a reaction of the nerve centres, as we stated the hypothesis at the outset, we believe, in company with M. Roux, that we ought to be able to suppress the anaphylactic shock by lowering the nervous sensibility of the animal.

Experience has shown us, indeed, that when the sensitised guinea-pig is anæsthetised with ether, and that when, during the narcosis, 0·25 c.c. of serum—a maximum dose, undoubtedly lethal—is injected into the guinea-pig intracerebrally, no reaction is observed: the animal awakens sound and unhurt.

The same thing happens when the guinea-pig is rendered insensible with alcohol. Take, for instance, a sensitised guinea-pig and make it imbibe alcohol, or, better still, administer alcohol by rectal lavage. Then let it sleep off the effects of the alcohol for an hour or two, until it returns by degrees to its nearly normal condition. At this moment inject into it one lethal dose of serum intracerebrally. The animal will not react, any more than will a fresh guinea-pig; that is to say, it will not manifest the slightest anaphylactic symptom. This experiment shews, therefore, that in lowering the sensibility of the animal by alcohol, the sensitised guinea-pig is unaffected by the lethal injection of serum. This immunity lasts, at least, for twenty-four hours following the absorption of the alcohol.

Besides ether and alcohol, other anæsthetics have been tried. Ethyl chloride gave us the best results; but it is not equal to ether, and still less to alcohol, because the manipulation of it is difficult. Ethyl chloride anæsthetises the guinea-pig with extreme rapidity, but it is also eliminated from the organism very rapidly. This is both an advantage and at the same time a disadvantage. We only succeed in maintaining the narcosis by an almost uninterrupted administration of the drug; the guinea-pig must be watched very nearly all the time, and it is necessary to make it breathe the ethyl chloride diluted with air. With urethane and chloralose the animal will survive several hours (as many as sixteen hours); we have not, however, succeeded in obtaining complete recovery with these substances. Banhoff obtains good results with chloral hydrate.

On the contrary, morphine hydrochloride and extract of opium have given us definitely negative results. These substances leave the hypersensibility of guinea-pigs completely intact, and the animals

succumb to the test injection, as do sensitised and non-anæsthetised controls.

In order to lower the sensibility of guinea-pigs, we have tried, besides anæsthetics, many toxic products. Thus, we have injected into sensitised guinea-pigs, the day before the test, a dose of atoxyl slightly less than the lethal dose. The experiment has shown that the guinea-pigs whose sensibility has been thus deadened afterwards remain resistant to a definitely lethal dose of serum. This has likewise been the case with sensitised guinea-pigs weakened by prolonged starvation, as Lesne and Dreyfus[1] and our collaborator Konstansoff have observed.[2]

By producing, with the aid of narcotics or chemical reagents, a transient refractory condition which protects the animal from anaphylactic shock, we can thus obtain, for an extended period and by a totally different means, a true immunity. Here we are required to handle a problem which is as important from the practical point of view and as pregnant with possibilities as is anti-anaphylaxis.

It was in this way, indeed, that the phenomenon was interpreted by observers who had first applied themselves to serum anaphylaxis in the guinea-pig: by Rosenau, Anderson, and Otto, on the one hand, and our collaborator Steinhardt on the other. This was so much the view of Rosenau and Anderson that these authors began by attacking the so-called serum toxin by means of the most varied chemical reagents. When they saw that they did not succeed, they then proceeded to vaccinate against this toxin, and in order to do this they went to work exactly as if they had to vaccinate the guinea-pig against a genuine toxin. They submitted their animals to a series of injections, each injection being separated from the

[1] *Comptes rend. Soc. de Biol.*, lxxi., p. 153, 1911.
[2] *Ibid.*, lxxii., p. 263, 1912.

next by a regular interval of six days, and each consisting of a massive dose of serum (5 c.c.). After having given three or four inoculations in this way, they again waited six days, and then proceeded to the test injection. As this test did not lead to the death of the animal, they believed that they had effected active vaccination against the toxin of the serum.

In the same manner we ourselves made our first attempts at obtaining passive immunity. In order passively to immunise against anaphylactic symptoms—that is to say, against the supposed toxin of horse serum—we began by inoculating guinea-pigs with a series of massive injections of horse serum; and when we considered the animals to be well immunised, we bled them, and mixed their sera with the supposed toxic horse serum in the hope of neutralising its effect.

Our hope was not realised, for a very good reason, as we ultimately understood. The horse serum remained quite as toxic after this operation as before. It might, strictly speaking, be thought that the poison contained in the serum was one of those which did not yield antibodies easily. But, in reflecting on this, we had our doubts. After all, we said to ourselves, perhaps a toxin does not exist in horse serum, as our predecessors Rosenau, Anderson, and Otto have thought. Even admitting that it does exist, why should we apply to anaphylaxis ideas borrowed from immunity, especially those relating to active or passive vaccinations ?

Our doubts assumed a more tangible form when, from experience, we saw that to confer immunity against anaphylaxis one injection only of serum was needed, and not a whole series of separate injections, as in the method of Rosenau and Anderson, or of Otto. Finally, when we discovered with surprise

that the animal was for practical purposes vaccinated against anaphylaxis as early as the day after the inoculation, we became convinced that we—together with Rosenau, Anderson, and Otto—had gone astray.

From that time it was clear that the hypothesis of toxin in serum must be rejected, and that the procedure of immunisation such as Rosenau and Anderson had employed, and such as we ourselves employed at the beginning of our researches, could not be upheld. We were forced to make a clean sweep of all we knew about vaccination, and to pursue an altogether different line. One fact had been acquired, however, namely, that in vaccinating guinea-pigs against anaphylaxis in the way one vaccinates against a toxin—that is to say, repeating the injections at given intervals—Rosenau, Anderson, and Otto had made use of a technique which in no wise responded to the end which they had in view. We would even go further, and say that this technique of vaccination was in direct opposition to the end in view, because, in multiplying the injections of serum, not only was the appearance of the anaphylactic state temporarily deferred, but there was no immunisation. We therefore found ourselves in the presence of an extremely curious and quite inexplicable phenomenon; a guinea-pig rendered anaphylactic with horse serum, after having received a non-lethal dose of this serum subcutaneously, was thereafter in a position to tolerate, some hours after, one or even two lethal doses of serum. From the point of view of prevailing conceptions on immunity, it is a fact which has not its parallel in biology. Reduced to its simplest terms, it may be summed up thus: a toxin—granted, pending further information, that there is one in the serum—injected in a dose which is not lethal protects the animal against the lethal dose of this same toxin when the latter is injected one to two hours after.

In other words, the addition of two doses of toxin, of which one is definitely lethal, made at one to two hours' interval, destroys all the harmful action of this toxin. There was here a kind of phenomenon of interference which till then was only recognised by physicians. Setting aside the hidden mechanism of this phenomenon, which appeared very obscure to us at that time, we immediately set to work to utilise the facts with a view to defence against anaphylactic shock. This was the starting-point for our procedure of vaccination by small graduated doses. But before explaining this process it will be useful rapidly to recapitulate the experiments which, historically, have preceded it.

In the course of researches on the relations between the toxicity and temperature of sera, we have proved that heated sera are so much the less toxic in proportion as they are brought to a higher temperature; and, furthermore, that an intimate relation exists between the toxicity of the sera and the duration of the heating. In studying concurrently the action of the temperature on the vaccinating power of sera from the point of view of anaphylaxis, we observed that the sera could be raised to a considerable temperature without perceptibly deteriorating the vaccinating power. Without entering into the detail of these experiments, we may say that the serum, even when heated to 80° C., is yet shown to be endowed with the peculiar quality of protecting against anaphylactic shock. It has the advantage over unheated serum that it possesses a minimum toxicity; even in increased doses this heated serum does not give rise to the slightest symptoms in sensitised guinea-pigs. It is sufficient, then, to inject prophylactically, for example, 3 c.c. of horse serum heated to 80° C. into a hypersensitive guinea-pig, subcutaneously or intraperitoneally, to render it absolutely immune to the

consecutive injection of a really lethal dose of serum made intracerebrally.

This process of vaccination by heated serum has been applied in practice. It has been utilised, amongst others, by Stanculeanu and Nita[1] in the following manner: These authors, having instilled horse serum into the conjunctivæ of patients with eye affections, had observed in certain individuals amongst them symptoms of local anaphylaxis—redness of the conjunctiva and of the eyelid, weeping of the eyes, œdema of the bulbar conjunctiva and of the two eyelids, conjunctival ecchymoses, œdema of the face with enlargement of pre-auricular and submaxillary glands. In two patients who presented particularly serious symptoms, these authors had recourse to what they called "the vaccine of Besredka," which is nothing else than serum diluted with distilled water (1 : 4) and heated to 83° C. The authors noted that in the patients thus vaccinated the injection of normal serum, made twenty-four hours afterwards, set up no further symptoms. Again, nothing happened when, three hours later, they repeated the injection of serum. They inferred from this that it was possible to vaccinate the conjunctiva against local serum anaphylaxis.

In the course of these researches on vaccination with sera rendered atoxic by heat, we arrived gradually at the conclusion that a vaccinating or antiphylactic effect could be obtained by the use of doses of unheated serum, so weak that they could be said to pass unnoticed by the animal vaccinated.

We have observed, indeed, that the guinea-pig, in the fully developed condition of anaphylaxis, tolerates without the least discomfort a lethal dose of serum given intracerebrally (0·125 c.c.) if it be first injected, for example, with 0·02 c.c. or even 0·01 c.c. of serum

[1] *Comptes rend. Soc. de Biol.*, lxvi., p. 1112, 1909.

intraperitoneally—that is to say, a quantity which is 200 to 500 times below the dangerous dose.

It is important to note that this vaccination by weak doses is extremely rapid; it can be effected in a few hours or even in a few minutes, according to the case. Let us take an example: Suppose a guinea-pig to have been rendered anaphylactic with horse serum. We inject it subcutaneously with 0·05 c.c. of this same serum, which is an amount at least fifty times less than a toxic dose. Of course, the guinea-pig tolerates the injection without symptoms. The animal immediately begins to be immunised, so effectively that, three-quarters of an hour later, it can be injected with a really lethal dose, or even two lethal doses, in the nerve centres, or in the general circulation without its manifesting the slightest symptom. The small dose of serum injected subcutaneously (0·05 c.c.) performs, therefore, the function of anti-anaphylactic vaccine.

After the introduction of this small injection of serum anaphylactic immunity is produced with more or less rapidity. The rate of production depends on whether the serum is introduced subcutaneously, intraperitoneally, intrathecally, intravenously, or intracerebrally. Thus, in the guinea-pig it is acquired, on an average, four hours after the subcutaneous injection, one or two hours after the intraperitoneal or intraspinal injection, and is, so to speak, instantaneous after the intravenous or intracerebral injection. The following illustration, drawn from veterinary practice, is an example of this method:

In 1909, Alexandrescu and A. Ciuca[1] had to administer anthrax antiserum to 180 head of cattle (150 milch cows and 30 bull calves). These animals had exhibited, at the time of the previous inoculations, particularly serious anaphylactic symptoms. The

[1] *Comptes rend. Soc. de Biol.*, lxviii., p. 687, 1910.

authors, fearing the recurrence of these symptoms, decided first to vaccinate their animals by our method. In order to test the efficacy of the latter, they divided the animals into two groups. The first group, composed of ninety animals, was given subcutaneously a preventive inoculation of 1 c.c. antiserum. The second group, which served as controls, was given no preliminary inoculation. Five hours after, all the animals of the two groups were given an injection of 5 c.c. of antiserum and 0·5 c.c. of bacillary emulsion.

The results of this experiment are quoted verbatim from the author's account: "No symptom of anaphylaxis, however slight, was observed during the twenty-four hours following sero-vaccination in the ninety animals which had undergone the preliminary anti-anaphylactic injection. On the contrary, ten animals (out of ninety) in the group used as controls exhibited anaphylactic symptoms, which consisted in œdema of the muzzle with hypersalivation, or in œdema of the mucous membranes of the vulva and anus, accompanied by colic."

The surprising rapidity with which the anti-anaphylactic state is established is a point of the greatest utility. It has enabled us to carry out, in a short time, a whole series of vaccinations, to which we attach the name of "subintrant," which confer on the animal an immunity that is proof against every test.

The small dose of serum which, in the experiment quoted above, did duty as vaccine, only safeguards, as we have remarked, against one or two lethal doses of serum.

But there are some cases where it is necessary to provide protection against several lethal doses. In a good number of serious infections we find ourselves compelled to administer massive doses of serum to subjects sensitised by former injections, and in order

to achieve our end we have recourse to intravenous injection. In such cases the use of graduated (*i.e.*, "subintrant") vaccination is certainly indicated. In place of one injection of serum only, two, three, or even four are made. At each fresh injection, which follows the preceding by a few minutes (three to five), the dose of serum is increased, and, as each new injection further strengthens resistance, we succeed in creating very rapidly a state of anti-anaphylaxis of remarkable stability. The following are some examples:

A sensitised guinea-pig is given, for the purposes of vaccine, 0·025 c.c. of serum intravenously, the lethal dose being 0·05 c.c. After this first injection, which gives rise to no trouble, the animal is able to tolerate, five minutes later, 0·1 c.c. serum, double the lethal dose. This second injection does duty in its turn as vaccine, and enables the animal to tolerate, two minutes later, 0·25 c.c., or five times the lethal dose. If we wait two minutes more we shall see that the animal can tolerate an intravenous injection of 1 c.c.—that is to say, twenty lethal doses—and that without the least trouble. All these injections can be made one after the other, without even withdrawing the cannula from the vein. Therefore, in less than ten minutes we succeed by this process in vaccinating against twenty times the amount of the lethal dose. We have been able to satisfy ourselves subsequently that we can vaccinate in this manner against as many lethal doses as we desire.

Another example may also be cited of guinea-pigs passively sensitised—that is, animals injected with serum from a guinea-pig or a rabbit already sensitised. The degrees of hypersensitiveness of the guinea-pigs in the experiments were such that they succumbed to the intravenous injection of 0·0125 to 0·025 c.c. We gave one of the guinea-pigs thus passively sensitised graduated inoculations at regular intervals

—at 12.10 a.m. 0·05 c.c. serum intraperitoneally, at 1.30 p.m. 5 c.c. by the same route, at 3.30 p.m. 0·1 c.c. injected into the jugular vein, at 3.35 p.m. 0·5 c.c. again by the jugular vein, at 3·45 p.m. a final injection of 5 c.c. into the vein. By this method an animal for which 0·025 c.c. would have been in excess of the lethal dose is able to tolerate, after four graduated injections in less than four hours, 5 c.c. of serum inoculated intravenously—that is, more than 200 times the lethal dose given in one injection—without any symptoms of anaphylaxis.

We give also other examples taken at random from our notebook of experiments. On October 10, 1910, a series of guinea-pigs was actively sensitised with egg-albumen. On October 28 one of the animals was inoculated with 0·01 c.c. by the jugular route. It was seized at once with anaphylactic symptoms and died in two minutes. Another animal of the same series was similarly injected with 0·002 c.c. egg-albumen. One minute after the injection it was likewise seized with anaphylactic symptoms and died within four minutes. The lethal dose was therefore 0·002 c.c. This fact having been ascertained with certainty, we gave a third guinea-pig of this series graduated injections of egg-albumen, beginning with 0·0005 c.c., by the jugular route. There was no reaction. Two minutes later we injected it with 0·002 c.c.—that is to say, an absolutely lethal dose. It did not react. Ten minutes afterwards it was given 0·02 c.c. without reaction. Again, ten minutes later, we injected its jugular vein on the opposite side with 0·2 c.c. without reaction. After another interval of ten minutes, we injected it with 2 c.c. of undiluted egg-albumen. The animal was obviously embarrassed by the injection, but it recovered immediately.

