Toxines and antitoxines / Tr. from the German by C. Ainsworth Mitchell.

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AND

ANTITOXINES.

BY

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TRANSLATED FROM THE GERMAN

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PREFACE TO THE GERMAN EDITION.

THE author's object in writing this work was to bring together all that was known on the subject of toxines, the definition of a toxine being based on chemical grounds and on the side-chain theory, quite independently of the origin of the poison.

Hence it follows that although this book may be regarded as complete in one respect—viz., that it does, in fact, give a comprehensive idea of toxines as a whole—yet, at the same time, it was not part of my plan to deal with all the toxic substances of a doubtful nature that are formed in animals and plants. For this reason many animal and vegetable poisons, the toxine character of which is open to question (*e.g.*, fish venoms), have only been described very cursorily, while no mention has been made of others (*e.g.*, *bee-poison*, which is probably of a basic nature).

In my attempt to give an exact outline of the toxines themselves and their antitoxines, I have had practically no previous work of importance at my disposal.

Therefore, so far as it was possible, I have obtained my facts solely from the original papers. Yet I cannot but fear that my book remains a *torso*, since much that has been written on toxines is scattered through different journals, many of which seem unlikely to deal with such a subject, and it is therefore probable that many facts may have escaped my notice.

CARL OPPENHEIMER.

NOTE BY THE TRANSLATOR.

THIS book is in many respects a companion volume to "Ferments and their Actions," by the same author. As in that case, I have made numerous additions to the text, so as to include the more important facts that have been made known since the appearance of the German Edition, and the selection from the voluminous mass of new matter has been made by Dr. Oppenheimer himself.

C. A. M.

GRAY'S INN,
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TOXINES AND ANTITOXINES.

GENERAL.

Introduction.—Not long after the principles of bacteriology had been established by Robert Koch, it became the general belief that it was not, in the main, to the bacteria themselves that the chief symptoms of infectious diseases were to be attributed. It was soon recognised that the living micro-organisms were, for the most part, only indirectly harmful, and that their *chemical products* were to be regarded as the immediate cause of the disease.

Brieger, in particular, pointed out in the very early days of bacteriology that search ought to be made for the *specific poisons* of bacteria, and he himself endeavoured to discover and isolate these hypothetical poisons.

He first separated from culture media that had been altered by the growth of bacteria, and notably from the mixed products of putrefaction, a series of well-defined chemical substances which he termed *ptomaines*—nitrogenous bases, some of which were virulent poisons. These substances, however, proved *not* to be the real bacterial poisons. They were not the weapons used by the parasites in living bodies. They were not the *specific bacterial poisons*, which were first grouped under the collective name of "Toxines." Then by degrees the conception of a toxine underwent a natural process of specialisation, so that the term began no longer to connote the poison isolated from any given products of decomposition formed in the vital processes of bacteria, but to be limited to the *specific* bacterial poison that caused specific illness. Hence this tendency to limit the connotation developed without being the result of conscious thought, or much less of being formulated in set terms. The confusion in the definition was still further increased by the fact that a series of bacterial poisons, apparently related to the proteids, were termed *toxalbumins*. Under this name were included, not only certain poisons that we must to-day regard as

true toxines, but also others that had nothing in common with them except their apparent proteid nature.

On the other hand, one very important and far-reaching theoretical result of these researches was that a comparison was drawn between these bacterial toxalbumins and other toxalbumins of the animal and vegetable kingdom, such as snake poison and the like on the one hand, and on the other the poisonous vegetable proteids *ricine*, *abrine*, and *crotine*, first investigated by KOBERT and his pupils. The most important deduction from this point of view was that first drawn at a later period by EHRLICH, CALMETTE, and others—viz., that the specific bacterial poisons do, as a matter of fact, enter into a fundamental relationship with the cell products in question of higher organisms to form specific "anti-bodies" in the organism of the attacked animal, so that EHRLICH'S side-chain theory was applicable in general to their action. These poisons are *haptines* in EHRLICH'S conception, and, as regards the theory, it matters little what was their origin. BRIEGER'S great service lies in the fact that he was the first to show a relationship between bacterial poisons and other known poisonous substances. Hence it is not a very essential point that BRIEGER'S views require modification in two respects, in consequence of later researches, of which his own were not the least important. In the first place, the relationship towards other toxalbumins does not hold good in the case of all the bacterial poisons isolated by BRIEGER, for very many of these are substances of non-specific character, not comparable with *ricine*, &c., since they are not haptines. In point of fact, there remain practically only the poisons of diphtheria, tetanus, *Bacillus botulinus*, and *B. pyocyaneus* as typical *true* toxines (*e.g.*, tetanolysine, staphylolysine, and staphyloleucocidine, with probably also the blood-solvent poisons of other bacteria), and some others, such as the poisons of cholera and typhus. Moreover, BRIEGER himself, at a later period, stated that his diphtheria toxine was not a proteid, and the proteid nature of ricine is also very doubtful, whereas snake poisons, even in the present condition of our knowledge, appear to be proteins. In this respect, however, toxines resemble the closely analogous enzymes, among which, in addition to, presumably, true proteids (trypsin, diastase (?)), there are substances of high molecular weight that belong to another class (pepsin, invertase). The definition of a true "toxine" is, therefore, arrived at in the following manner in the case of bacterial poisons :—

All bacteria produce certain chemical substances in the media in which they develop.

Although many of the bacterial substances formed in the most different ways are products of secondary decomposition due to obvious chemical reactions, yet beyond doubt some of them are *primary* products of bacterial metabolism.

Some of these metabolic products are more or less virulent *poisons*. In this respect there is, in general, no difference between pathogenic and non-pathogenic micro-organisms.

Thus, even when substances of this kind are poisonous, they have certainly nothing to do with the poisoning of an organism through the invasion of bacteria, even assuming they are produced by pathogenic micro-organisms. Such poisons, for instance, as *neurine* have their specific effects, whether produced by bacteria or by purely chemical reactions. Hence they must be eliminated first of all from the definition of a *toxine*.