A sensitised guinea-pig, therefore, which has been given four graduated inoculations in the space of

forty minutes is thereby enabled to tolerate without anaphylactic symptoms an injection one thousand times greater than the lethal dose.

On November 12, 1910, a series of guinea-pigs was passively sensitised with the serum of a rabbit (1·5 c.c.) which had been given several injections of egg-albumen subcutaneously. On November 13 the lethal dose was established as being 0·002 c.c. of egg-albumen intravenously and 0·005 intracerebrally. In two guinea-pigs of this series we injected in successive doses intravenously, 0·001 c.c., 0·01 c.c., 0·25 c.c., 1 c.c., and finally 2 c.c. These injections were made at five minutes' intervals. Two other guinea-pigs, sensitised in the same manner (*i.e.*, passively), were given first 0·001 c.c., then 0·01 c.c. by the jugular vein. Afterwards 0·5 c.c. was injected intraperitoneally. One hour later 0·25 c.c. was injected into the jugular vein, followed ten minutes after by 1 c.c. Then, three minutes after this, 2 c.c. of egg-albumen was injected into the jugular vein on the opposite side. These guinea-pigs did not shew the slightest symptoms after the injection of 2 c.c. of egg-albumen (diluted with an equal quantity of normal saline solution) whilst the controls succumbed in two or three minutes to an injection of 0·002 c.c.—that is to say, a dose 1,000 times weaker.

The guinea-pigs which have been vaccinated in the way we have just indicated straightway resist all tests, however severe they may be. We have injected them with 100 or 1,000 times the lethal dose of serum or egg-albumen intraperitoneally, intracerebrally, intrathecally, or intravenously, but they evince an absolute indifference.

In the examples quoted the guinea-pigs were vaccinated intravenously or intraperitoneally, but they can be vaccinated just as well, following the same principle, by any of the other routes. Thus,

we had animals vaccinated several times subcutaneously which afterwards withstood the most severe inoculations made intraperitoneally, intracerebrally, intrathecally, or intravenously.

If desirable, we can make use of several routes together for purposes of vaccination. We can begin, for example, with a subcutaneous injection, then an intravenous injection, and finally an intraspinal injection. An animal thus vaccinated, no matter to what point of economy we go in use of the serum, afterwards resists multiple lethal doses.

Are the phenomena of local anaphylaxis liable to occur if the same process be used? The first researches in this direction were carried out by our collaborator Grineff.[1] This author sensitised rabbits with heated egg-albumen, these animals being, as we know, the most suitable for local anaphylaxis. Starting from the fourth subcutaneous injection, Grineff observed characteristic cutaneous lesions. These lesions were most marked at the time of the final injections. In order to prevent these lesions, the author injected the auricular vein of two rabbits, the day before the fourth subcutaneous injection, with 2 c.c. of solution of egg-albumen. The day following this vaccination he made the fourth injection of 10 c.c. egg-albumen, inoculating at the same time two control rabbits with the same quantity of egg-albumen. The two control rabbits exhibited a few days after marked infiltration of the skin, while the two other vaccinated rabbits exhibited nothing abnormal. The same phenomenon was observed after the fifth or the sixth subcutaneous injection. The two controls after each injection had a large amount of œdema proceeding to necrosis, whilst the two other rabbits, which had been given the day before an anti-anaphylactic intravenous injection, remained unhurt.

[1] *Comptes rend. Soc. de Biol.*, lxxii., p. 974, 1912.

From these experiments Grineff concluded that "local anaphylaxis, just the same as general anaphylaxis, can be checked by the process of Besredka's small doses."

Some analogous experiments have been made by two of our collaborators, Manoukhine and Potiralovsky. They sensitised rabbits by repeated injections of horse serum subcutaneously. In order to preserve the rabbits against local anaphylaxis, these authors preceded the subcutaneous injections by quite a small injection intravenously. In this way they were able to prove satisfactorily, as Grineff had done in his experiments with egg-albumen, the distinctly beneficial influence of these preliminary anaphylactic vaccinations.

The method of vaccination with small doses has been found to have other uses besides those connected with serum-therapy. We may quote, purely because of its historical interest, the method of immunisation with blood-corpuscles. Further, there is the method of immunisation of horses with bacterial cultures, which is now constantly used because of its practical economic value.

It was, indeed, whilst experimenting with red blood-corpuscles that we came to understand all the advantages that can be reaped from this method of small doses. We noted that when, fifteen days after a first injection of foreign blood (sheep, goose, or fowl) into a rabbit, we reinjected the same blood intravenously, the rabbit often manifested an uneasiness which went on increasing minute by minute. In fact, the injection was barely completed before the animal became violently convulsed, paralysis supervened, and death ensued in a few minutes.

Whilst studying this phenomenon, we noted the curious fact that it was sufficient to inject intravenously the day before, or an hour before, or even

a few minutes before, a weak dose of the same blood (0·2 to 0·5 c.c.) to make sure of preserving the rabbit from death.

This experiment, we may say in passing, permits of very powerful hæmolytic sera being obtained (1 : 6,000) with 3 to 4 injections of blood intravenously, without the animal running the least danger. What is most important is that by this means we can obtain, without any loss of animals, antibacterial and anti-endotoxic sera[1] by injecting bacterial cultures intravenously. Those who are in the habit of immunising horses do not ignore the serious risks which attend the intravenous method of injection. It is by no means rare to see horses fall to the ground a few minutes after the injection, and to experience afterwards much difficulty in recovering from the shock. Very often they do not recover. Cases of sudden death of horses are no longer taken into account. Under certain conditions precisely similar symptoms are observed in rabbits.

We tried at first to prevent anaphylactic mishaps in rabbits[2] by applying in their case the method of small doses. We very soon demonstrated the fact that rabbits injected with meningococci intravenously were with certainty protected from fatal consequences by the use of this method.

On the strength of this result, the constancy of which we have had occasion to verify a number of times, we commissioned our collaborator L. Cruveilhier[3] to apply this process to some female goats in immunising against the gonococcus and the diphtheria bacillus.[3] Experience shewed that female goats, when immunised intravenously, after a preliminary injection, have never exhibited grave symptoms. The

[1] *Annales de l'Institut Pasteur*, xxvi., p. 83, 1912.
[2] *Comptes rend. Soc. de Biol.*, lxvii., p. 266, 1909.
[3] *Ibid.*, lxix., p. 38, 1910.

intravenous injections were carried out twice, the first injection being nearly ten times weaker than the second. The interval between the two injections was, at the beginning of our experiments, fixed at twenty-four hours; later, we reduced it at first to three hours, then to one hour, and finally to ten minutes. An interesting fact is that the reaction that follows the second injection of bacteria, however massive, is always relatively weak. Thus, six hours after the second injection, made with a very large quantity of bacteria, the temperature reaction was not perceptibly greater than six hours after the first injection, made with a dose of bacteria ten times less. The temperature the next day was almost always normal, even when the quantities of culture injected intravenously were extremely large.

The method of small doses has consequently been applied by Briot and Dopter[1] in the immunisation of horses against meningococci. "The contrast," say these authors, "is surprising between the results of these injections practised twice and those in which the emulsion has been injected entirely at the first trial. Not only have these horses been able to tolerate with impunity the dose injected the previous week, but, moreover, progressively increasing doses.

M. Ciuca has utilised the method of small doses in horses intended for the preparation of antistreptococcic and antidysenteric sera. At our advice, he introduced, ten minutes before the injection of the total dose of virus, a tenth or a twentieth of the dose. Before the use of this method three horses out of five succumbed to anaphylactic symptoms in the space of seven to ten minutes. Since M. Ciuca[2] employed the process in question, he has never had any deaths. According to his statement, not only are the horses

[1] *Comptes rend. Soc. de Biol.*, lxix., p. 174, 1910.
[2] *Zeitschr. f. Immunitätsf.*, t. xix., p. 174.

saved, thanks to this process, but immunisation is effected in a more regular manner. Thus, on former occasions the temperature rose slowly and remained high for three or four days. After the use of anaphylactic vaccinations the temperature rises immediately, but falls almost invariably within eighteen hours. The general condition is good; the animal preserves its appetite and vivacity, which was not the case before the application of this process. Ciuca noted that anaphylactic symptoms were particularly frequent in dysenteric horses in the course of immunisation with whole cultures. Out of seven horses thus immunised, six died with classical symptoms. Each injection was followed by a rise of temperature (39° C.), anorexia, and occasionally diarrhœa. Since the employment of anti-anaphylactic injections, none of these troubles have been observed. The temperature rises to 39°-40° C., but it does not remain at that level for more than twelve hours. The general condition remains good during the whole time. To sum up, the method of small doses constitutes, according to Ciuca, a most efficacious means of preventing lethal symptoms in the course of immunisation; it much reduces the febrile period, and almost completely abolishes the bad general condition which ordinarily persists some days after the injection of large doses of bacteria. Such are the facts relating to anti-anaphylactic vaccination in animals.

How can anti-anaphylactic accidents be avoided in man?

We will not here lay stress upon the numerous attempts made by different workers to destroy the so-called "toxic substance" of sera. We have already spoken of it elsewhere, and the question is now settled.

Let us here recall the method recommended by Auer. It consists in a preventive administration of

atropine. Without entering into the details of these experiments, we may say that Friedberger and Mita, then Mita alone, were unable to demonstrate any appreciable effect,[1] although they employed increased doses of atropine, and confirmed the indications laid down by Auer. Ascoli[2] proposed to vary the sera in order to avoid anaphylactic symptoms. If an individual had been injected once with horse serum, he should the second time be injected with goat serum. If a fresh serum treatment was considered necessary, he should be injected with dromedary serum. In proceeding thus, we should be sure, says Ascoli, of screening him from serum symptoms.

It is to these sera that the Italian scientist gives the name of "anallergetic," thus emphasising their peculiar quality of not giving rise to any allergetic or anaphylactic symptoms. Most certainly the means recommended by Ascoli is reliable, but it does not appear very practicable. First of all, it is very difficult, supposing it to be only for the purpose of a single serum—antidiphtheritic, for example—to have at one's disposal all at one time horses, goats, sheep, or dromedaries possessing an increased and accurately titrated antiserum. Admitting that this difficulty, which is not to be lightly regarded, can be surmounted, it is necessary, to avoid any confusion, that each person should possess a kind of memorandum book or serum *dossier*, in which is jotted down the kind of animal purveyor of serum, and this would be presented to the doctor every time he judged it expedient to make a new injection of serum.

One would agree that it is infinitely simpler to have recourse to the procedure of small or graduated doses, which is now sanctioned by long practice.

We have already observed that in France serum

[1] *Zeitschrift f. Immunitätsf.*, I. Orig., xi., p. 501, 1911.

[2] *Deutsche Med. Woch.*, xxxvi., p. 1215, 1910.

mishaps are rare, and we have indicated the principal reason. But, though this was the case a few years ago, it is not so since the introduction of intraspinal and intravenous injections. Anaphylactic mishaps are likely to multiply, considering the increasing number of individuals that have now been injected with serum at some time in their lives. It is in the course of cerebro-spinal meningitis that the injection of serum exhibits the most serious dangers. We endeavoured to study this question first from the experimental point of view.

In collaboration with Mlle. Lussofsky, we have been able to shew[1] that the classical picture of anaphylaxis in guinea-pigs can be reproduced by means of intraspinal injections. Guinea-pigs sensitised fifteen days previously with horse serum are inoculated with injections of 0·066-0·5 c.c. in the intervertebral space just above the sacrum. This intrathecal injection often causes shock immediately in the guinea-pig, but this should not be confounded with true anaphylactic shock. In most cases the animal recovers quickly, and it is only after an interval of five minutes that true anaphylactic symptoms appear.

As soon as we had established the possibility of producing the anaphylactic syndrome by the intrathecal route, we applied ourselves to the task of looking for the means of preventing it—that is to say, producing a condition of anti-anaphylaxis. We soon became convinced that the method of vaccination by small doses, which had been already contemplated, in these cases likewise, ensured an absolute immunity to the animal.

Without entering into the details of these experiments, it will be sufficient to remark that whatever be the route chosen for vaccination, be it subcutane-

[1] *Comptes rend. Soc. de Biol.*, lxviii., p. 1099, 1910.

ous, intrathecal, or intravenous, we are always certain of being able to protect the animal against spinal anaphylaxis. The only difference that is observed in the selection of the various routes is the rapidity with which anaphylactic immunity is established.

From this point of view, the subcutaneous route is the least favourable of all—that is to say, anti-anaphylaxis takes the longest time to establish. Thus, the guinea-pig, vaccinated subcutaneously, only acquires immunity against intraspinal injection after some five hours. Intraspinal vaccination is manifestly more rapid, and we have recourse to it in man whenever practicable. Experiments on guinea-pigs shew that intraspinal vaccination confers anti-anaphylaxis at the end of an hour, or of two hours at the maximum. Immunity is established, then, in this case, at least twice as quickly as by the subcutaneous route.

The most rapid vaccination route is the intravenous. It is not only the most rapid, it is also the surest, as numerous experiments have shewn us; an animal intravenously vaccinated is already in a state of anti-anaphylactic immunity at the end of ten to fifteen minutes.

What should be the attitude of the physician at the bedside from the point of view of the anti-anaphylactic measures? Let us point out that while it may be a good thing to be informed of the past serum history of the patient, it is not entirely indispensable. First, the patient is not always able to give information as to whether he has been previously injected with serum or not; then, there are some subjects who, without having had a serum injection, yet react to the first injection in a violent manner, for reasons which have hitherto eluded us. We advise, therefore, that the sensibility of the patient should be tested in every case, and that a weak dose of serum

should always be given first, as though the person injected were in an anaphylactic condition. This recommendation is particularly useful in cases where the serum must be introduced by intravenous or intrathecal inoculation.

Having established this fact, which route shall we choose for vaccination? The choice should be dictated solely by the state of the patient and by the necessity for more or less rapid intervention. Take, for example, a patient attacked with cerebro-spinal meningitis; it is in this case, in practice, that there is most to be feared from serum mishaps. Several cases may be brought forward as instances. You are called to a patient who presents the symptoms of meningitis. If, for the sake of accuracy in diagnosis, you prefer to wait for the laboratory tests before intervening, and put off the injection of serum into the spinal cavity till the next day, do not go away without having injected 10 to 20 c.c. of serum subcutaneously. The patient will be none the worse for it. His meningitis, if such it be, will not be relieved, but he will benefit by the subcutaneous injection, from the anti-anaphylactic point of view. If the next day you have decided to perform a lumbar puncture and to inject antimeningococcic serum, he will be vaccinated against anaphylaxis, and will be able to tolerate there and then, without untoward symptoms arising, 30 to 40 c.c. of serum injected into the spinal canal.