In the second place, a series of substances has been prepared by different processes from the dead cells of many species of pathogenic micro-organisms. These are of a proteid nature, as, for example, BUCHNER's bacterial proteins, and are more or less *poisonous*. But their toxic effects differ but little whatever their origin. They never show a specific character, and never produce symptoms resembling those of specific diseases. In addition to this, the *cell protoplasm* of many bacteria contains toxic proteids that cannot be isolated from the protein, and these are also non-specific in their action.

Excluding these, what is left on which to base the definition of a toxine? Certain pathogenic bacteria, when grown in a pure cultivation, produce poisons that *dissolve* in the liquid nutrient media, and can only be obtained in an undecomposed, concentrated, and imperfectly purified condition by the most careful treatment—substances which are *not ptomaines* and *not proteids* (*vide infra*).

Such substances have been isolated, in particular, from pure cultivations of the bacilli of *diphtheria* and *tetanus*, and they are the *true bacterial toxines* in the narrower sense of the word. In like manner all those more definite chemical poisonous substances formed in the cells of higher animals and plants must be separated from the toxines.

The latter form a class of substances whose definition is found in their nature and characteristic mode of action, irrespective of their origin.

Toxines are characterised in the first place by a sum of *external properties*. Their chemical structure is absolutely unknown. They are extremely unstable, and very sensitive towards even slight chemical influences, and especially towards the action of

heat. They are not proteids and not *toxalbumins*, and they show many remarkably close analogies with ferments.

Physiologically, they are distinguished by being under suitable conditions extremely poisonous, far more so than any other known poisons. Nearly all toxines have also the peculiar characteristic of not acting *immediately*, but only after a latent period, a *time of incubation*, in which respect their action is analogous to the poisoning by living bacteria. In spite of their being so extremely poisonous to many animals, exceeding in this respect the most active of the simple poisons, such as hydrocyanic acid, they yet exhibit in only a few cases (*e.g.*, snake poison) the immediate action that is characteristic of the simpler poisons. Above all, they are characterised by the *specific nature of their action.* Toxines have a peculiar form of activity characteristic of the whole group, as we shall subsequently see. In addition to this, each individual toxine has its own particular mode of action, which, in the case of bacterial toxines, shows a close relationship with the disease produced by the parent cells, being completely analogous in the case of tetanus poison. They are also strictly specific in the narrower sense of the word—*i.e.*, they are only able to injure certain living organisms, whilst they have absolutely no effect upon other organisms (some of which are closely allied to them), with which they stand in the fundamental highly important relationship of *natural immunity.* Nor are their relationships of *acquired immunity* less important, for it is a fundamental property of toxines to produce in the organism attacked antidotes of a strictly specific nature, which render the poison harmless *in vivo*, and, when separated from the parent organism, can also exercise their neutralising activity *in vitro*, each on its respective toxine, and *only* on that. *Thus, each true toxine has also its corresponding true antitoxine.*

Incidentally, it may be mentioned that hitherto all attempts to prepare true antitoxines to the simple crystalloid poisons have been unsuccessful. Even the recent statement by HIRSCHLAFF,[1] who claimed to have prepared an antimorphine serum, has been shown by MORGENROTH[2] to be completely unsupported by the facts, and to have been due to want of accuracy in the preparation of the minimum lethal dose.

Not only have we data for forming a conception of what a toxine is chemically and physically, but we also have a theory based upon them. According to EHRLICH'S *side-chain theory, a toxine is a poison which possesses at least two specific atomic*

[1] Hirschlaff, "Antimorphinserum," *Berl. klin. Woch.*, 1902.
[2] Morgenroth, "Zur Frage d. Antimorphinserum," *ibid.*, 1903, 21.

groups,—a haptophore group, whose function it is to enter into combination with the attacked cells, and a toxophore group, which produces the toxic effects. Every substance that possesses a specific affinity, a suitable haptophore group, for definite complex groupings of protoplasm, is a *haptine*, and every *poisonous* haptine that also possesses a toxophore group is a *toxine*. We must therefore base our definition of a toxine in accordance with this, and rigorously exclude from the toxines any poisonous substance that is not a haptine and that does not produce an antitoxine.

This has been much more easily done in the case of mineral and vegetable poisons, for no one would dream of including among the poisons, in the narrower sense, the alkaloids, &c., of plants, or the few poisonous crystalline substances that can be isolated from the secretions and organs of animals, such as, for example, the alkaloids in the skin of the toad, adrenaline, &c.

But it is of much more importance to effect a classification of bacterial poisons by means of this sharp definition. We must, in the first place, separate from the toxines all *non-specific* substances as defined above, from whatsoever bacteria obtained.

But a still further classification is introduced by the fact that there are probably *specific bacterial poisons*, which are only produced by definite groups of bacteria, and exert specific activity, but which are not haptines, do not form anti-bodies, and are therefore not *toxines*. Poisons of this nature appear to play a part—*e.g.*, in tuberculosis. They will be shortly discussed in the special part. In addition to these there is a whole series of very imperfectly known bacterial poisons, whose specific nature and toxine character there are strong reasons for doubting.

Most difficult of all is the question of the poisons produced by certain bacteria, notably those of cholera and typhus. Their toxines are scarcely known in the free state, and the poisons *excreted* by them do not appear to be the true toxines. On the other hand, we find among them another type, which presents great difficulties in the way of closer investigation. These are the *endotoxines*, which are firmly attached to the living cells, and are thus comparable with the *endo-enzymes* of yeast and animal organs. We shall discuss these more fully in a subsequent chapter.

On Toxines in General.—The true toxines, as defined above, are characterised, we repeat, by their physical and chemical properties in the aggregate, which we have now to describe more closely, combined with their fundamental property of

splitting off free haptophore side-chains in suitable organisms —*i.e.*, of producing *antitoxines*.

Although each individual toxine possesses its own characteristics, which can only be dealt with properly in the special part, yet all true toxines have a number of properties in common which justify us in speaking of them collectively.

Bacterial toxines share these properties with all the other toxines known to us, such as *snake venoms*, the poison of the *blood of the eel* and *muræna*, *spider* and *toad venoms*, *ricine*, *abrine*, *crotine*, &c.

In the first place, the *mode of production* is common to the bacterial toxines. They are to be regarded not, it would seem, as the products of culture media altered by the invasion of bacteria, but, as was demonstrated by BUCHNER,[1] as the actual products of the cell protoplasm, as secreted products of bacterial cells. Just as the cell of the pancreatic glands produces and secretes its *trypsin* and the starch cell of wheat endosperm its *diastase*, so do the bacterial cells secrete their specific toxines. The fact that in the case of certain micro-organisms—*e.g.*, of *cholera*, &c.—the toxines may, under suitable conditions, be retained firmly in the protoplasm, has also its analogy among *ferments*, for we find the yeast enzymes possessing exactly the same characteristic.

When grown upon suitable culture media, pathogenic micro-organisms that produce toxines usually develop their characteristic poisons within a very short time. Thus, SPRONCK[2] obtained a very active diphtheria toxine within forty-eight hours.

The virulence increases, however, with the age of the cultivation. ROUX and YERSIN[3] found that a filtered diphtheria cultivation of seven days' growth killed a rabbit in six days, but that after forty-two days' growth an equal dose of the same cultivation caused death in a much shorter time. SPRONCK's diphtheria toxine was ten times more virulent after five or six days' than after forty-eight hours' growth. Still, the virulence attains its maximum after a certain time, and then begins to diminish owing to the decomposition of the toxine (*vide infra*, *Toxoids*), so that old cultivations become less poisonous. Then, after a fairly long time, the degree of virulence usually becomes constant.

[1] Buchner, "Die Bedeutung der aktiven löslichen Zellprodukte, &c.," *Münch. med. Woch.*, 1897, 12.

[2] Spronck, "Prépar. de la tox. dipth.," *Ann. Past.*, xii., 701, 1898.

[3] Roux and Yersin, "Contribution à l'étude de la diphthérie," *ibid.*, iii., 273, 1889; iv., 385, 1890.

The kind of *culture medium* naturally has the greatest influence on the production of the poison.

In general, *bouillon cultivations* are employed, usually with the addition of a certain proportion of peptone; but culture media are also frequently prepared from meat extracts, yeast extracts, &c.

Agar and other nutrient media can hardly be advantageously employed. Interesting experiments have been made to produce toxines in *proteid-free* culture media, as, for instance, in asparagine solutions containing suitable salts (ARMAND and CHARRIN [1]), and in dialysed urine. Satisfactory results, however, have not yet been obtained (GUINOCHET,[2] USCHINSKY[3]). ZINNO'S[4] explanation of apparently successful attempts to produce toxines in such proteid-free culture media is, that only traces of proteid are necessary for the production of detectable amounts of poison. As a matter of fact, the quantities of toxine thus produced are extremely small. He concludes, from his own experiments, that the presence of a small amount of proteid is *indispensable*.

Speaking generally, this factor varies so greatly with the kind of toxine that the reader must be referred to the special part, where a full description is given of the different methods that have been employed to obtain the largest possible yield of toxines.

Here we will only point out briefly that too great acidity or alkalinity of the medium must always be avoided, and that, in general, the same precautions with regard to temperature, &c., must be observed as are usually taken to obtain the most active and virulent cultivations of bacteria.

One point, however, of special interest may be mentioned here. The production of a very active and virulent *growth* of the bacteria themselves is not invariably accompanied by the development of very energetic cultures of their *toxines*. For, on the one hand, it would seem that the production of poison by the bacteria is not a direct function of their activity of growth or their high degree of virulence. Indeed, in the case of *diphtheria*,

[1] Armand and Charrin, "Transformation de la Matière organique azoté, &c.," *Bull. Med.*, 1891, 356; 1892, 957. Absts. in *Centralbl. f. Bakt.*, xi., 248, 1892. *Cf.* Buchner, "Bakteriengifte und Gegengifte," *Münch. med. Woch.*, 1893, 449.

[2] Guinochet, "Contrib. à l'étude de la toxine du bacille de la diphthérie," *ibid.*, 1893, 293.

[3] Uschinsky, "Les Poisons de la diphthérie et du choléra," *ibid.*, 1893, 293.

[4] Zinno, "Beitr. z. Stud. d. Entstehung der Toxine," *Centralbl. f. Bakt.*, xxxi., 42, 1902.

there may be very energetic growth of bacteria, whilst the culture medium is absolutely *non-toxic* (LUBOWSKI [1]).

Moreover, in the case of vegetable and animal toxines, the production of poison depends on manifold physiological conditions, such as age, nutrition, &c., with which points we will deal more fully in the special part.

On the other hand, it is beyond question that certain agencies increase the growth, and eventually the virulence, of the bacteria, but *reduce* the yield of toxines. This is due to the fact that they partially destroy the toxine already formed. Even when agencies of this kind *increase the production of toxine* simultaneously with the vigour of growth of the bacteria, yet, if employed too freely, the amount of toxine destroyed exceeds that of new-formed toxine, so that the final result is a diminished yield of toxine. Thus, when such agents are employed (*e.g.*, the admission of air to diphtheria cultivations), it is possible to plot a curve, the abscissæ of which represent the increasing quantities of the agent, and the ordinates the final yields of toxine. So long as, for example, the introduction of air causes the diphtheria bacilli to produce an abundance of toxine and the simultaneous destruction by the current of air of toxine already formed keeps within narrow limits, the curve will rise. But by degrees the destructive effect of the air outweighs its favourable influence on the production, and the curve falls again. There is thus an intermediate point at which, with a definite intensity of air current, there is a maximum yield of toxine, and its position obviously depends on numerous conditions, such as the nature of the growth, the culture medium, temperature, &c. This *optimum* is hardly ever realised in practice, the result being, as we shall see later, that conflicting statements are made as to the benefit or injury produced by the same agents.

In addition to the introduction of air, other factors may have a similar effect. Thus, an increase of temperature may influence both the production and the decomposition of a toxine. On the other hand, there are apparently agents that do actually increase the final yield of toxine. A considerable amount of work has been done in these experiments to obtain larger quantities of toxines by the use of the most suitable culture media and temperatures, by the addition of special substances, &c., so that highly poisonous cultivations of the most important toxines can now be prepared. These methods, however, are of an entirely special character, and it is hardly possible to give at this stage

[1] Lubowski, "Ueber einen atoxischen und avirulenten Diphtheriestamm," *Zeit. f. Hyg.*, xxxv., 87, 1900.

any general method of importance that is universally applicable to *all* toxines.