Let us take another case. You are in the midst of an epidemic of cerebro-spinal meningitis, and there is no doubt about the diagnosis. You have decided to inject intrathecally 20 to 30 c.c. of serum. If it is not a very urgent case, begin by giving an intraspinal inoculation of 2 c.c.; allow one or two hours to elapse, then reinject by the same route the total dose of serum—that is to say, 20 to 30 c.c. If the

case is very urgent and if you think that each hour which passes robs the patient of the chance of recovery, carry out the anti-anaphylactic vaccination intravenously. Begin by diluting the serum (5 c.c., for example) in six times its volume of physiological saline solution; inject 1 c.c. of this solution intravenously at the bend of the elbow. According as the patient reacts or not, wait three to five minutes; if he does not react, then inject 3 c.c. of this same solution. If he does not exhibit any untoward symptom, you reinject 10 c.c. two minutes afterwards: finally, after another interval of two minutes, you will make the last injection, employing 25 c.c. of solution. From that time your patient may be regarded as vaccinated against anaphylactic mishaps. As we have been able to assure ourselves in a great number of cases, the patient is in a position to receive, ten minutes afterwards, an intravenous or intrathecal injection of 10 to 30 c.c. of pure undiluted serum.

These graduated small injections, which follow at a few minutes' interval, can be performed without its being necessary to withdraw the needle from the vein. These few examples are sufficient to enable the physician to familiarise himself with regard to the mode of anti-anaphylactic vaccination to be adopted in each particular case.

If the case is one of cerebro-spinal meningitis or any other disease, he has only to remember that it requires—

(*a*) Three or four hours to obtain anti-anaphylactic vaccination subcutaneously;

(*b*) One to two hours for intraspinal injection;

(*c*) Ten minutes to a quarter of an hour for intravenous inoculation.

We have now to say a few words on anti-anaphylaxis by way of the digestive tract.

In the course of our researches on anaphylaxis with

milk we observed that guinea-pigs sensitised with milk are easily rendered anaphylactic if the milk is administered to them by the rectum or the mouth. We were able later to prove the same fact in guinea-pigs sensitised with horse serum: after vaccination *per rectum*, the animals resisted a lethal dose of serum given intracerebrally. We have not, on the other hand, succeeded in vaccinating guinea-pigs by introducing serum by the mouth.

However, certain facts observed later in the course of researches on anaphylaxis with egg-albumen, have made us resume the experiments of vaccination by the oral method. The following are the facts: In studying anaphylaxis with egg-albumen, we proved that sensitised guinea-pigs could be submitted to anti-anaphylactic vaccination by the mouth under certain conditions. Thus, when we take a guinea-pig which has been sensitised with egg-albumen, and cause it to ingest 5 c.c. of egg-albumen, it remains, at the end of twenty-four hours, as sensitive as before the ingestion of the meal. When submitted to intravenous or intracerebral injection, it is immediately attacked with anaphylactic symptoms and dies in a few minutes. But if, instead of proceeding to the test inoculation the day after the ingestion, we wait two days, or, better still, three days after the vaccinating meal, it is proved that the anaphylactic state has disappeared and has given place to an anti-anaphylactic condition.

In presence of these facts relating to egg-albumen, we asked ourselves whether in the old experiments of vaccination by the mouth against serum anaphylaxis, we should not have been equally fortunate if we had waited longer before proceeding with the test injection. And, indeed, from experiments started along these lines, we have shewn that what is true of egg-albumen holds good in a great number of

cases for serum. When we wait forty-eight hours, reckoning from the injection of serum, we find an anti-anaphylactic state which did not exist the evening before.

We can therefore assert, on the basis of our experiments on guinea-pigs, that, whatever may be the sensitising substance, whether it be milk, egg-albumen, or even serum, the anaphylactic state can be abolished by the administration of this substance, either by the rectum or by the mouth.

After these experiments we tried to effect anti-anaphylaxis by the mouth with other substances as well. Our collaborator Grineff has succeeded in obtaining it with heated egg-albumen;[1] Ch. Richet[2] has obtained it with crepitin.

We have seen above that egg-albumen, when heated, acts from the point of view of anaphylaxis in quite a different way from raw egg-albumen. It became, therefore, interesting to see how heated egg-albumen acted from the point of view of anti-anaphylaxis. Acting on our advice, Grineff proceeded to vaccinate by the oral method with heated egg-albumen, and he has arrived at the same results as those obtained by us with milk, serum, and raw egg-albumen.

Ch. Richet, to whom we are indebted for the information on the subject of alimentary anaphylaxis, likewise stated that the animal could be rendered anti-anaphylactic *per os*. He related the case of a dog which received crepitin by the mouth, and which two days after withstood a toxic dose injected intravenously without symptoms.

To sum up, we can produce an anti-anaphylactic condition—that is to say, we can prevent anaphylactic shock from occurring—by the following different

[1] *Comptes rend. Soc. de Biol.*, lxxii., p. 344, 1912.

[2] *Ibid.*, lxx., p. 252, 1911.

methods of injection—oral, rectal, subcutaneous, intraperitoneal, intracerebral, intrathecal, and intravenous.

The oral method is the least practical of all, because it requires at least one or two days before anti-anaphylactic immunity is established.

The rectal method is more prompt in action, but it is subject to some risks, the reabsorption of the antigen by the mucosa being delayed according to individual idiosyncrasy and the nature of the antigen.

The intraperitoneal and intracerebral methods—above all, the latter—confer immunity in a very short time, varying from a few minutes to an hour at the most. This immunity is the most effective and reliable; but it is to be understood that these methods may be impracticable in the case of man.

There remains vaccination by the subcutaneous, intrathecal, and intravenous routes. From these routes the physician will have to make his choice.

Vaccination by the subcutaneous method, in view of the slow absorption, may be of service in cases in which the injection of therapeutic serum is not urgent.

According to the sensitiveness of the individual, for the purpose of vaccination, 1 to 5 c.c. of serum should be injected subcutaneously; then, four hours later, the whole of the intended dose (20 to 30 c.c.). It must, however, be pointed out that there are individuals who are extremely sensitive to subcutaneous injections; in those the only route of vaccination is, beyond a doubt, the intravenous path, which will be dealt with farther on. Vaccination by the intrathecal route is above all indicated in cerebrospinal meningitis, in the course of which disease anaphylactic mishaps are frequent. In order to protect the patient from these, we should begin by introducing into the spinal cavity 1 or 2 c.c. of serum.

Then we should wait an hour. In a subject sensitised by previous injections, it is not unusual to observe after this small dose (1 to 2 c.c.) some slight symptoms —"abortive anaphylaxis." We should wait for these symptoms to cease completely, and after that we shall be able to inject with impunity into the spinal cavity, then and there, 20 to 40 c.c. of serum.

Vaccination by the intravenous route is the one we should prefer above all the other methods; it is rapid, certain, and also protects against local anaphylaxis as well as general. As soon as the anti-anaphylactic immunity is acquired, which requires ten to fifteen minutes, a strong dose of serum can be injected equally well intrathecally, intravenously, or subcutaneously, without the patient running the least danger of anaphylaxis. The intravenous method has, moreover, this advantage, that it permits one to observe, with the needle still in the vein, the sensitiveness of the patient. We begin by testing this sensitiveness by introducing intravenously as weak a dose as is desired—0·1 c.c. of serum, for example. (The serum is diluted to ten times its volume with physiological saline solution, and 1 c.c. of this dilution is injected.) If the patient does not react at the end of three to five minutes, another injection of 0·3 c.c. of serum is given (3 c.c. of the dilution) without withdrawing the cannula. We again wait two minutes, and if nothing happens we inject 1 c.c. of serum (10 c.c. of dilution). At this moment anti-anaphylactic immunity is acquired; but for greater security, after a further interval of two minutes, we make a last injection of 2·5 c.c. of serum (25 c.c. of dilution). Whatever may have been the degree of the patient's hypersensitiveness before this vaccination, we can be certain that he will now tolerate, without the least trouble, 20 to 40 c.c. of undiluted serum at any stage of the illness.

What is the mechanism of this anti-anaphylactic vaccination?

The following passage was written on this subject in 1907, in one of our first memoirs dealing with anaphylaxis:[1] "Anti-anaphylactic vaccination, which may be effected either intraperitoneally or intracerebrally, is very probably a phenomenon closely allied to that of neutralisation of tetanus toxin *in vitro* with antitetanic serum. Vaccination should amount, therefore, to a desensitisation, and should have the effect of restoring the guinea-pig to its original state; anti-anaphylactic immunity should therefore be nothing else than that natural immunity which every normal guinea-pig possesses in the presence of intracerebral injection of serum."

We have nothing further to add to this at the present date. Indeed, from the time when we discovered that the sensitised guinea-pig became forthwith vaccinated by a single injection (and that in an exceedingly short space of time) we grasped the fact that anti-anaphylactic immunity had nothing in common with other recognised processes of immunity —that is to say, with those against bacteria and their toxins. In fact, ordinary antitoxic or antibacterial immunity is not established till after the lapse of eight days at the least. It becomes more effective as the number of injections is increased, and the immunisation is of longer duration. It is accompanied by the appearance of antibody in the serum. It never protects against the inoculation of virus into the nerve-centres.

On the other hand, anti-anaphylactic immunity is established after a single injection. It is, so to speak, instantaneous. It is not accompanied by the appearance of antibody, but, quite on the contrary, by the disappearance of antibody or of the sensibilisin. It

[1] *Annales de l'Institut Pasteur*, April, 1907.

extends to the nerve-centres, the brain, and the spinal cord.

The only feature in common between these two immunities, so diametrically opposed, consists in their specificity.

It is simply to synthesise in a single word the general effect of characters so curious and so opposed to current ideas on immunity that we have coined the phrase "anti-anaphylaxis." In order not to allow it to be deflected from its proper meaning, it will be necessary to reserve it solely for cases of rapid vaccination, either by a single injection in a weak dose or by a series of graduated small injections following one another at very close intervals.

It may, perhaps, not be without interest to recall the fact that our conception of anti-anaphylaxis as being a desensitisation and a mere return to the normal state has been strenuously and universally opposed by all those who have given their attention to the question. Various theories, such as absorption of complement or some particular change in condition of the animal, have been brought forward, to combat our way of thinking, which, after all, is based on experiment. In order to demonstrate the small foundation of our conception, our opponents have gone so far as to deny the specific character of anti-anaphylaxis, and to aver that it can be effected by various methods, other than that which consists in employing the homologous serum.

It is needless now to insist farther on the fact that researches made in different directions have had the effect of rallying almost all our opponents on our side.

Let us quote in evidence of this the comparatively recent experiments carried out by Richard Weil and Arthur Coca.[1] We may recall that one of the arguments which we adduced in favour of the theory of

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., xvii., p. 141, 1913.

desensitisation was the fact that sensitised guinea-pigs, when once rendered anti-anaphylactic, could be submitted to a fresh sensitisation. Weil and Coca have taken up the same idea, investing it with a quasi-mathematical expression. They likewise experimented with guinea-pigs which had already been sensitised, and were then at a much later date rendered anti-anaphylactic by one of our processes. They put the following question: Are these guinea-pigs to be considered as having reverted to their former state? In order to ascertain this, they sensitised them passively by means of an antiserum.[1]

The experiment shewed that to effect this procedure it was necessary to employ the same quantity of antiserum as one would have to employ in the case of sensitising fresh guinea-pigs. In other words, the animals rendered anti-anaphylactic behave at the time of passive resensitisation exactly like control animals that have never undergone any injection.

Weil and Coca have thus been led to conclude that the term which best expresses the mechanism of anti-anaphylaxis is that of "desensitisation" which we proposed in 1907.

Upon setting out with this conception of anti-anaphylaxis, we learn what should guide the practitioner in his choice of dose or vaccinating doses.

The anaphylactic state being due to the presence of specific antibody or sensibilisin, the duty of the physician is to neutralise it as much as possible by the addition of the maximum of antigen (the latter is represented in the majority of cases by horse serum).

If for the purpose of vaccine a very slight quantity of antigen (horse serum) be added, the end is only partly attained: a little antigen neutralises a little

[1] For the description of the technique of passive sensitisation, see Chapter III.

antibody; and it follows that the anaphylactic state is only slightly diminished.

If we carry out a series of graduated small injections, we end in neutralising the sum total of the sensibilisin in the circulation; thus desensitised, the subject acquires that anti-anaphylactic immunity which is peculiar to every normal individual.

In order to effect this immunity it is therefore in our interest, for the purpose of vaccine, to inject as much antigen as possible, without, however, injecting too much, for anaphylactic shock supervenes very rapidly.

When should we stop? What is the optimum dose of antigen that should be injected? To this question, which has often been put to us, the reply is as follows: To be certain that anti-anaphylactic immunity is acquired it is necessary to proceed to the dose which we term "precritical," the effect of which is shewn in man by an onset of anxiety and a redness of the face lasting some minutes. It is a sure indication, and from this point of time onwards the patient is desensitised, and is in a position to tolerate with impunity as much serum as it is desired to administer.

This precritical dose may be varied in different subjects according to the degree of hypersensitiveness manifested by the particular individual. The principle is that we should act in practice as if we were always dealing with individuals who are most hypersensitive; and commencing with weak doses, as above indicated, proceed rapidly without stopping if we see that the patient does not react to them, and go on injecting stronger and stronger doses till the precritical dose is attained.

CHAPTER VI

ANAPHYLAXIS IN THE PRESENCE OF VARIOUS SUBSTANCES

Anaphylaxis in the presence of tissue extracts—Researches on crystallin; on spermatic fluid. Congestin, Crepitin—Vegetable albumens, etc.—Bacterial anaphylaxis, active and passive—Tuberculin reaction—Therapeutic anaphylaxis—Applications of anaphylactic reactions.

THE facts detailed in the preceding chapters are for the most part derived from the experimental study of sera, milk, and egg-albumen. These facts, supported by very careful and indisputable experiments, constitute the basis of our present knowledge relating to anaphylaxis and anti-anaphylaxis.

But it is not only these substances that set up the state of anaphylaxis; every substance that contains an animal or vegetable albumen possesses this power. Perhaps this latter also belongs to other substances; at the present time we can make no definite pronouncement on this point.

At all events, it is the case that agreement respecting these substances is not yet as fully established as in the case of those so far studied. So, while waiting for the points in dispute to be cleared up, we think it will be useful to summarise in this same chapter an account of the experiments relating to tissue extracts, bacteria and their products, vegetable albumens, etc.

After the study of sera, that of tissue extracts is fully demonstrated.[1]

[1] We omit the discussion of anaphylaxis in the presence of the red blood-corpuscles on account of its complex character.

Guinea-pigs are injected with tissue extracts of the horse, of the sheep, or of man. After the prescribed interval, they are tested intravenously. The test is carried out with the organic extract that has been used at the first injection or with extracts of other organs, or, better still, with serum from the corresponding animal.

The result of these experiments, which were first carried out by Ranzi,[1] shews that guinea-pigs can be sensitised by tissue extracts, but that the anaphylactic state thus produced is not specific. For instance, the animals sensitised with a given organ react, at the time of the intravenous test, not only to the extract of this organ, but also to that of another organ. They also react equally well to the serum of the same animal.

Ranzi observed, moreover, that animals sensitised to sheep's serum are equally hypersensitive to the tissue extracts of the sheep.

In view of these facts our late lamented co-worker Ohkubo[2] debated whether in the experiments indicated it was not simply a question of anaphylaxis in the presence of serum contained in the organs rather than of anaphylaxis in the presence of the organs themselves. This idea appeared to him as all the more probable from the fact that guinea-pigs, when sensitised with organic extracts of the rabbit, reacted in consequence more vigorously to rabbit's serum than to the organic extracts in question.