On the other hand, we must not omit to mention briefly here that toxine solutions do not invariably have a *uniform strength.* This phenomenon manifests itself notably in the case of *tetanus poison.* Apart from the fact that Nicolaier's bacillus produces two totally different poisons—viz., that which causes the characteristic symptoms of tetanus, and *tetanolysine* (*q.v.*)—very great differences are also to be observed in individual solutions of the poison as regards their specific activity. Although, as a general rule, tetanus poison is considerably more toxic for guinea-pigs than for rabbits, yet there are also some preparations of the poison that are almost as deadly for rabbits as for guinea-pigs (Tizzoni).

Behring has confirmed this statement with regard to Tizzoni's poison, and has also found in the case of his own cultivations that certain parts were, relatively, extremely poisonous to rabbits. We must therefore conclude that tetanospasmine is not an individual substance, but consists of different active constituents. Similar phenomena have also been observed in the case of the poisons of diphtheria. Over-neutralised poisons are known which are absolutely without effect upon guinea-pigs, but are still poisonous to rabbits.[1]

Assuming now that we have prepared cultivations of living bacteria rich in toxines, it is necessary to treat them in such a way as to eliminate the action of the living cells, so as to be able to examine the poisons separately. For this purpose we can either kill the bacteria, or attempt to remove them completely from the poison.

The first method, in which no attempt is made to remove the dead cells, cannot, by itself, lead to any definite conclusion as to the action of the poison, inasmuch as the dead bodies still possess definite chemical and physiological properties, which must render all deductions uncertain. Fortunately, this method, which was formerly employed, can be dispensed with in the investigation of true toxines, and, as a matter of fact, has fallen into complete disuse.

For it is possible to separate true toxines from their parent cells by *filtration through filters impervious to bacteria.* The chief substances used for the filtration, in addition to infusorial earth and chalk, are porcelain filters or Chamberland's candles. During the filtration the greater portion of the toxine passes completely into the filtrate, the residual cells retaining only as much viru-

[1] For further particulars see Ehrlich, *Münch. med. Woch.*, 1903, 33.

lence as corresponds to the amount of toxine mechanically adhering to them, and from this they can be freed by washing with a physiological solution of salt. But the cells no longer contain any true toxine, which might presumably be extracted from them by breaking up the cell structure (treatment with alkali), as H. KOSSEL,[1] for example, was able to prove in the case of diphtheria bacilli. It is true that these dead cells may still contain poison of quite another kind (*bacterial proteins*), but these have nothing to do with the *specific* poisoning (*vide infra*).

The *phytotoxines* are found in the most different organs of plants, and especially in the seeds, from which they can be isolated by extraction with dilute solutions of salt.

The *zootoxines* are produced in the secretions and blood of animals. It follows from all this that *typical toxines* are *free secretions*—substances that are phsiologically thrown off by the cells into the surrounding media. In this respect they are analogous to the true enzymes. Just as the pancreatic glands secrete *trypsin*, or the glandular cells of the starch layer secrete *diastase*, so does the cell of the diphtheritic micro-organism secrete diphtheria toxine.

This can only be stated with certainty, however, for the typical toxines, notably diphtheria and tetanus. In other cases the facts are much more obscure.

As we shall see later, it is still quite open to discussion whether, for example, the micro-organisms of cholera and typhus produce true toxines in the sense of our definition. Even granting that this is the case, the toxines are certainly not secreted in a free state in any appreciable amount, but are firmly retained, at least, by the living cell. Only when the cell decomposes after death are they liberated to a limited extent, as in the case of older cultivations. But even then the poisonous substances have undergone radical changes, having been converted into secondary, more stable products that no longer show the characteristics of a true haptine. We shall return to this point later.

A close analogy to this retention of the active substances to the living cells may be observed in the case of certain *ferments*.[2] We know that the *yeast cell*, in addition to secreting a small amount of free *diastase*, also contains a series of other enzymes —*invertase*, *maltase*, &c.—which can only be set free after the

[1] H. Kossel, "Zur Kenntnis des Diphtheriegiftes," *Centralbl. f. Bakt.*, xix., 977, 1898.

[2] Oppenheimer, *Ferments and their Actions*, English translation by C. Ainsworth Mitchell. London, 1902 (Griffin & Co.).

death or injury of the cell protoplasm, or, as in the case of *zymase*, after breaking down the cell wall; and we also know that *Monilia candida* will not give up its invertase to the surrounding medium, under any conditions whatsoever.

When toxine solutions have once been obtained by filtration of the cultures, or analogous preliminary means, such as extraction of seeds, &c., they can at once be used, although containing numerous impurities, for physiological experiments. Moreover, even in this impure condition some rough experiments can be made with them to determine the behaviour of the respective toxines towards physical and chemical agents.

Moreover, in order to have it in a more suitable form for keeping, this solution may also be concentrated with various precautions, or may even be evaporated to dryness without materially injuring the toxine. The main conditions are that the temperature must never exceed 45° C. (whence it is best to carry out the evaporation *in vacuo*), and that any strong acids or bases that may be present must be partially neutralised.

For a closer investigation of toxines, however, tedious *processes of purification* are necessary to remove all impurities as completely as possible. The simplest method is *dialysis*, which, however, only separates the toxine from the salts and peptones also present in the solution, but does not eliminate the proteids.

Hence, complicated methods have had to be devised to isolate the toxines in the purest possible condition. The favourite methods employed consist of precipitation by means of ammonium or magnesium sulphate with subsequent dialysis, and of precipitation by means of the salts of heavy metals with subsequent decomposition of the resulting double compounds. The first of these methods yields, eventually, only solid concentrated preparations of toxines which are still impure, although capable of being used for practical purposes, and the second method is the only one that gives approximately pure toxine preparations. A fuller account of its details, which have been worked out, notably by Brieger and his pupils, is given in the special part. They include exceedingly tedious manipulations requiring close attention, and consisting, in the main, of precipitation with zinc, lead, or mercury salts. The double compounds of the toxines with these salts that are precipitated are again decomposed by means of hydrogen sulphide, or of alkali carbonates, or phosphates. Then by means of filtration or dialysis solutions are obtained, which on evaporation *in vacuo* yield preparations which in favourable cases are very rich in toxine. They invariably, however, still contain considerable quantities

of impurities, either of inorganic nature (ash) or of organic nature (albuminous substances). A *pure* toxine is, up to the present, as little known as a pure enzyme, and it is scarcely to be expected that it will be successfully prepared in the immediate future. Even in the case of preparations, which were still not pure although containing relatively very few impurities, BRIEGER and BOER obtained such minute quantities that further purification was out of the question. Moreover, attempts to prepare toxines on proteid-free culture media (USCHINSKY, *loc. cit.*) have given very unpromising results.

As regards the chemical nature of toxines there is, therefore, practically nothing known. Just as in the case of enzymes, to which they stand in such close relationship, they were at first regarded as *albuminous substances* and termed *toxalbumins*. The more thoroughly, however, the preparations were purified, the stronger became the idea that the albuminous substances, although very difficult of removal, were only impurities, and that the pure toxines themselves were in all probability *not* proteids in the ordinary sense. And BRIEGER himself, to whom we owe the conception of toxalbumins, succeeded in preparing toxine preparations which no longer gave the ordinary reactions of proteids (*cf. Tetanus Poison*) any more than did toxines produced in albumin-free culture media.

In the case of other toxines, too, successful attempts have been made to reduce, very considerably at least, the albuminous impurities.

JACOBY[1] succeeded by means of digestion with trypsin in obtaining preparations of ricine that were practically free from albuminous substances. The active principle itself is not attacked by this enzyme, whereas the albuminous substances present are decomposed by it. Since then these decomposition products, like trypsin itself, cannot be precipitated by ammonium sulphate, even in a 50 per cent. solution, whereas ricine is readily precipitated at that degree of concentration, it is possible by this means to obtain from the mixed products of the digestion preparations of ricine that no longer give the proteid reactions.

That is the only—negative—knowledge that we have of the constitution of toxines. With this exception we have to content ourselves with stating that they are bodies of high molecular weight, probably allied to the proteids with which they correspond in certain properties, and still more closely related to the ferments, whose constitution is equally a matter of hypothesis,

[1] Jacoby, "Die chemische Natur des Ricins," *Arch. exp. Path.*, xlvi., 98, 1901; *Hofm. Beitr.*, i., 51, 1901.

and to whose properties they offer the closest analogies in their reactions and activity.

These analogies stand out in the sharpest relief, when we compare the influence of external factors on bacterial toxines with their effect upon ferments. There is the closest correspondence in almost every particular.

A special characteristic of toxines is their extreme sensitiveness to the action of heat. In their normal solutions they soon perish at temperatures above 50° C.; at 80° C. their activity is at once destroyed, and even at 45° C. they are slowly decomposed. Individual ferments differ but little in this respect. On the other hand, they offer great resistance to the action of dry heat. Solid preparations can be heated to over 100° C. without suffering injury; but even these appear to be destroyed by a temperature of 150° C.

It is interesting to note that in liquids devoid of water, such as *amyl alcohol*, they can also be heated far above 80° C., and that many salts, such as, *e.g.*, anhydrous sodium sulphate, increase their resistance to the action of heat (Buchner [1]). Low temperatures check their activity, but do not otherwise injure them.

They dialyse to a slight extent through parchment, but not through *collodion* (Rodet and Guéchoff [2]), and through animal membranes, such as those of the œsophagus, bladder, large intestine, and especially the small intestine (Chassin and Moussu [3]).

In all these respects they behave exactly like enzymes.

Toxines are more sensitive than enzymes to the action of *light*. Diphtheria or tetanus poison in aqueous solution is very rapidly destroyed by both sunlight and diffused daylight (tetanus poison within forty-eight hours according to Kitasato [4]). Light is without action upon them when they are in the dry condition, or suspended in liquids devoid of water.

An *electric current* can also be injurious to toxines, though only when continuous, for alternating currents of high tension are quite harmless to tetanus poison (Marmier [5]). Even when merely allowed to stand in solution in the dark, with every precaution, the poisons gradually become weaker, being decomposed, at any rate, in the case of diphtheria and some other

[1] Buchner, "Bakteriengifte und Gegengifte," *Münch. med. Woch.*, 1893, 449.

[2] Rodet and Guéchoff, *Soc. Biol.*, lii., 965, 1900.

[3] Chassin and Moussu, "Influence de la dialyse, &c.," *ibid.*, lii., 694, 1900.

[4] Kitasato, "Exper. Unt. üb. d. Tet. Gift.," *Zeit. f. Hyg.*, x. 287, 1891.

[5] Marmier, "Les toxines et l'électricité," *Ann. Past.*, x., 469, 1896.

poisons, into toxoids (*vide infra*). In the case of other poisons the existence of toxoids has not been established with certainty.

All toxines are very sensitive to the influence of nearly all chemical agents.

Oxygen, even when as dilute as in the atmosphere, has a pronounced injurious effect. When exposed to the air, especially when light is also present, toxines speedily lose their virulence, notably so in the case of *tetanospasmine*, which is greatly weakened by simple filtration.

Speaking generally, all *oxidising agents*, including hydrogen peroxide, are very injurious.

Sieber[1] found that *calcium peroxide* completely destroyed the virulence of diphtheria and tetanus poison within a few hours (1,000 times the lethal dose), and also that of *abrine* (5,000 times the lethal dose being rendered inert by 0·5 gramme of calcium peroxide). He also discovered that the *oxydases* of animal tissues acted upon bacterial toxines, but not upon *abrine.* Moreover, on simultaneous injections of oxydase and toxine, the animal on which the experiment was made remained healthy. A vegetable oxydase (from the yam) also proved effective, although *peroxydases*, which only turn guaiacum blue when hydrogen peroxide is also present, were inert. An interesting statement made by him is that *fibrin* from the blood of a highly-immune horse contains an oxydase that destroys diphtheria virus, which is not the case with ordinary fibrin. But it is open to question whether this was not some remnant of unseparated antitoxine.