In order to verify this hypothesis, Ohkubo made

We bear in mind the fact that the procedure of anti-anaphylactic vaccination in small doses, applied to the red corpuscles, permits of the avoidance of accidents which are often such a troublesome obstacle to the preparation of hæmolytic sera when the injection is made intravenously. For details as to this point, see *Comptes rend. Soc. de Biol.*, lxvii., p. 266, 1909.

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., ii., p. 12, 1909.

[2] *Ibid.*, vi., p. 176, 1910.

use of extracts of organs completely deprived of blood. He introduced a cannula into the portal vein of a living rabbit, and carefully washed the organs with physiological saline solution until the fluid discharged from the carotid was no longer coloured. Then he ground up the organs into fine particles—liver, spleen, kidney—macerated them in physiological saline solution, and the next day injected the extracts, thus prepared, subcutaneously into guinea-pigs.

When, three weeks later, Ohkubo put these animals to the test, he discovered that they did not react to the injection of the macerated organs. Therefore, the conclusion drawn from the previous experiments of Ranzi—namely, that of specific anaphylaxis in the presence of tissue extracts—was found to be erroneous.

Minet and Bruyant,[1] Calmette's collaborators, have in their turn endeavoured to eliminate the cause of error due to the presence of blood in the organs. Instead of driving the blood from the organs by means of lavage, they freed them from it by biological means, vaccinating the animals against the serum by the procedure of administering small doses. If in spite of this anti-anaphylactic vaccination, the guinea-pigs, having been sensitised with the extract of organs, reacted upon reinjection of the same extracts, it would be proved that anaphylaxis to organs was an undoubted fact, the anaphylaxis to serum having been eliminated.

The experiments thus conducted induced Minet and Bruyant to conclude, in agreement with the opinion of Ranzi, and contrary to that advanced by Ohkubo, that anaphylaxis to tissue extracts does exist, and that it is independent of that attributable to serum contained in the organs.

Crystallin occupies a place by itself from the point

[1] *Comptes rend. Soc. de Biol.*, lxxi., p. 166, 1911.

of view of anaphylaxis, just as it is peculiar also from the point of view of precipitation. The animal possesses, as is well known, the power of manufacturing a precipitin with the crystallin from the same species.

Is the guinea-pig capable of manufacturing sensibilisin—that is to say, of becoming sensitised with the crystallin of the guinea-pig?

Kruscius[1] answers in the affirmative; Romer and Gebb[2] in the negative. In order to decide this difference of opinion, Kapsenberg,[3] after weighing the evidence, finds that the two opponents are equally right. The result of his experiments shews that the guinea-pig is capable of being sensitised with crystallin of the same species when the dose of the test injection is raised. When crystallin of another species is injected into the animal under experiment, a minimal dose of the substance is sufficient to determine the anaphylactic state.

In the two cases, therefore, there is production of anaphylactic antibody or of sensibilisin; the difference only relates to the quantity of antibody formed.

Another fact has confused the question of crystallin. Supported by a comparative study of the crystallin of the pig, the ox, and the ass, Andrejew, has gone as far as to deny the specificity. According to this author, a guinea-pig sensitised with the crystallin of one species reacts in the presence of the crystallin of another species. The reaction, it is true, is less pronounced than in the case of homologous crystallins, but it exists none the less.

Fresh experiments were necessary to clear up the

[1] Gräfe's *Archiv. f. Ophthalmologie*, lxxxii., p. 180, 1912 (quoted by Morax and Bollak).

[2] *Archiv. f. Augenheilkunde*, p. 6, 1910.

[3] *Zeitschr. f. Immunitätsf.*, xv., p. 518, 1912.

[4] *Arbeiten a. d. kaiserl. Gesundheitsamte*, xxx., p. 450, 1909.

question of crystallin—namely, those of Morax and Bollak.[1] The result of their study goes to shew that (1) an animal sensitised with the crystallin of another species reacts almost always to reinjection of crystallin of this species, and, furthermore, very often reacts quite as well to this as to the homologous crystallin of its own species; (2) that the animal does not react to the injection of serum of the same species.

From the point of view of anaphylaxis crystallin, therefore, seems to be in possession of quite special properties; it possesses the specificity of the organ and not of the species. In other words, the animals injected with the crystallin of another species do not react to serum of the same species; on the contrary, they are hypersensitive to all crystallin irrespective of species.

Animals sensitised with human semen[2] react anaphylactically to semen of the same species. In order to produce shock, the reinjection should be made with a massive dose, and directly into the heart. The reaction fails when the injection is made with semen of another species or with serum of the same species.

In this anaphylaxis, in the presence of semen, there exists, therefore, in the case of sera, simultaneous specificity both of organ and of species.

To sum up, from the point of view of anaphylactic reaction, in the case of organs such as the liver, spleen, and heart, there does not appear to be specificity; in the case of crystallin there is specificity of organ and not of species; in that of semen there is simultaneous specificity of both organ and species.

In the course of this account allusion has already been made to the question of congestin, to which

[1] *Annales de l'Institut Pasteur*, xxviii., p. 625, 1914.

[2] Minet et Leclercq, *Comptes rend. Soc. de Biol.*, lxx., p. 506, 1911.

the first researches of Charles Richet, henceforth to become classical, were devoted.

Important researches by the same author were next carried out on *crepitin*,[1] which is the toxin of *Hura crepitans*. This is a nerve poison with an extremely slow action which acts on the secretion and vasomotor innervation of the stomach and intestines. In the case of the dog a second injection of crepitin sets up symptoms of anaphylaxis comparable in every respect with those observed with actino- or mytilo-congestin.

We have succeeded with crepitin better than with any other substance in realising what Charles Richet calls anaphylaxis *in vitro*. By mixing this poison with serum derived from a sensitised dog, and by next injecting this mixture into a fresh dog, he succeeded in then and there producing anaphylactic shock. It is in this experiment that Charles Richet demonstrates the newly formed poison—apotoxin—which in his opinion is the cause of anaphylaxis. We shall return to this later, when discussing the various theories of anaphylaxis.

Anaphylaxis experiments have been conducted with the fluid of *hydatid cysts*, the fluid of *cœnurus*, extract of *mussels*, of *rice*, of *kidney beans*, of *wheat*, of *maize*, etc.

Karasawa[2] made a study of vegetable proteins in this connexion. He triturated wheat, kidney beans, and rice finely, and prepared from them watery extracts, with which he sensitised guinea-pigs. Ten to thirty days later the animals were tested, either with the extract which had been used for sensitisation or with some other extract.

These experiments shewed that the substances in question were capable of giving rise to the same

[1] *Annales de l'Institut Pasteur*, xxiii., p. 745, 1909.
[2] *Zeitschr. f. Immunitätsf.*, I. Orig., v., p. 509, 1910.

anaphylactic disturbances as the albumen contained in serum, milk, or egg-albumen. They shewed, moreover, that the anaphylactic reaction was strictly specific. Thus, the guinea-pigs injected with extract of rice tolerated the injection of other extracts (wheat, sago, kidney beans) very well. Those that were sensitised with extracts of kidney beans did not react when they were injected with extracts of lentils, walnuts, or peas.

We may note in passing that, according to Césa-Bianchi and Vallardi,[1] animals which have consumed a large quantity of maize afterwards evince a very great degree of sensitiveness in the presence of maize, when injected even in slight doses intravenously or intraperitoneally. This sensitiveness is manifested by excitation phenomena, followed by paralysis, by respiratory troubles, hypothermia, etc., exactly the same as in classical anaphylaxis.

A large number of researches have been devoted to the study of *bacterial anaphylaxis*. In spite of the very animated discussions on the subject, the question even to-day presents more than one point of obscurity. We submit the stages of the process to the reader, so that he may draw his own conclusions from them.

The symptoms that have been observed to follow repeated injections of bacteria unquestionably recall those which characterise the shock produced by the albumens of serum, milk, or egg-white, the anaphylactic nature of which could never have been called in question. But is this resemblance to the classical symptoms sufficient to justify the inclusion of the bacterial proteins within the clinical picture of anaphylaxis?

One of the outstanding features of anaphylaxis is its specificity, which is in each case more or less

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., xv., p. 370, 1912.

rigorously defined. Does bacterial anaphylaxis comply with this postulate?

Kraus and Doerr, who have experimented with typhoid and dysentery bacilli and with the cholera vibrio, maintain that the reaction is rigidly specific. According to them, guinea-pigs sensitised with a typhoid culture only respond to the second injection of typhoid; on the other hand, they remain unaffected by the injection of paratyphoid or cholera toxin.

Delanoë,[1] who has observed guinea-pigs sensitised with typhoid cultures reacting to the injection of paratyphoid A and B bacilli and even of *B. coli*, holds the opposite opinion. The reaction is most certainly less violent, according to this author, than when the homologous extract is injected; but it is none the less real in cases of injection of heterologous bacterial extracts.

In the opinion of Holobuth,[2] the cause of this divergence resides in the technique employed by these authors. With a view to unifying the results, Holobuth proposes his own technique. This consists in sensitising guinea-pigs subcutaneously with weak doses of bacteria ($\frac{1}{100}$ of a loopful of bacilli heated to 70° C.) repeated during the next ten days. The test injection should be carried out intravenously. According to this author's directions, it should be made, three weeks after the last subcutaneous injection, with a massive dose (0·5 c.c. of bouillon culture in 10 to 15 c.c. decinormal soda). If this technique be strictly adhered to, in Holobuth's opinion, bacterial anaphylaxis, with all the known characters, even with a fatal issue, can with certainty be obtained in the majority of cases.

In the opinion of this author bacterial anaphylaxis is specific. In some cases, however, he has seen

[1] *Comptes rend. Soc. de Biol.*, lxvi., pp. 207, 252, 248, 389, 1909.
[2] *Zeitschr. f. Immunitätsf.*, I. Orig., iii., p. 639, 1909.

guinea-pigs sensitised with the typhoid bacillus react to the injection of *B. coli;* but he adds that this is due to the fact that *B. typhosus* and *B. coli* are members of the same class. It must be admitted, however, that a reaction which shews no distinction between the bacillus of Eberth and *B. coli* cannot be specific.

After these researches, Kraus[1] thought it would be of use to reopen the question. As a result of these fresh experiments carried out in collaboration with Amiradzibi, the specificity of bacterial anaphylaxis has emerged more victorious than ever. These workers specially noted that the specificity was not only rigidly fixed for the species of bacterium, but that in the same species—in the case of *B. coli*, for example—it extended to the strain of the bacterium. Guinea-pigs sensitised with a certain strain of *B. coli* only responded anaphylactically to that strain and not to any other strain of *B. coli*. The same was found to be the case with the typhoid bacillus, the cholera vibrio, and the dysentery bacillus of Flexner.

Our co-worker Studzinski,[2] in his turn, has sensitised guinea-pigs with two strains of *B. coli* by carefully following the technique of Kraus. He has most certainly succeeded in rendering the guinea-pigs hypersensitive, but he has not been able to prove either the fixed specificity observed to be present in the experiments of Kraus, or even the constancy of the phenomenon peculiar to all true anaphylaxis.

Another of our collaborators, Nefedoff,[3] has sensitised guinea-pigs with cholera vibrios. It is a curious fact that in his experiments the anaphylactic state appeared to be more marked in proportion as the initial sensitising dose was stronger. Now we know

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., iv., p. 607, 1910.
[2] *Comptes rend. Soc. de Biol.*, lxx., p. 173, 1911.
[3] *Ibid.*, lxxiv., p. 672, 1913.

that it is the reverse of this in the really characteristic anaphylactic process.

Another characteristic, none the less important, which may serve as a criterion of an anaphylactic condition, consists in the facility with which anti-anaphylactic immunity is obtained. But Nefedoff has not succeeded in conferring this immunity on guinea-pigs which he had sensitised with cholera vibrios. We may note, by the way, that in this latter report Nefedoff's experiments do not agree as to results with those of Delanoë.

These few facts are sufficient to shew how indefinite the subject of bacterial anaphylaxis is, and how much it needs to be supported by fresh researches.

Passive anaphylaxis has likewise been the subject of numerous researches. It is to Kraus[1] and his fellow-workers that we are indebted for the first experiments.

A rabbit received increasing doses of typhoid bacilli. Fifteen days after the last injection it was bled, and it was as interesting as it was unexpected to find that its serum appeared to be capable of conferring passive anaphylaxis on a fresh guinea-pig in the presence of typhoid bacilli.

Indeed, it was only necessary to inject a fresh guinea-pig with 3 c.c. of this serum the day previous for this animal the next day to be in a state of bacterial anaphylaxis; one dose of the typhoid bacilli injected intravenously, which only killed the control after the lapse of several hours, immediately set up in the injected guinea-pig symptoms of anaphylaxis which terminated fatally in a few minutes.

The same phenomena can be observed when a mixture is made *in vitro* of bacilli and the serum in question, and the whole is injected subcutaneously into a fresh guinea-pig.

This anaphylaxis which is transmitted to the

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., iv., p. 607, 1910.

guinea-pig by the serum of the prepared rabbit is strictly specific, and on that point opinions are not divided, contrary to what obtains in the case of passive bacterial anaphylaxis.

Passive anaphylaxis has been produced by Briot and Dopter[1] in the case of the meningococcus with antimeningococcic serum; by Briot and Dujardin-Beaumetz[2] in the case of the plague bacillus with antiplague serum; by Nefedoff[3] in the case of the cholera vibrio with the corresponding serum.

Having had occasion to witness the sudden death of horses in the course of immunisation by intravenous injection, we have been compelled to establish, even before Kraus,[4] an analogy between these mishaps and those which characterise anaphylactic shock. We have even gone further, and have asked ourselves whether in the case in which our interference would be justified it would not be possible to avoid these mishaps by the application of the procedure of anti-anaphylactic vaccination. The experiments made, as has already been seen, have justified our conjectures.[5]

The special sensitiveness of tuberculous subjects to the injection of *tuberculin* is a well-known fact. Is this anaphylaxis? At first sight one would be strongly inclined to believe it. But upon reflection we find that several features, and these by no means of the least importance, are here lacking.

Our present knowledge tells us that an animal which is in a state of anaphylaxis contains an anaphylactic antibody or sensibilisin. Now, all attempts to discover the presence of this antibody in the serum of tuberculous subjects were unavailing up to the time when Bail[6] carried out his experiments.

[1] *Comptes rend. Soc. de Biol.*, lxix., p. 10, 1910.
[2] *Ibid.*, p. 14.
[3] *Ibid.*, lxxiv., p. 672, 1913.
[4] *Ibid.*, lxvii., p. 266, 1909.
[5] See Chapter V.
[6] *Zeitschr. f. Immunitätsf.*, I. Orig., iv., p. 470, 1910; *Ibid.*, xii., p. 451, 1912.

This worker states that when an emulsion of tuberculous tissues is injected into a fresh guinea-pig the animal rapidly becomes hypersensitive to the tuberculin; if twenty hours afterwards, or even later, this guinea-pig is injected with tuberculin, grave symptoms are set up which may terminate fatally. Control experiments made with emulsion of normal, non-tuberculous organs remained negative. The same result is obtained when a guinea-pig is injected with an emulsion of normal organs to which tubercle bacilli are added. In neither of these two cases is so much as a trace of hypersensitiveness to tuberculin proved. Consequently it is only tuberculous organs that possess the power of transmitting passive anaphylaxis to tuberculin; these organs are therefore the carriers of the anaphylactic antibody or sensibilisin.

Bail's experiments appeared to be decisive, and the problem would have been considered as settled once for all had not other experimenters been impressed with the impossibility of reproducing these experiments.

Thus, Joseph[1] has endeavoured to produce passive anaphylaxis with the serum of tuberculous sheep. It is known that these animals are particularly sensitive to tuberculin; it is sufficient to inject them with a minimal dose (0·0001 c.c. of tuberculin) to witness a rise of temperature to a marked degree. Now, in spite of this great sensitiveness peculiar to tuberculous sheep, it was found possible to inject their serum at will into nine guinea-pigs without giving rise to the appearance in the latter of even the slightest degree of sensitiveness to the tuberculin.

We may add that Bail's experiments have been confirmed by Onaka, but they have completely failed at the hands of Kraus, Loewenstein, and Volk.[2]

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., iv., p. 575, 1910.
[2] *Deutsche med. Wochenschr.*, xxxvii., p. 389, 1911.

There is therefore reason, in the interim, for not being too dependent upon them, and for considering that, till proof to the contrary is forthcoming, the anaphylactic antibody has not yet been demonstrated in the case of tuberculin.

It may be remarked, moreover, that the symptomatology of the tuberculin reaction is not typical of anaphylaxis. Tuberculous guinea-pigs have been in vain directly injected with tuberculin subcutaneously; that rapid succession of excitation and paralysis which is a characteristic of anaphylactic shock has never been witnessed in them.

Moreover, the tuberculin reaction only originates in tuberculous animals. It has not been found possible so far—and we ourselves have made numerous attempts without success—to sensitise guinea-pigs either with fluid tuberculin or with that contained in the bodies of killed tubercle bacilli. It is only infection by living bacilli that renders the animal hypersensitive to tuberculin.

If it were really of the nature of anaphylaxis, the tuberculin reaction should be capable of arrest by the procedure of small doses.

Setting out with this idea, Bruyant[1] injected tuberculous guinea-pigs with a weak dose of tuberculin intraperitoneally, then three hours later he submitted them to a test injection with a strong dose. The experiment shewed that guinea-pigs thus quasi-vaccinated exhibited a febrile reaction as strong as guinea-pigs not submitted to anti-anaphylactic vaccination.

In another set of experiments carried out by the same author tuberculous guinea-pigs were injected intraperitoneally with 0·01 gr. of Koch's tuberculin as an anti-anaphylactic vaccine. Three hours afterwards they were injected with a dose ten times

[1] *Comptes rend. Soc. de Biol.*, lxx., p. 782, 1911.

greater. As a result of this second injection the mortality was as great among the guinea-pigs that had been vaccinated as among those that had not been so treated.

To sum up: The tuberculin reaction shews no clinical resemblance to anaphylactic shock; its appearance does not follow the injection either of fluid tuberculin or of the bodies of tubercle bacilli; it is not accompanied by the appearance of the anaphylactic antibody; and, lastly, it cannot be influenced by anti-anaphylactic measures.

For all these reasons, in spite of the phenomena which favour its anaphylactic nature, the tuberculin reaction should be considered, pending fresh information on the subject, as dependent on a poison *sui generis* of special activity in tuberculous subjects.

Before bringing this chapter to a conclusion we have yet to mention some researches bearing upon the so-called drug anaphylaxis.

Clinicians have long been aware of cases of intolerance for certain drugs without being able to explain them save by bestowing upon them the specious title "idiosyncrasy." The arrival of the era of anaphylaxis has opened up a fresh horizon to their view.

It is to Bruck[1] that we are indebted for pioneer experiments dealing with this question. He reported the case-history of an individual who was peculiarly susceptible to *iodoform*. An application of iodoform in ether had the effect of producing a swelling and redness of his scrotum and penis, and a rise of temperature (39·7° C.), followed by a hæmorrhagic eruption covering the pubic region and the upper part of the thighs.

This patient's serum was injected into three guinea-pigs. Two other control guinea-pigs were used, one

[1] *Berl. klin. Wochenschr.*, xlvii., p. 1928, 1910.

being given the same dose of serum derived from a normal subject, and the other horse serum. The next day the author injected five guinea-pigs with an equal quantity of iodoform (0·33 gr. per kilogramme for each animal). Whilst the two control guinea-pigs failed to shew any abnormal symptom, the three others injected with the patient's serum exhibited very pronounced symptoms of anaphylaxis.

The serum of the patient in question therefore contained, in Bruck's opinion, anaphylactic antibody to iodoform, and this enabled him to transmit passive anaphylaxis to iodoform to fresh guinea-pigs.

A similar observation on the subject of intolerance to *antipyrin* has been published by the same author. The person who suffered from this intolerance—he was a medical man—was bled, and his serum injected into a guinea-pig. Another guinea-pig was injected under the same conditions with the serum of a normal subject. The next day the two guinea-pigs, and two others as well, were injected with antipyrin. Only the guinea-pig which had been injected with the serum of the medical man exhibited anaphylactic symptoms (which ended fatally); the other three guinea-pigs remained in a perfect state of health.

This experiment, the nature of which is so curious, has but one fault: it is unique.

Our collaborator Cruveilhier[1] has endeavoured to bring this problem within the region of experiment.

He sensitised guinea-pigs by injecting them intraperitoneally with 6 centigrammes of antipyrin. A fortnight or three weeks later he tested them by intracerebral injection (25 centigrammes). Out of twenty-two guinea-pigs thus treated, seventeen died in less than twelve hours; in five of these death was preceded by violent convulsions, restlessness, dyspnœa, and the passage of urine. Out of nineteen

[1] *Comptes rend. Soc. de Biol.*, lxxi., p. 223, 1911.

control guinea-pigs injected subdurally with the same dose of antipyrin, three died in less than eighteen hours, but with symptoms that differed unquestionably from anaphylactic shock; all the others survived.

In addition, Cruveilhier was successful in sensitising guinea-pigs passively; this was effected by injecting them with rabbit's serum which had been prepared by repeated injections of antipyrin.

Lastly, in four cases sensitised guinea-pigs were enabled to resist the test injection by means of anti-anaphylactic vaccination (method of small doses) carried out shortly before the test.

Manoiloff's[1] experiments have been carried out with the serum of six persons who manifested a pronounced intolerance to sodium bromide and of three individuals peculiarly sensitive to quinine sulphate.

Blood was withdrawn from these various individuals at the particular time of their exhibiting symptoms of intolerance. Their serum was injected into guinea-pigs and rabbits in doses of 3 to 5 c.c. The experiments shewed that doses of bromide and of quinine which were harmless in the case of normal animals proved toxic and sometimes rapidly lethal in the case of animals injected with sera derived from the individuals in question. This was especially marked in the case of quinine, which had a fulminating action on animals passively anaphylactised.

It need hardly be stated that the animals injected with the serum of normal individuals suffered from no untoward symptoms upon receiving the same dosage of sodium bromide or of quinine sulphate.

Practical applications of the anaphylactic reaction have not been made on an extensive scale. In certain cases such application may be of real service. From the outset of our researches we pointed out the advantage which may be derived from it. We

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., xi., p. 425, 1911.

expressed our opinion on the subject as follows: "Having in view the great specificity of the anaphylactic reaction on the one hand and the minimal dose required to sensitise the guinea-pig on the other, there is every reason for hoping that this reaction will prove of service in medico-legal practice, in the same way as the precipitin reaction and the reaction which is based on deviation of the complement."[1]

A little later (December, 1908) Uhlenhuth expressed the same opinion, adding that the anaphylactic reaction could be utilised in cases in which the precipitating reaction failed, particularly in cases of heated albumens; he laid special stress on our earlier experiments dealing with the thermostability of sensibiligen—that is to say, on the property of albumens exhibited in sensitising the guinea-pig, even after the albumens had been brought to boiling-point.

The anaphylactic reaction has enabled us to determine the human or animal nature of mummies many thousands of years old. We may remark that the precipitin reaction as well as that of fixation of complement proved ineffectual in these cases.

What is of more direct utility is the fact that the anaphylactic reaction can be employed with success in the examination of the products of secretion and excretion, such as milk, egg-albumen, hæmoglobin, gastric juice, sweat, oils, etc.

In certain cases it supplies information as to specificity where precipitins fail; in that of crystallins, for example.

Karl Schern[2] has employed the anaphylactic reaction to discover adulteration of earth-nut oil-cakes with rice grains, field mustard, etc.

Minet and Leclercq[3] have carried out interesting

[1] *Bull. de l'Institut Pasteur*, March 15, 1908, vi., p. 236.
[2] *Berlin. thierärztl. Wochenschr.*, February 16, 1911, p. 113.
[3] *Comptes rend. Soc. de Biol.*, lxxii., p. 602, 1912.

experiments on sausages manufactured from different kinds of meat—veal, pork, and horse-flesh. They sensitised guinea-pigs with macerations of these different kinds of sausages; then, some time afterwards, they submitted the guinea-pigs to the intravenous test with sera from veal, pork, and horse-flesh. They demonstrated the fact that so long as one is dealing with the meat of a single species sensitisation takes place on normal lines and specificity appears to be complete. But when one is in the presence of a mixture of several kinds of meat, sensitisation ceases to take place regularly.

Thus, Minet and Leclercq have noted that guinea-pigs sensitised with mixed pork and horse-flesh sausages are sometimes insensitive to horse serum, and react strongly to that of the pig; other guinea-pigs of this series failed to react to either of the two sera. It follows, therefore, that the anaphylactic reaction, while it is quite valuable when it is employed to determine the nature of boiled meats, must be used with caution in cases which deal with a mixture of meats.

CHAPTER VII

THEORIES RELATING TO ANAPHYLAXIS

Theory of Charles Richet—Theory of Friedberger—Its extension to passive anaphylaxis by Doerr and Russ—Bacterial anaphylotoxins—A critical review of the interpretation advanced by Friedberger and his school—Theory of Kraus and Biedl—Theory of Auer and of Lewis—Theory of M. Nicolle—Theory of Vaughan—Physical theory of Doerr—The author's theory.

If the material conditions necessary to bring about the appearance of the anaphylactic state are at the present day known in all their details, the mechanism which governs the production of this condition, and, above all, the production of shock, is far from being elucidated. In default of hard-and-fast explanations, we are compelled to fall back upon theories. It is only fair to add, however, that the domain of theory becomes more and more narrowed each day, and that, taken altogether, the edifice of anaphylaxis reposes upon facts with a certainty and accuracy such as are rarely met with in biology.

We already know that after a first sensitising or preparatory injection there is seen to appear in the blood of an animal a specific anaphylactic antibody (sensibilisin or toxogenin).

There is another fact no less firmly established; the animal endowed with this antibody appears to be attacked with the symptoms with which we are now familiar as soon as the injection of antigen is renewed.

Such are the facts. But the point at which the play of imagination needs to intervene is when we

have to connect these facts one with the other and explain why the second injection is so dangerous. This is just the point at which theory commences—that is to say, where the breach in the anaphylactic structure makes its appearance, a breach which each author seeks to fill up for the time being from the resources of his own imagination.

According to Charles Richet the syndrome for which we proposed the name *anaphylactic shock*, a term universally adopted at the present day, is not a shock at all in the proper sense of the word such as we generally understand it, but a true intoxication by a poison, to which he gives the name of *apotoxin*.

Charles Richet has given us a satisfactory explanation as to how he conceived the genesis of apotoxin, but our knowledge as to its properties is still far from being well defined.

We should note that as soon as it was demonstrated that the serum of animals in a state of anaphylaxis contained a specific antibody (sensibilisin) it became clear to everyone that anaphylactic mishaps were due to the combination of this antibody with antigen. What was less clear on the one hand was the nature of this antibody, and on the other the conditions under which this combination was accomplished.

According to Charles Richet, the combination of antibody with antigen has the effect of setting up a toxin, just as amygdalin, when it combines with emulsin, forms prussic acid.

It is in order to emphasise this point of view that Richet designates the anaphylactic antibody by the name of toxogenin—that is to say, a producer of toxin.

"The phenomena of anaphylaxis," he says,[1] "are the phenomena of intoxication. The poison is a special substance the modes of production of which

[1] "Anaphylaxis," p. 236.

we know—that is to say, it is formed by the combination of toxogenin with antigen; we thus have the chemical reaction:

Toxogenin+antigen=apotoxin."

So much for the genesis of this chemical poison apotoxin. As to its properties, Charles Richet remarks that it is almost impossible at present to pursue the study of apotoxins further; that the various apotoxins are probably substances extremely similar, if not identical; that they are rapidly destroyed and cannot accumulate in the blood.

In his identification of apotoxins with the anaphylotoxins of Friedberger—to the subject of which we shall return presently—Richet has enabled us to penetrate still farther into the foundation of his conception of anaphylaxis.

It was at the beginning of 1910 that the idea of anaphylotoxin was first promulgated in microbiology.[1] It is a curious fact that the theory of Friedberger had the good fortune, rather rare in matters of science, to rally at once the forces of the great majority of bacteriologists.

According to Friedberger, the anaphylactic antibody is none other than the antibody precipitin. At the time of the second injection this antibody combines with the antigen and gives rise to a precipitate. It is not, however, the precipitate itself which directly gives rise to anaphylactic symptoms, but a new substance which is formed at the expense of the precipitate and the complement of the circulating blood. This is the substance to which Friedberger has given the name of anaphylotoxin.

What was particularly fascinating in this theory was the fact that one was not led to waste time over abstractions. All the postulates were liable to immediate

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., iv., p. 636, 1910.

control, and, in fact, all the conjectures contained in the theory appeared at once to find confirmation by experiment.

As soon as he had formulated his theory, Friedberger set himself the task of synthesising his anaphylotoxin *in vitro*. As we have just indicated, this is a reaction product by three substances:

Antibody =precipitin.
Antigen =precipitogen.
Complement =fresh serum.

Friedberger then mixed precipitogen (sheep's serum) with precipitin (rabbit's antisheep serum). After the precipitate thus obtained had been well washed he added guinea-pig's complement, and left this in contact with the precipitate for twelve hours. The next day he centrifuged the mixture. Upon testing the supernatant fluid, he found that it was markedly toxic, and that when injected intravenously into fresh guinea-pigs it set up anaphylactic symptoms in a few minutes, with rapid fall of temperature, delay in coagulation of the blood, leucopenia, etc.

If in this experiment serum heated to 55° C., and not the fresh serum of the guinea-pig, is brought into contact with the precipitate, anaphylotoxin, in Friedberger's opinion, will not be formed. Consequently, he considered that complement is necessary for the production of anaphylotoxin.

This experiment with heated serum led up to another conclusion, none the less important. It was known, indeed, from the time of the experiments of Doerr and Russ that the precipitate alone was capable of setting up grave symptoms when injected intravenously. It might therefore be asked whether, in the course of preparation of the anaphylotoxin, there did not survive, in spite of the centrifugalisation, a small quantity of precipitate in the super-

natant fluid portion, which conferred its toxicity on this fluid.

Now, the fact that the substitution of heated serum for fresh serum is sufficient to arrest the production of anaphylotoxin excluded this hypothesis. This fact proved that the precipitate, if it still remained, did not count for anything in the production of the phenomenon. It is certainly, therefore, the anaphylotoxin which possesses a definite toxic power.