A little is also known with regard to the action of other chemical substances. Strong bases and acids naturally have a destructive effect, and weak bases are injurious, while very dilute acids, especially those of organic nature, have probably a stimulating influence. The influence of neutral salts and of various other substances, notably upon tetanus poison, has been studied by Fermi and Pernossi.[2] Some have a stimulating and others an injurious effect upon the toxic activity.

Indifferent gases, such as carbon dioxide, hydrogen, and carbon monoxide, have no influence. Only in the case of hydrogen sulphide did Brieger[3] observe any injurious effect upon tetanus

[1] Sieber, "Ueber die Entgiftung der Toxine durch die Superoxyde," *Z. physiol. Chem.*, xxxii., 573, 1901.

[2] Fermi and Pernossi, "Ueber das Tetanusgift," *Zeit. f. Hyg.*, xvi., 385, 1894.

[3] Brieger, "Weitere Erfahrungen über Bakteriengifte," *ibid.*, xix., 111, 1895.

poison after the toxine and gas had been kept in contact for four days in a sealed tube.

Protoplasmic poisons, such as carbolic acid, chloroform, &c., have no *materially* injurious effect. Alcohol is very injurious. According to SALKOWSKI,[1] salicylic aldehyde is extremely harmful, and he makes the same statement as to the action of chloroform and formalin.

Iodine and carbon bisulphide have probably a quite distinctive mode of action, inasmuch as they appear to attack only the toxophore group, and to tend to the formation of toxoids (EHRLICH[2]). Thymus extract has apparently a similar action (BRIEGER, KITASATO, and WASSERMANN.[3]

Fate of Toxines in the Organism.—Toxines disappear fairly rapidly after their introduction into the circulatory system of susceptible animals. In a short time the blood is completely *free from toxines*, as has been proved by the experiments of BOMSTEIN, CROLY, and BRUNNER on diphtheria (*q.v.*), the virus having entered into close combination somewhere or other in the latent stage of its activity, as has been shown by the researches of DÖNITZ[4] and others. DÖNITZ found that he could not save infected animals by the injection of antitoxines, even when only a few minutes had elapsed after the poisoning, since the toxine was no longer in a free condition when it encountered the antidote. Only by the injection of very large doses is it possible after a certain time to break up the combination of the toxine with the cells of the body, and so *destroy* the effects of the latent poisoning. Yet even then there is a time limit, and after its expiry, notably in the case of tetanus, even huge doses of antitoxine are no longer of any avail. Herein lies one of the causes of the defective therapeutical results in the serum treatment of tetanus. According to the latest views on tetanus, the antitoxine cannot follow the toxine in the nerve tracts. (For further particulars see *Tetanus*.)

A toxine escapes detection as such when small doses are injected into the body. Thus, if a single lethal dose, or a small multiple thereof, be injected into a susceptible animal, the poison rapidly disappears from the blood, nor can it then be detected in

[1] Salkowski, "Ueber die Wirkung der Antiseptica auf Toxine," *Berl. klin. Woch.*, 1898 (No. 25), 545.

[2] Ehrlich, "Die Wertbemessung des Diphtherieheilserums," *Klin. Jahrbuch*, vi.

[3] Brieger, Kitasato, and Wassermann, "Immunität u. Giftfestigung," *Zeit. f. Hyg.*, xii., 137, 1892.

[4] Dönitz, "Ueber des Tetanus-antitoxin," *Deutsche med. Woch.*, 1897, 428.

the organs of the animal.[1] The poison has then become firmly attached to the organs specifically susceptible to it. Moreover, it is *not* excreted with the urine (GOLDBERG[2]).

When, however, the doses are very large, the poison takes some time to disappear, and can then appear in the urine.[3] This, too, is perfectly explicable by the theory that the *receptors* are not adapted for such a sudden invasion of enormous quantities of poison, and hence a small proportion of the toxine breaks through the barrier of the kidneys and appears in the urine. The fact that the toxine disappears in the system furnished one of the supports for the conception of the so-called "*fermentation theory*" *of tetanus*. According to this, the true toxine first splits off by a secondary reaction within the organism another poison, upon which the anti-body can no longer act (whence tetanus is not curable *after* the poisoning [*vide supra*]), and this having no incubation period, acts with the rapidity of an alkaloid, such as *strychnine*. COURMONT[4] and others claim to have sometimes detected such a poison in the organs of the victims of tetanus. We will critically examine this theory in its proper place, and endeavour to show that it is at least superfluous. The disappearance of the toxines on the one hand, and the incurability on the other, can be readily explained by the side-chain theory without the aid of additional hypotheses.

But all this only applies to susceptible animals. The fate of toxines introduced into the circulatory system of *refractory*, naturally *immune*, animals is materially different.

The question of natural immunity has not yet been completely elucidated in all its details. It is, undoubtedly, an extraordinarily complex phenomenon, and, in particular, its forms of manifestation and its causes show essential differences as regards natural immunity against *poisons* on the one hand, and against living bacteria on the other. In the case of toxines, we have only to deal with natural *antitoxic* immunity.

This can be due *a priori* to two causes. Either the body of the naturally-immune animal contains *antidotes* which neutralise the action of the intruding poison, or the *cells* of the animal are

[1] Salter's assertion (*Lancet*, 1898, i., 152) that toxines pass into the sweat is not supported by sufficient evidence.

[2] Goldberg, "Ueber Ausscheidung des Tetanusgiftes durch die Nierensekretion," *Centralbl. f. Bakt.*, xxvi., 547, 1899. But *cf.* Cobbett ("Excretion of Diphtheria Toxine in the Urine," *Brit. Med. Journ.*, 1900, i., 21), who claims to have detected the toxine in the urine.

[3] See in particular Brunner, "Z. Kenntnis d. Tetanusgiftes," *Z. f. klin. Med.*, xxxi., 367, 1897.

[4] *Cf.* v. Leyden-Blumenthal, *Der Tetanus*, Vienna, 1901.

immune against the attack of the poison, which is thus for them a completely indifferent substance.