In order to complete the immunological picture of anaphylotoxin, we may add that it resists heating to 58° C. for half an hour; and that its toxic power only disappears at 65° C. It is capable of being precipitated by alcohol. It can be dried without losing its properties; and becomes very toxic again when redissolved in a small volume of water.

Two of its peculiarities have appeared perplexing to us from the outset of these researches, and our especial attention has been drawn to them. The first is that when injected directly into the brain the anaphylotoxin does not produce any toxic effect; the second, that anaphylotoxin is produced even when complement is brought into contact with precipitate that has been heated to boiling-point.

We shall return to the mention of these facts when discussing the rôle of anaphylotoxin in anaphylaxis.

The theory of anaphylotoxins has found, as we have already said, enthusiastic acceptance, especially at the hands of Doerr and Russ. Not content with espousing the theories of Friedberger so far as active anaphylaxis is concerned, these workers have extended their application to passive anaphylaxis. We have already shewn in Chapter III. that in order to produce passive anaphylaxis to horse serum in a guinea-pig, for example, one has only to inject into it serum from a rabbit that in its turn has received several injections of horse serum.

We know, on the other hand, that rabbit's serum thus prepared contains a precipitin in the presence of horse serum.

Now, Doerr and Russ have stated, without the slightest reservation, that experiment with the anaphylactic antibody (sensibilisin) and the precipitating antibody only forms one out of many experiments that shew the existence of an entire parallelism between these two substances.[1] In another contribution[2] these workers have demonstrated that the anaphylactic antigen (or sensibiligen) content of any serum goes hand in hand with its precipitogen content. They have seen, moreover, that the anaphylactic antibody appears and disappears in the serum at exactly the same time as the precipitating antibody.

In other words, in the opinion of Doerr and Russ, these substances are incapable of dissociation in serum.

There are authors, however, who have thrown doubt on the identity of these substances, and have shewn that certain sera are capable of producing a condition of anaphylaxis without exhibiting precipitation.

Doerr and Moldovan[3] made a vigorous reply to this objection, remarking that the assumed lack of parallelism only shewed defective technique on the part of those who found these differences. Satisfactory technique consisted, in the opinion of these authors, in adding variable quantities of antiserum to a fixed quantity of antigen. There were cases, they said, in which the precipitate passed unnoticed because it was redissolved in the excess of antigen. But if only this technique were complied with to the

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., iii., p. 706, 1909.
[2] *Centralbl. f. Bakt.*, I. Orig., lix., p. 73, 1911.
[3] *Zeitschr. f. Immunitätsf.*, I. Orig., v., p. 161, 1910.

smallest extent, one would never fail to witness an absolute parallelism between the power of precipitation and that of anaphylaxis.

Up to this point the only question under discussion was that of serum precipitins and the anaphylotoxins which depended on them.

It was not long before Friedberger extended his theory to bacteria. In collaboration with Goldschmid,[1] he prepared bacterial anaphylotoxins, invariably starting from this principle, that the three following substances should participate in their constitution:

Antigen	=bacteria.
Antibody	=specific serum.
Complement	=fresh serum.

The bacteria on which these first experiments were made were *V. Metchnikovi*, *B. typhosus*, *B. prodigiosus*, and *B. tuberculosis*. In accordance with the technique already described for serum anaphylotoxins, the bacteria were mixed with the corresponding specific serum. The precipitate obtained was washed in physiological saline solution and allowed to remain in contact with fresh guinea-pig's serum for a period of twelve hours. Finally, the next day it was centrifuged.

The fluid portion separated from the precipitate constitutes the bacterial anaphylotoxin. Indeed, the latter, when injected into guinea-pigs intravenously, sets up characteristic symptoms, which most frequently terminate in death at the end of from three to five minutes.

The bacteria employed first of all exhibited a markedly infective nature. Friedberger and Reiter[2] eventually extended their researches to toxin-producing bacteria, such as the dysentery bacillus.

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., ix., p. 398, 1911.

[2] *Ibid.*, xi., p. 493, 1911.

Seitz[1] obtained anaphylotoxins with the pneumococcus, the diphtheria bacillus, the streptococcus, and the staphylococcus. Marcora[2] obtained it with *Trypanosoma nagana*. Lastly, Friedberger[3] has brought complement into contact with tetanus toxin, and has obtained a tetanus anaphylotoxin lethal to the guinea-pig, when injected intravenously, in from three to eleven minutes.

According to Friedberger and his numerous skilled assistants, all these anaphylotoxins play a primary part not merely in anaphylaxis, but even in the course of bacterial infections in general. In the opinion of these authors it is the anaphylotoxins and not the endotoxins, as has hitherto been believed, that dominate the whole symptomatology of infectious diseases, including tetanus.

Neufeld and Dold,[4] experimenting on similar lines with the typhoid bacillus, the cholera vibrio, and the pneumococcus, have not been slow in arriving at this point of view.

In his monograph devoted to the study of bacterial anaphylotoxins,[5] Dold has summarised all these researches as follows: "The researches that have been carried out on anaphylotoxins have contributed greatly to the comprehension of infective processes." A little farther on he says: "We agree with Friedberger in considering that it is to these toxic products that are due in large measure the general phenomena observed in the course of various infections." Lastly, at the end of his description of the anaphylotoxins this author comes to the conclusion that, "thanks to these recent researches, we are in a position to

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., xi., p. 588, 1911.

[2] *Ibid.*, xii., p. 595, 1912.

[3] *Berl. klin. Wochenschr.*, xlviii., p. 1880, 1911.

[4] *Ibid.*, xlviii., p. 55, 1911.

[5] "Das Bakterien-Anaphylotoxin und seine Bedeutung für die Infektion," Jena, p. 80, 1912.

explain at the present day a large number of phenomena connected with infection and immunity that have hitherto been enshrouded in obscurity."

If we have considered it worth while discussing this question in some little detail, it is because eminent bacteriologists have based, and are still basing, their greatest hopes on the study of anaphylotoxins.

Are we really in the presence of a discovery capable of enlightening us upon the phenomena of anaphylaxis, and, in addition, upon those of infection and immunity ?

Such is not our opinion. In describing the properties of serous anaphylotoxin we mentioned the curious fact that, though highly dangerous as an intravenous injection, anaphylotoxin produces no disturbance when injected beneath the dura mater. Now, the cerebral route, as we have seen in the course of this treatise, is to be preferred as the most efficient, and if anaphylotoxin were really what Friedberger and his school think it to be—that is to say, the anaphylactic poison—it is certain that the intracerebral injection would soon have decided the matter for us.

The same reservations obtrude themselves upon our notice when we look into the manner of preparing anaphylotoxins. In Friedberger's first conception, so attractive at first sight, an anaphylotoxin is produced by (1) the antigen encountering the antibody, and (2) the normal serum of the body coming into play and acting through its complement on the combination thus formed.

Now, experience shews that one can deviate considerably from this scheme and still obtain active anaphylotoxins.

Thus, the activity of the anaphylotoxin is the same, if not more efficient, when the complement acts on the precipitate, which has been heated to boiling-point,

as we have previously shewn. Now, the toxin formed in this case is evidently very different from the one which might possibly be formed in the organism.

But what should especially make one reflect is the fact that an active anaphylotoxin is obtained when, of the three substances, one, the antibody, is completely suppressed. Thus, simply by making the complement act on the antigen, as in the case of the tubercle bacillus or a bacillus of such slight virulence as the *B. prodigiosus*, a highly toxic anaphylotoxin is obtained.

That is not all. Keysser and M. Wassermann[1] have shewn that the anaphylotoxin can also be prepared by suppressing the antigen and making the complement act on a substance as inert as barium sulphate or kaolin.

Finally, Doerr and Russ,[2] as well as Seitz,[3] have gone still further; they have discovered that complement is not indispensable to the constitution of an anaphylotoxin.

Thus, Doerr and Russ mix horse serum with the serum of a rabbit immunised against horse serum. After twenty-four hours the precipitate is separated by centrifugalisation from the fluid part. Now, whether one injects into the guinea-pig's veins the precipitate or the fluid, the animal manifests the symptoms which are produced by the anaphylotoxin prepared with complement.

Seitz has arrived at the same result in a more convincing manner; he prepares dysentery anaphylotoxin by treating the dysentery bacillus with guinea-pig serum, which he heats beforehand for an hour at 65° C. Now, in spite of the destruction of the

[1] *Folia Serologica*, vii., pp. 243, 593, 1911; *Zeitschr. f. Hygiene* lxviii., p. 535, 1911.

[2] *Centralbl. f. Bakt.*, I. Orig., lxiii., p. 243, 1912.

[3] *Zeitschr. f. Immunitätsf.*, I. Orig., xiv., p. 91, 1912.

complement, the fluid obtained after an hour's contact with the bacteria shews itself as deadly in intravenous injection as an anaphylotoxin prepared by the ordinary procedure.

In brief, neither the antibody, nor the antigen, nor the complement, is indispensable to the production of an anaphylotoxin.

Have we, then, the right to maintain that the anaphylactic poison, if such a thing exists, is represented by Friedberger's anaphylotoxin? We have still less authority for so doing, since, in our desire to investigate the typhoid anaphylotoxin, we have established some most curious facts. Thus, by planting fresh serum on the surface of a sterile peptone-agar slope culture, we have obtained, the next day, a toxic fluid with characteristics absolutely identical with those of the typhoid anaphylotoxin.

In collaboration with Stroebel and Jupille, we have seen that a preliminary injection of peptone into the veins of a guinea-pig protects the animal from the toxic effect of the typhoid anaphylotoxin.[1] The peptone protects in the same way from the serous anaphylotoxin. Now when it is borne in mind how rigidly specific anti-anaphylactic vaccination is, it is impossible not to conclude that all anaphylotoxins can have little in common with anaphylaxis.

More recently Bordet[2] has obtained a toxic substance by mixing guinea-pig complement and a weak emulsion of agar (0·5 gr. agar to 100 c.c. of physiological saline solution). By studying this substance closely, our collaborator Tchernoroutzky[3] found that it was identical with that which we had previously obtained with peptone agar slope tubes, and which we referred to under the name of peptotoxin.

[1] *Comptes rend. Soc. de Biol.*, lxxi., pp. 413, 599, 691, 1911.
[2] *Ibid.*, lxxiv., p. 225, 1913.
[3] *Ibid.*, lxxiv., p. 1213, 1913.

Further, Friedberger's theory as it was first enunciated—that is to say, founded on the reaction of precipitin as its starting-point—is faulty at its base.

Kraus has observed that the parallelism noted between the power of precipitation and of sensitisation often fails, contrary to what is maintained by Friedberger, Doerr, and Russ, and the others of their school. Thus the guinea-pig, which is easily sensitised, is a mediocre producer of precipitin; the rabbit, which contains, after suitable preparation, a powerful anaphylactic antibody, does not necessarily possess precipitin; finally, the goat, which readily produces precipitins, has a serum which completely lacks the power of conferring passive anaphylaxis.

We may add that Doerr, who was at first an ardent believer in the theory of anaphylotoxins, was afterwards constrained to abandon it in favour of the physical theory which we shall put forth later.

Kraus and Biedl,[1] when studying anaphylaxis in the dog, were especially impressed with the fact that the second injection of serum, or the trial injection, is always followed by a lowering of arterial pressure.

According to immunologists, this arterial depression is the keystone to anaphylaxis, and explains by itself all the symptoms: excitement followed by depression, vomiting, defæcation, anuria, etc.

Another fact which has struck the Vienna workers with regard to the anaphylactic dog is the diminution in coagulability of the blood almost to the point of non-coagulability.

Now, when peptone is injected into the veins of a dog, a lowering of arterial pressure and non-coagulability of blood are both observed. From thence, to establish a connexion between the two phenomena was a temptation that Kraus and Biedl could not

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., vii., p. 408, 1910.

resist. However, before announcing it, they wished to find out how a dog sensitised with serum, then "vaccinated" with peptone, would behave at the trial injection made with the same serum.

The experiment thus performed shewed that the peptone did vaccinate—that is to say, that it conferred a state of anti-anaphylaxis at the time of the second injection of serum; the dog shewed none of the above-mentioned symptoms. In the same way a dog sensitised to the serum, then submitted to anti-anaphylactic vaccination by means of a small dose of serum, became refractory to the injection of peptone.

From all these facts, Kraus and Biedl have concluded that anaphylactic intoxication is brought about by a poison which, physiologically speaking, is identical with peptone (de Witte).

In our opinion, Kraus and Biedl ought, before formulating their theory, to have tried other animals than the dog. That these things take place in the dog, as they say, nobody doubts; but what is arguable is their interpretation of them.

For the theory of Kraus and Biedl to be true, it should apply equally to the guinea-pig—the anaphylactic reagent *par excellence*. Even if the symptoms of anaphylaxis may slightly differ in the dog and the guinea-pig, the mechanism of anaphylaxis should always be the same, and should not vary with the animal species.

Starting from this idea, we have asked our collaborator Werbitzki[1] to sensitise guinea-pigs to horse serum, next to inject peptone as anti-anaphylactic vaccine, and then to make the trial injection by introducing horse serum subdurally.

If the peptone is equivalent to the serum, as Biedl and Kraus think, what is true of the dog must be true

[1] *Comptes rend. Soc. de Biol.*, lxvi., p. 1084, 1909.

of the guinea-pig. Now, the experiment shews that peptone confers no immunity from anaphylactic accidents upon the guinea-pig sensitised to serum.

We must, then, conclude that the immunity which Biedl and Kraus observed when injecting the dog with peptone is a special immunity, an immunity against the lowering of arterial pressure, but in nowise an anti-anaphylactic immunity. In other words, the symptoms that these authors note in the dog are dependent on changes of vascular equilibrium, and not on anaphylaxis.

As Charles Richet has rightly observed, the authors have taken for the cause of anaphylaxis what is in reality only the effect.

Whilst Kraus and Biedl pay special attention to what takes place in the blood, the attention of Auer and Lewis[1] has been drawn chiefly to the lungs. The view of these American authors regarding anaphylactic shock is as follows: As soon as the trial injection is made, a tetanic contraction of the muscles of the bronchi is produced; occlusion follows, and prevents the entrance of air to the bronchi. According to them, the cause of asphyxia is in the bronchi, and is not of central origin. They rely on the appearance of the lungs, which, at the autopsy, are distended, bluish-pink in colour, do not collapse on incision, and are free from œdema.

According to M. Nicolle and Abt,[2] the hypersensitiveness of guinea-pigs is explained by the development of lysin, and the absence of coagulation is purely an example of hypersensitiveness. Anti-anaphylaxis results from the reduction of lytic power. "The excess of serum not split up at the time of the Besredka-Steinhardt experiment is sufficient to produce (in time) enough supplementary

[1] *Journal of Experimental Medicine*, xii., p. 151, 1910.
[2] *Annales de l'Institut Pasteur*, xxii., p. 143, 1908.

lysin for the organism to recover its original albuminolytic titre.

Nicolle believes that the nerve-cell does not play any part in the production of hypersensitiveness, and that it only suffers passively the effects of the real poison liberated by the albuminolysin.