Both cases occur. As we shall see later, normal sera, and especially horse serum, contain antitoxines which afford protection against small doses of toxines. And it is particularly interesting that, according to WASSERMANN,[1] from 80 to 85 per cent. of the human race have appreciable amounts of a diphtheria antitoxine virus in their serum. These facts, however, are insufficient in themselves to explain the origin of natural antitoxic immunity, for such antitoxines are found exclusively in the sera of susceptible living organisms. On the other hand, *the normal sera of refractory animals contain absolutely no trace of antitoxines.*

It was difficult to account for this fact until EHRLICH succeeded, by means of his side-chain theory, not only in explaining it, but in making it one of the chief arguments in support of his view. Where there are no corresponding receptors (receptive groups in the body cells), there can be no attack on the part of a toxine, and the result is immunity against the poison. But in such cases the *splitting off of side-chains to form an antitoxine* is equally out of the question. Hence, according to this view, it is *impossible* for the blood of *absolutely* refractory animals to contain antitoxines.

But it is interesting to know what happens to the toxines introduced into the circulatory system of such organisms. It is quite conceivable that such unstable and extraordinarily sensitive substances might be rapidly decomposed in the blood without having produced their injurious effects, or that they might be very rapidly expelled from the body with the excretions.

Neither is the case. It is a remarkable phenomenon that these extremely active substances, which under favourable conditions can produce results of astounding intensity, should, when introduced into the blood of refractory animals, behave like the most harmless inert chemical substances remaining unaltered for a relatively long period, until finally they are slowly drawn into the metabolic changes, and gradually undergo complete oxidation.

Hence it follows that, in the case of these animals, the *mutual attraction between the poison and body cell* must be much smaller than in the case of susceptible animals. There is no absolute difference, however, between susceptible and refractory animals,

[1] Wassermann, "Ueber die persönliche Disposition u. Prophylaxe gegen Diphtherie," *Zeit. f. Hyg.*, xix., 408, 1895.

but only a relative one. The attractive power of the body cells (*receptors*) shows a gradual decrease from the most susceptible to the least susceptible animal. Thus tetanus poison, in a dose many hundred times greater than is sufficient to kill a mouse, circulates unchanged through the blood of a pigeon. If, however, still larger doses are given, the pigeon becomes ill. In this case then there is not complete immunity, but only a very slight power of attraction on the part of the receptors. According to METSCHNIKOFF,[1] and FERMI and PERNOSSI (*loc. cit.*), certain cold-blooded animals have still smaller powers of attraction.

METSCHNIKOFF found that in the case of *fishes*, *tortoises*, and *alligators*, and also of *arthropoda*, the toxine remained unaltered in the blood without producing antitoxine. Only in the case of alligators, after longer action (fifty-eight days), did he obtain any antitoxine, while he was able to bring about this formation of antitoxine in old *crocodiles* by keeping the reptiles at a temperature of 30° C.

Even then, however, he could not observe symptoms of illness in the animals. Similar results were obtained by FERMI and PERNOSSI in experiments on snakes, tritons, and turtle doves.

Moreover, METSCHNIKOFF detected active tetanus toxine after the lapse of a month in the livers of scorpions, in which there had been neither symptoms of poisoning nor any formation of antitoxine.

The *hen* has been a particularly favourite subject for experiments with tetanus poison, because although offering extreme resistance she is not completely immune against it. METSCHNIKOFF asserts that the toxine can be recovered from the *blood* and the *ovaries*, and that eventually slight traces of antitoxine appear. ASAKAWA[2] found that the toxine introduced into hens' blood remained almost unaltered until the seventh day, and then slowly disappeared without being excreted.

ASAKAWA could not detect any toxine in the brain or spinal cord of the hen, although he found it in all the other tissues. This may, of course, be due to the presence of blood in these tissues leading to some erroneous conclusion as to presence of toxine, while there is but little blood in the central nervous system. But, on the other hand, it is also very probable that slight traces of toxines disappear there by combining with

[1] Metschnikoff, "Influence de l'organisme sur les toxines," *Ann. Past.*, xi., 801, 1897; xii., 81, 1898. Also, *Immunität*, Germ. translation by Meyer, p. 264. Jena, 1902.

[2] Asakawa, "Die Basis der natürl. Immun. des Huhnes gegen Tetanus," *Centralbl. f. Bakt.*, xxiv., 166, 1898.

isolated receptors. For the hen is not absolutely proof against tetanus, and it is also possible to demonstrate a slight antitoxic activity in the brain, &c., of that bird. Corroborative evidence of this is afforded by the fact that direct intercerebral injection of tetanus poison produces the symptoms of tetanus in the hen.

Hence, according to the views of EHRLICH and WASSERMANN, the *defective attraction of the toxine for the body cell (receptor) is the main cause of natural antitoxic immunity.* If the poison circulates in the free state and does not combine with the receptors, or does so only to an insignificant extent, the toxophore group is unable to act energetically, and so no serious injury is done.

Defective attraction, however, is not invariably the cause of natural immunity. Thus, MORGENROTH [1] found that in the case of the *frog* tetanus poison enters into firm combination even in the cold, without making the animal ill. Under these conditions the toxophore group is inactive; but it acts immediately when the frog is warmed to about 30° C.

These theories of the attraction of the poison for the living cell and their specific combination are supported by experimental proofs. WASSERMANN [2] found that fresh substance from the central nervous system of *susceptible* animals combined with considerable quantities of tetanus poison. This was in agreement with the results of METSCHNIKOFF and ASAKAWA, who found that, in the case of less susceptible animals, the smaller the degree of susceptibility the less, too, was the amount of combination in the brain, &c. Thus, the brain of the hen only enters into feeble combination with the toxine, and that of the tortoise not at all. An additional support of this theory is furnished by experiments which show that, in the case of those animals (*e.g.*, *rabbits*) in which the tetanus poison combines *intra vitam* with receptors which are *not* attached to the cells of the central nervous system, emulsions of other organs—*e.g.*, the *spleen*—also combine with the tetanus poison (WASSERMANN).