According to Vaughan and Wheeler,[1] the first injection of the albuminoid substance is followed by a sort of excitement or stimulation of certain cells, then by the appearance of a ferment specific for the substance injected. This ferment, which is found in the interior of the cells in the form of zymogen, reacts at the time of the second injection. It follows that the albuminoid substance, injected for the second time, becomes rapidly digested. The effect of this is the setting free of a toxic group, and the appearance of the usual anaphylactic phenomena.

To shew the foundation for this theory, Vaughan,[2] with his collaborators (Vaughan Jun. and Wright), tries to extract from the organs of sensitised guinea-pigs the ferment in question. For this purpose the organs, finely macerated, are emulsified in physiological saline solution, and the supernatant fluid which should contain the ferment is brought in contact with egg-albumen.

When this contact is of short duration (thirty minutes) the product thus obtained is not toxic. But when the contact is prolonged, and especially when the organs are removed at the moment when the animal is clearly sensitised, a product is obtained which, injected into the veins of a fresh guinea-pig, kills it very rapidly, with all the classical symptoms of anaphylaxis.

The ferment in question, which exerts a proteolytic action on the egg-albumen, passes through the

[1] *Journ. Infect. Dis.*, vi., p. 476, 1907.

[2] *Zeitschr. f. Immunitätsf.*, I. Orig., xi., p. 673, 1911.

Berkefeld filter. It is rendered inactive by heating at 56° C. for thirty minutes. It can be made active again by the addition of fresh extract from the organs.

The same result is obtained by bringing horse serum into contact with the ferment which is contained in the extract from the organs.

Giving up this theory of precipitins as soon as he discovered that it did not tally with the facts, Doerr conceived a physical theory.[1] According to this, the reaction between the antigen and the antibody brings with it physical modifications of the blood, and these modifications produce the usual anaphylactic symptoms. He admits the existence, in the fresh serum of the guinea-pig, of a toxic substance and of another antagonistic to it which masks the first substance. When bacteria or precipitates are brought into contact with complement, these bacteria or precipitates absorb the antagonistic substance. The toxic substance, no longer held in check, becomes free—hence the anaphylactic shock.

In other words, the anaphylactic poison is not formed at the expense of the antigen, as Friedberger thinks, but at the expense of the complement itself.

This physical theory, which explains, indeed, a great many phenomena, is more satisfying mentally than the anaphylotoxic theory. Thus it has attracted many followers (Muternich,[2] Bordet,[3] and others).

It must be remembered that the physical conception of anaphylaxis was first formulated by us ten years ago. We summed up our experiments on the mechanism of anaphylaxis and anti-anaphylaxis in the following terms: " In a general way, most of the facts reported seem to indicate that the phenomena of anaphylaxis and anti-anaphylaxis are reducible to the actions of precipitation and absorption which

[1] *Wien. klin. Wochenschr.*, xxv., p. 331, 1912.

[2] *Comptes rend. Soc. de Biol.*, lxxiii., p. 56, 1912. [3] *Loc. cit.*

govern the relation of the colloids to one another." The only object of this conception, formulated, it is true, in general terms, was at that time to put forward the idea of a physical process in opposition to that of a definite chemical poison.

In exposing, as we have just done, the principal theories of anaphylaxis current at the present moment, our idea has been to gather together all the evidence in order that the reader might be in a position to form his own opinion.

Before passing on to the statement of our own conception of anaphylaxis, the different elements of which have already been sketched in the previous chapters, we think it may be well to return to the nature of the disturbances caused by the anaphylotoxins, the poisons of Kraus and Biedl, Vaughan, Doerr, and other experimenters.

It is the similarity of these disturbances to those observed in the course of anaphylaxis, a similarity quite disturbing at first sight, which is, in our opinion, the principal cause of confusion. However, when one thinks about it, there can be no great choice in the mode of dying in a guinea-pig which dies in a few minutes from intravenous injection; the clinical picture which precedes its rapid death is always perceptibly the same. It is not so much the nature of the substance injected which determines the symptoms of death as the rapidity with which one injection follows another, and especially the intravascular manner of the injection, which stamps them with special character.

It is not, then, to be wondered at that, side by side with the real anaphylactic disturbances, others are observed so similar as to be mistaken for them, but not arising from the same cause.

Let us take, to settle matters, the anaphylotoxins, which, of all the poisons called anaphylactic, are the

best known. As we have already indicated above, their actual manner of formation has nothing in common with the conditions which govern the production of the anaphylactic shock.

That is not all. It must be remembered that the phenomenon of anti-anaphylaxis, which is the specific attribute of all anaphylactic phenomena, does not apply at all to the anaphylotoxins. Thus, our collaborator Sukiennikowa[1] has discovered that guinea-pigs sensitised to egg-albumen, then vaccinated by means of small doses, are as sensitive to the injection of the anaphylotoxin prepared with egg-albumen as the non-vaccinated control guinea-pigs.

Elsewhere we have seen that anaphylotoxins are only fatal in intravenous injections.

With Stroebel and Jupille we have observed that this deadly action of the anaphylotoxins is completely annulled by a previous injection of peptone; we have been able to prove it both for the serous anaphylotoxin and the typhoid anaphylotoxin.[2]

Must it not be concluded from all these facts that, like all the other so-called anaphylactic poisons brought forward, Friedberger's anaphylotoxins have a very different mode of action, and that their action depends, very probably, upon their properties of coagulation? The disturbances observed by Friedberger and others, which end very soon in death from asphyxia, would prove, in reality, symptoms of embolism and not of anaphylaxis.

Being unable, at present, to bring forward more direct proofs, we present the following hypothesis, which all the facts so far established seem to confirm.

It is implied in all the theories we have just reviewed that the anaphylactic shock is due to a special poison, this poison being, according to Charles

[1] *Zeitschr. f. Immunitätsf.*, I. Orig., xvii., p. 304, 1913.
[2] *Annales de l'Institut Pasteur*, xxvii., p. 185, 1913.

Richet, the apotoxin; to Vaughan and Wheeler the toxic group of protein; to Friedberger the anaphylotoxin; to Kraus and Biedl a peptonoid substance; to Doerr a complement derivative.

In our opinion, the anaphylactic poison does not exist.

Until we gain proof to the contrary, we hold to the first, purely physical conception of anaphylaxis which we formulated in February, 1907—that is to say, at the time when there was no theory of anaphylaxis in existence.

In order to presume nothing as to the nature of the antigen or its antibody, we have referred to them under the names of sensibiligen and sensibilisin.

What takes place at the time of the trial injection? The newly arrived antigen comes into contact with the already formed sensibilisin. The effect of their affinity is to produce an intense reaction. Whether this reaction disturbs the equilibrium of certain nerve cells where the combination takes place, or whether the latter is accompanied by the setting free or the absorption of energy, calorific or otherwise, we have presented to us a series of phenomena always the same, and which constitute the anaphylactic shock.

In adopting this term, our idea was to exclude carefully all idea of intoxication, and to indicate, on the contrary, that, in our opinion, it was simply a case of violent disturbance without the formation of a fresh chemical substance.

What governs anaphylaxis and anti-anaphylaxis is neither the toxin nor the antitoxin, but, on the one hand, the rate at which the sensibiligen and the sensibilisin come into contact; and, on the other, the place where they meet, which is probably the nervous system. That is why experiment by the vascular or cerebral method is the most severe of all. For the

same reason the intraperitoneal method is much less severe; while the subcutaneous route, which permits an extremely slow absorption of antigen, is the least effective for the production of anaphylactic shock.

These differences of toxicity have struck us particularly in our study of egg-albumen. Whilst the injection of 0·0025 c.c. of egg-albumen subcutaneously is enough to overwhelm the sensitised guinea-pig in a few minutes, the dose of 5 c.c. of egg-albumen—that is to say, 2,000 times as strong—does no harm when injected intraperitoneally.

What becomes, then, of the so-called anaphylactic poison in the last case? Where does it go?

It can be admitted, certainly, that the poison is destroyed as soon as it is formed; but if it is destroyed before exercising its toxic power, is it not useless to take into account this phantom poison?

It should be noted that the various poisons mentioned by Friedberger, Kraus and Biedl, and Doerr, are not at all weak and likely to disappear instantaneously without the animal reacting to them.

It is no more necessary to admit the existence of a toxin for the explanation of anti-anaphylaxis than for anaphylaxis.

In the case of anti-anaphylaxis also, it is the rapidity of the reaction which explains everything. When, to obtain anti-anaphylactic immunity, we employ the method of repeated small doses, we are only provoking a series of slight, successive anaphylactic shocks; the great shock is thus broken by the reaction being made slower and being divided into small doses.

Whether it is a question of the anaphylactic shock which kills in a few minutes, or of anti-anaphylactic vaccination which produces no apparent disturbance, the mechanism is always the same.

As we have previously seen (Chapter V.), the anti-

anaphylactic immunity depends upon the slow neutralisation of the sensibilisin by the antigen—that is to say, on the progressive desensitisation of the animal. Anaphylactic shock is also a desensitisation; only, instead of being slow, it is rapid: the whole difference is in the time of reaction.

Imagine a flask of sulphuric acid to which water is to be added. If all the water is poured in at once, a kind of shock or explosive discharge is produced, due to the rapid liberation of heat.

On the contrary, if the water is poured in in small quantities, even if these quantities are increased progressively at the cost of a series of insignificant shocks, the acid will in a very short time be weakened or desensitised, so that afterwards any amount of water can be added without the least risk of an accident.

CHAPTER VIII

RECENT WORK ON ANAPHYLAXIS

By S. Roodhouse Gloyne

The modern tendency to recognise anaphylaxis in almost every phenomenon of immunity has not only led to the production of an ever-increasing literature on the subject, but it has also caused a good deal of confusion as to the real meaning of the word. If the subject is to be a pathological entity at all, it must be something more definite than a vague hypersensitiveness to infection, and by no means the least contribution to the question in the present work of Besredka is the attempt to define clearly the symptoms and signs of the reaction. Obviously, all cases of acute illness or sudden death after inoculation cannot be attributed to anaphylaxis without careful investigation and, if possible, adequate proof.

The first difficulty encountered is that of bridging the gap which exists in almost all experimental work in pathology between phenomena artificially produced in animals and signs and symptoms observed in man. In either case the onset of symptoms is sudden and immediate. In animals three stages in the rapid progress of the condition can be recognised (Vaughan)[1]—(1) peripheral irritation, in which the animal becomes violently excited and scratches itself furiously; (2) paresis, most marked generally in the hind limbs; (3) general convulsions, with expulsion of urine and fæces, dyspnœa, collapse, and often death. As shewn by Besredka, these symptoms are most clearly

[1] "Protein Split Products," 1913.

defined and most constant in their occurrence in the guinea-pig. They are accompanied by fall of blood-pressure, diminished coagulability of the blood, disappearance of polymorphonuclear cells from the blood-stream, and spasmodic contraction of the muscles of the bronchioles. With regard to this last sign, Manwaring and Crowe[1] have recently carried the matter a step farther by means of perfusion experiments with isolated anaphylactic lungs. They note three types of pulmonary anaphylactic reaction—(1) bronchial anaphylaxis or spasmodic contracture of the muscles of the bronchioles; (2) vascular anaphylaxis or spasmodic contracture of pulmonary bloodvessels, usually accompanied by œdema; (3) pseudo-anaphylaxis or the plugging of the pulmonary bloodvessels with thrombi and agglutinate masses of corpuscles.

It appears to be a general rule that the symptoms and signs of anaphylaxis vary with the animal inoculated rather than with the protein used. In man these symptoms have been observed chiefly after injections of antitoxic horse serum in the treatment of diphtheria, tetanus, etc. Here we immediately encounter the question, Is the normal reaction of man to a primary injection of foreign serum an example of anaphylaxis? Or, in other words, is there any difference between the so-called serum sickness and anaphylaxis? Goodall,[2] who has recently published observations on 3,502 consecutive cases of serum sickness following injections of diphtheria antitoxin, considers that for the present at any rate it is advisable to keep the two conditions distinctly separate. He therefore divides his cases into three classes—(1) persons who exhibit ordinary serum sickness after a primary infection—the normal reac-

[1] Proc. Soc. Exper. Biol. and Med., xiv., p. 173, 1917.

[2] *Lancet*, i., p. 323, 1918.

tion to serum; (2) persons who have been reinjected after a lapse of at least ten days, and may shew a true anaphylactic reaction; (3) persons who have never been previously inoculated, but who nevertheless shew excessive severity of symptoms without any latent period—almost certainly a true anaphylaxis.

In the first group the signs noted by Goodall were rashes—usually urticaria or erythema marginatum—pyrexia, and joint pains. These are the usual symptoms of a normal serum reaction. The rashes occurred in rather more than one-third of the cases inoculated; they were most common at the site of the injection, but were frequent also on the exterior surfaces of the extremities, and varied in duration from a few hours to a few days. Goodall also noted that a combination of urticaria and erythema in the same patient was not uncommon, and suggests that it may have been due to a mixture of serum from two horses during manufacture. Pyrexia was usually transient, but occasionally lasted as long as a fortnight. The temperature was rarely very high, but was sometimes accompanied by enlargement of glands, tonsillitis, or albuminuria. The joint pains generally affected the wrists, elbows, ankles, and knees, and were seldom serious. An analysis of 464 consecutive cases shewed a latent period for normal serum sickness of three to twenty-two days—most commonly six to fifteen—between the first injection and the onset of symptoms.

In the second group of true anaphylactic reactions in persons reinoculated after a period of at least ten days, the latent period was much shortened and the symptoms of unusual severity. Besides profuse urticarial rashes with gigantic weals, the mucous membranes of the mouth, nose, pharynx, and larynx, were often involved; the tongue was swollen, respiration embarrassed, and prostration followed. In a

few exceptional cases rigors, convulsions, drowsiness, dyspnœa, collapse, vomiting, diarrhœa, abdominal pains, and high temperature, were noted. These symptoms were always part of an immediate reaction, and were not often of long duration. The shortest period noted between the primary and secondary injection was sixteen days and the longest seven years. Out of a total of 203 reinjected patients, 36 per cent. exhibited no symptoms of anaphylaxis at all.

The third group given by Goodall consists of a small collection of cases in which there is absence of latent period with sudden onset of severe symptoms which may rapidly terminate in death. Instances are met with in persons who have never been inoculated with serum before, whilst a considerable number of the patients have been found to be asthmatics. About forty fatal cases have now been recorded in medical literature (Kolmer).[1] Goodall considers that these cases have probably been born anaphylactic, or have been sensitised in some unknown way.

Up to the present time the most extensive observations made in man have been carried out with diphtheria antitoxin. In the case of tetanus a few records exist of serious anaphylaxis in man following prophylactic injections of antitetanic serum from immunised horses, and presumably further details will soon be forthcoming as the result of the prophylactic treatment of war wounds. Vernoni[2] has recently published a list of twelve cases of anaphylaxis following serum injections for tetanus, with one death. He considers that the antibodies responsible are diffused throughout the body, but do not appear to penetrate the meninges unless the latter are diseased. For

[1] "Infection, Immunity, and Specific Therapy," 2nd edition, 1917.

[2] *Rivista di Clinica Pediatrica*, xv., p. 337, 1917. Quoted in *Journal of American Med. Assoc.*, lxix., p. 949, 1917.

this reason he advises injecting serum by the spinal route.

True anaphylaxis in man therefore consists in a typical symptom-complex following a second injection of protein after an interval of at least ten days. These symptoms are urticarial and erythematous rashes, œdema of the skin and of the mucous membranes of mouth, nose, pharynx, and upper respiratory passages, vomiting, abdominal pain and diarrhœa, dyspnœa, collapse, and occasionally death. We have seen, also, that the normal serum reaction consists in slight and transient symptoms of much the same character. Until pathological investigations have carried us further, it is suggested that we should refer to this latter group with slight symptoms as serum sickness, and retain the word anaphylaxis for the more serious cases following second injections. It may be that the difference is only one of degree, in which case we must presuppose a sensitising dose or some equivalent, but the distinction will at any rate serve to emphasise certain points in the theory and practice of sensitisation.

Several other conditions in man shew symptoms very closely resembling true serum anaphylaxis—*e.g.*, (1) the so-called food anaphylaxis, (2) insect stings, (3) the state of hypersensitiveness to certain drugs, sometimes referred to as drug anaphylaxis.

Food anaphylaxis has been dealt with in Chapter V. of this work by Besredka. It is a question which raises numerous difficult problems still requiring elucidation. Vaughan,[1] for example, states that the sensitising group resulting from protein digestion is destroyed in normal digestion, and that it is only under abnormal conditions that protein sensitisation results through the alimentary canal. Whether or not all proteins contain this sensitising group, and

[1] Vaughan, *loc. cit.*

under what conditions it acts when present, are questions yet to be solved.

The relation of insect stings to anaphylaxis has not so far been investigated to any great extent, but occasionally cases are recorded[1] which suggest that there is some close connexion. The condition of hypersensitiveness to drugs, on the other hand, has received a fair share of attention. For many years it was regarded as a personal idiosyncrasy incapable of explanation, but recently the explanation has been sought for in anaphylaxis. The drugs most commonly associated with the condition are quinine, copaiba, iodoform, iodides, bromides, antipyrin, atropine, and various alkaloids. Quinine especially has been the subject of recent investigation (Boerner).[2] If the original view of anaphylaxis be true, that it is invariably due to the injection or ingestion of a foreign protein, one is faced with the difficulty of reconciling this so-called drug anaphylaxis with the true condition of protein sensitisation. Hence the introduction of the terms " indirect " or " secondary " anaphylaxis, which assumes that the drug acts upon the protein molecules and liberates a toxic product. There is some diversity of opinion regarding the source of the protein. It has been suggested (Jobling and Petersen,[3] and Doerr[4])—(1) that absorption of complement from the blood-serum may render the latter poisonous; (2) that the poison, whatever it is, is already pre-formed in the serum, but that its action is neutralised by some constituent of the serum which becomes absorbed at the time of the administration of the drug; (3) that the absorption

[1] Atkinson, T. R., *Brit. Med. Journ.*, London, ii., p. 1148, 1907; and Goodall, *loc. cit.*

[2] *Journal of American Med. Assoc.*, lxviii., p. 907, 1917.

[3] *Journ. Exper. Med.*, xix., pp. 459, 480, 1914.

[4] " Handb. d. path. Mikroorgan.," Kolle and Wassermann, 2nd edition, ii., p. 947, 1913.

of some constituent of the serum leads to a breaking down of serum proteins, with liberation of toxins. Animal experiments on this subject are quoted by Besredka in Chapter V. of this work. The whole subject needs a great deal of investigation. It may be that it will help to bridge the gap which at present exists between true serum anaphylaxis as we see it following serum inoculation and the state of hypersensitiveness to a specific toxin (the so-called "bacterial anaphylaxis") found in many of the infective diseases, such as tuberculosis, syphilis, and others. Indeed, this analogy has been carried yet a step further, and "it has been argued with some plausibility that a man may be sensitised by the degeneration products of his own tissues, and that this is an explanation afforded of some of the curious outbursts which are occasionally witnessed in such affections as nephritis, especially when they are chronic" (Goodall).[1] On the other hand, we are faced with phenomena, such as those of "serum fastness" and "drug fastness," which point to an acquired and generally specific resistance on the part of the animal body. It is clear, therefore, that we have not yet by any means solved this very difficult problem.

Bacterial anaphylaxis, a state of hypersensitiveness produced by the liberation of the foreign protein of bacteria by the process of bacteriolysis, has of late years been made responsible for the symptoms of the acute exanthemata. The incubation period (J. McIntosh)[2] and the presence and distribution of secondary rashes point in this direction, but certain wide differences between the clinical pictures of the various exanthematous diseases have still to be accounted for on this hypothesis. As the result of an analysis of 100 cases of secondary rash in acute

[1] *Loc. cit.*

[2] *Quarterly Journal of Medicine*, vii., p. 272, 1913-14.

infectious disease, Goodall[1] concludes that though anaphylaxis may explain "a very large number of these rashes and their accompanying symptoms," yet the most important incidents of an attack of acute infectious disease still remain unexplained.

The difficulties become still greater when we turn to a chronic bacterial infection such as tuberculosis. It was pointed out a good many years ago that sensitisation to the *B. tuberculosis* closely resembled anaphylaxis. Vaughan and his co-workers, who have made elaborate and extensive experiments with the split products of the tubercle bacillus, consider that the sensitisation in this case is a true bacterial anaphylaxis. A full résumé of the work done on this side of the question will be found in Vaughan's collected work already quoted. On the other hand, in Chapter VI. of the present work Besredka has advanced cogent reasons for believing that the tuberculous toxin is a thing apart, and in no way connected with true anaphylaxis.

It is impossible to close this brief résumé of the manifestations of anaphylaxis in man without reference to the condition of bronchial or spasmodic asthma. As long ago as 1909, Auer and Lewis[2] shewed that the lung of an anaphylactic guinea-pig presented changes resembling bronchial asthma, and even previous to this it had been recognised clinically that asthmatics were particularly sensitive to injections of horse serum. A. G. Auld[3] has recently advanced the question a step further by the use of hypodermic injections of peptone solutions in the treatment of asthma, on the principle that this disease is an auto-sensitisation, and that anti-anaphylaxis may be induced by peptone inoculations.

[1] *Loc. cit.*

[2] *Journal of American Med. Assoc.*, liii., p. 458, 1909.

[3] *Brit. Med. Journ.*, i., p. 580, 1917.

From the etiological point of view, however, it seems only reasonable at present to draw a clear line of distinction between these various conditions. True anaphylaxis consists in a series of well-defined and recognisable symptoms with sudden onset, arising as the result of the inoculation or, less frequently, the ingestion of foreign protein; and Besredka has clearly defined the limits of this reaction in the present work. Besides this condition, there are numerous diseases—acute exanthemata, bacterial infections, asthma, etc.—in which there is a varying amount of positive evidence that anaphylaxis is concerned in the causation of symptoms. At the same time, the evidence is by no means complete, and it is rather unfortunate that an alternative name —such as allergy—is not more frequently adopted. The term "anaphylaxis," however, appears to have become firmly fixed in our nomenclature, and its significance broadens every year.

Meanwhile, in the course of researches on the revived cellular theory of anaphylaxis, serum tests have been devised. Dale[1] has shewn, by means of the graphic method, that when the excised and washed muscle of a sensitised animal (*e.g.*, virgin guinea-pig's uterus) is placed in a bath of Ringer's solution, it will contract on the addition of the protein which had been used for sensitisation. Manwaring and Yoshio Kusama[2] have substituted the guinea-pig's lung for the uterus in the test; Schultz[3] and R. Massini[4] have used the guinea-pig's intestine. Schultz,[3] indeed, suggests that in serum anaphylaxis all smooth muscle becomes sensitised. It is interesting to note in passing that with the use of this test

[1] *Journ. Pharmacol. and Exper. Therap.*, iv., p. 167, 1912-13.
[2] *Journal of Immunology*, ii., p. 157, 1917.
[3] *Journ. Pharmacol. and Exper. Therap.*, i., p. 549, 1909-10.
[4] *Zeitschr. f. Immunitätsf.*, I. Teil Orig., xxv., p. 179, 1916.

Massini has been unable to detect any sensitisation of the guinea-pig's intestine with tuberculin.

The actual mechanism of the production of anaphylaxis still eludes the grasp of the bacteriologist. In the present work Besredka has shewn how physical conditions such as heat, dilution, precipitation, etc., affect the protein used as antigen. It was originally suggested by Wells[1] that nothing less than the intact protein molecule will produce anaphylaxis, and a good deal of recent research has centred round this point. Zunz[2] claims to have obtained typical reactions with proteoses, and Abderhalden with a synthetic polypeptid.[3]

Pick and Yamanouchi[4] and Bogomoletz[5] also believe that lipoids will serve the same purpose, while Thiele and Embleton[6] obtain contrary results. Gideon Wells[7] has noted antigenic differences in this connexion between α and β nucleoproteins. Finally, Dale and Hartley[8] have shewn that every sensitisation with a whole serum is in reality a complex multisensitisation, and that each of the three proteins separated from horse serum, for example—euglobulin, pseudo-globulin, and albumen—can act as anaphylactic antigens. They further suggest that the successive crops of serum rash obtained in certain patients as the result of only one injection may "represent the successive appearances at different intervals of sensitiveness to the different serum proteins." The balance of opinion appears to be in favour of the view that only proteins are con-

[1] *Journ. Infect. Diseases*, xii., p. 341, 1913.
[2] *Zeitschr. f. Immunitätsf.*, I. Teil Orig., xvi., p. 580, 1913.
[3] *Zeitschr. Physiol. Chem.*, lxxxi., p. 315, 1912.
[4] *Zeitschr. f. Immunitätsf.*, I. Teil Orig., l., p. 676, 1909.
[5] *Ibid.*, I. Teil Orig., v., p. 121, and vi., 1910.
[6] *Ibid.*, I. Teil Orig., vi., p. 160, 1913.
[7] *Journ. Biol. Chem.*, xxviii., p. 11, 1916.
[8] *Biochem. Journ.*, x., p. 408, 1916.

cerned in the production of anaphylaxis. In view of the work of Vaughan on protein split products and of various workers on "drug anaphylaxis," it seems probable, however, that some alteration in the protein molecule is necessary for the production of the reaction.

It is perhaps rather unfortunate that up to the present time the morbid histology of this subject has not produced any great interest amongst workers in immunity. Recently, however, Broughton[1] has made microscopic examinations of the post-mortem findings of guinea-pigs rendered anaphylactic with egg-albumen or with beef serum. He found the most marked changes in the small arteries of the liver, kidneys, spleen, and heart. These changes, which were confined to the small arteries, were (1) degeneration and regeneration of endothelium, (2) œdema and fissuring of intima and media, (3) sometimes splitting of the internal elastic lamina. The subject is now becoming important in relation to the recently developed cellular theory. Weil[2] has also demonstrated changes in the livers of anaphylactic dogs (congestion and degeneration of parenchyma cells).

This aspect of the question leads us to a consideration of the various theories which have been advanced in explanation of the phenomena of anaphylaxis. Unfortunately, the terminology, like that of immunity work in general, has become extremely confusing. Most workers have produced theories based upon the interaction of antigen and antibody, and in almost all cases new terms have been coined for the purpose. Many of these terms presuppose conditions which it is impossible to prove at the present time, and for this reason are unsuitable. Besredka in the present work has been careful to avoid this by the use of the

[1] *Journal of Immunology*, i., p. 105, 1916; ii., p. 501, 1917.
[2] *Ibid.*, ii., pp. 429, 525, 1917.

terms sensibiligen and sensibilisin, which merely indicate the plain fact of sensitisation.

In analysing these theories, Kolmer[1] divides them into two main groups—(1) those based on the humoral or chemical theory of anaphylaxis, which assume that antigen and antibody meet and interact in the blood-stream; (2) those which assume that the antibody is within the tissue cells and that the reaction takes place in this position—the "cellular" theory. As Besredka has already given a short abstract of these theories in the previous chapter, it will not be necessary to recapitulate them here. It will be noted that almost all the earlier work on the subject was based on the humoral or chemical theory.

Recently, however, largely owing to the work of the late R. Weil,[2] a good deal of investigation has been carried out on the cellular theory.

Of the earlier humoral theories, probably that of Friedberger on anaphylotoxins has received the most attention. It has been extended from time to time by Friedberger himself and by others in order to explain the various steps of the reaction. Recently Novy and Drekmif[3] have extended the researches on anaphylotoxin still further. They believe that the matrix of this poison is not located in the nitrogenous constituents of the antigen, as Friedberger suggested, but that it is always present in circulating plasma, and can be changed by a catalyser into anaphylotoxin itself by the action of an inert substance such as agar, kaolin, etc. These authors suggest that the reaction is analogous to the catalytic change of fibrinogen into fibrin in coagulation.

The part played by the body cells in the phenomena of anaphylaxis has become more and more the subject

[1] *Journal of Immunology*, ii., pp. 429, 525, 1917.
[2] *Ibid.*, iii., p. 1, 1918.
[3] *Journal of American Medical Association*, lxviii., p. 1525, 1917.

of investigation of late years. It is assumed that the anaphylactic antibody is in some way closely connected with the body cells, and that the interaction with antigen takes place in this position—hence the symptoms and signs of "shock," which is probably a cellular phenomenon. In a series of papers extending over the last four or five years, Weil[1] has shewn that—(1) antigen and antibody may coexist in the blood and in the cells of the living animal, and that even if in combination with antigen the antibody may still be capable of reacting with fresh antigen; (2) anaphylaxis consists simply in the cellular reaction due to fixation of antigen by the cellular antibody; (3) blood taken from dogs at the height of anaphylactic shock fails to produce any effect when injected into normal animals, whereas the liver of these dogs is enormously congested, the parenchyma cells are degenerated, the blood is incoagulable, and the blood-pressure low. This author therefore finds no direct evidence of anaphylotoxin in the circulating blood, but rather evidence of a cellular reaction, chiefly to be found, apparently, in the liver. Manwaring and Crowe[2] have also recently shewn by means of perfusion experiments with the livers of normal and of anaphylactic guinea-pigs that the evidence points to the explosive formation or liberation of vaso-dilator and broncho-dilator substances by the sensitised liver cells.

The relation of bacterial anaphylaxis to the cellular theory is obviously of considerable importance. Zinsser and Parker[3] have studied the question by means of Dale's anaphylactic guinea-pig uterus test. They find that bacterial anaphylaxis is strictly analogous to serum anaphylaxis. For the success

[1] *Journal of American Medical Association*, lxviii., p. 1525, 1917.
[2] *Journal of Immunology*, ii., p. 517, 1917.
[3] *Journal of Experimental Medicine*, xxvi., p. 411, 1917.

of the experiment it appears to be necessary that solution or extraction of the bacteria should first take place. The antigen produced from these disintegrated bacteria reacts with antibody, which remains an integral part of the cell protoplasm, and the authors consider that the entire process takes place within the body cell.

The study of anaphylaxis has become more and more extensive since Richet's original observations on Actiniæ. The difficulties surrounding the question are great. A few have been overcome, but a large number still remain. No theory of anaphylaxis will explain all the facts, any more than any theory will entirely explain immunity in general. Indeed, anaphylaxis is so intimately connected with the general problem of immunity that a solution of the former would probably go far towards explaining the latter. The general trend of immunological research at the present day appears to be along the lines of physical chemistry, more especially as it relates to the chemistry of the colloids. Hitherto this has been the "No Man's Land" between the physicist and the biochemist on the one hand and the bacteriologist on the other, and it may be that further exploration of this little-known territory will add greatly to our knowledge of those problems which are so intimately connected with immunity and infective disease.

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