It is not a general rule that receptors only occur in the particular organs in which the poison produces its injurious effects. It frequently happens that combination and formation of antitoxine take place in other organs where the toxine does little injury. The action of the poison and formation of the

[1] Morgenroth, "Zur Kenntnis des Tetanus des Frosches," *Arch. Internat. d. Pharmacodyn.*, vii., 265, 1900.

[2] Wassermann and Takaki, "Ueber tetanusantitoxische Eigenschaften des Centralnervenssystems, *Berl. klin. Woch.*, 1898, 5; Wassermann, "Weitere Mitt. über Seitenkettenimmunität," *ibid.*, 1898, 209.

antitoxine are two distinct processes which may, under certain conditions, follow widely different courses.

Fate of Toxines in the Digestive Tract.—The question of what becomes of toxines when they are introduced into the stomach or intestinal canal has a special interest. It is the unanimous opinion of observers that all toxines, including snake poisons, &c., with the single exception of *ricine*, are absolutely without action upon the system from the stomach. And it has been shown by GIBIER[1] that these toxines do not act by way of the anus.

CHARRIN and CASSIN[2] assert that absorption of toxines through the intestine takes place when the mucous membrane is injured.

It was proved by NENCKI and SCHOUMOW-SIMANOWSKI[3] that even large doses were not absorbed in the least through the digestive tract, and that only when huge doses, more than 100,000 times the lethal amount, were given, did symptoms of poisoning finally appear.

These results conclusively show that toxines are *not* absorbed into the system through the normal intestinal tract. They must therefore either pass unchanged into the excreta, or they must be completely destroyed. RANSOM[4] concluded that the first alternative happened in the case of tetanus poison, but later workers, notably NENCKI and SCHOUMOW-SIMANOWSKI (*loc. cit.*) and CARRIÈRE,[5] were unable to detect any trace of toxine in the excreta, even when doses as large as 100,000 times the lethal amount were given; whilst CARRIÈRE was also unable to discover any antitoxic function in the serum after the introduction of toxine *per os*. Although RÉPIN[6] identified *abrine* in the fæces he was unable to detect either *diphtheria poison* or *cobra venom*.

Hence, toxines are *destroyed;* and in this process three factors must be taken into account—viz., the *living intestinal wall*, the

[1] Gibier, "Effets produits par les toxines, &c., injectées dans le rectum," *Sem. Med.*, 1896, 202 (abst.).

[2] Charrin and Cassin, "Fonctions protectrices actives de la muqueuse intestinale," *ibid.*, 1895, 545.

[3] Nencki and Schoumow-Simanowski, "Die Entgiftung der Toxine durch die Verdauungssäfte," *Centralbl. f. Bakt.*, xxiii., 840, 1898.

[4] Ransom, "Das Schicksal des Tet. Giftes nach seiner intestinal Einverleibung," *Deutsch. med. Woch.*, 1898, 117.

[5] Carrière, "Toxines et digestion," *Ann. Past.*, xiii., 435, 1899 (gives Bibliography); also, "Du sort de la toxine tetanique introduit dans le tube digestif," *Soc. Biol.*, li., 179, 1899.

[6] Répin, "Sur l'absorption de l'abrine par les muqueuses," *Ann. Past.*, ix., 517, 1895.

intestinal bacteria (FERMI and PERNOSSI), and the *secretions of the intestine.*

From the concordant results of experiments made by NENCKI (*loc. cit.*) on PAWLOW's dogs and of those by CARRIÈRE (*loc. cit.*) with preparations of enzymes, it is beyond question the *digestive ferments* that render toxines innocuous. Thus, CARRIÈRE (*loc. cit.*) found that even the *saliva diastase* had an injurious influence, that *pepsin* was not very active, but that *trypsin*, and, above all, the *bile*, had the greatest effect. NENCKI obtained the following results in experiments with pure sterilised fluid from fistulas :— *Pepsin*, by itself, destroyed bacterial poisons (but not *abrine*). The presence of acid was not essential, since the same result was obtained after nearly complete neutralisation, as had also been stated previously by CHARRIN.[1] *Pancreatic juice*, by itself, had a greater destructive effect upon diphtheria toxine than upon tetanus toxine, the latter being particularly sensitive to the action of a mixture of 3 parts of pancreatic juice and 1 part of bile.

Attempts to produce immunisation by the simultaneous injection of bile with the toxine were unsuccessful. CHARRIN and LEVADITI[2] injected diphtheria poison (100 times the lethal dose) into freshly extirpated pancreas, and found that it was completely destroyed after twenty-two hours. Muscle plasma or pancreas heated to 70° C. were without action. According to CARRIÈRE, no destructive effect is to be attributed to the mucous membrane of the intestine or the intestinal bacteria.

BALDWIN and LEVENE[3] also proved that pepsin, trypsin, and papayotin destroyed diphtheria toxine.

Notwithstanding this, CANO-BRUSSO[4] still asserts that the destruction of the tetanus poison in the intestine is to be attributed to the action of the mucous membrane itself.

Mode of Action of Toxines.—Toxines, as shown above, do not act through the digestive tract, and it is necessary to introduce them by other means into the organism. The most convenient method is *subcutaneous injection*, just as in cases of poisoning by living bacteria.

[1] Charrin, "Action des sucs digestifs sur les poisons microbiens," *Arch. de Phys.*, 1898, 67. Charrin and Lefèvre, "Action de la pepsine sur la toxine diphth.," *Soc. Biol.* xlix., 830, 1897 ; *Sem. Med.*, 1897, 296.

[2] Charrin and Levaditi, "Action de pancréas sur la toxine diphth.," *Soc. Biol.*, li., 215, 1899.

[3] Baldwin and Levene, "Action of proteolytic ferments on bacterial toxines," *J. Med. Research*, vi., 120 ; *Malys Jb.*, 1901, 953.

[4] Cano-Brusso, "Untergang d. Tetanusgiftes im Darm," *Malys Jb.*, 1901, 914.

Direct introduction into the circulatory system (intravenous) is still more effective than intraperitoneal, intercerebral, or subdural inoculation, as has been used in the case of tetanus and gonococci poisons, or yet HOMÉN's method of injection into the nerves, which is sometimes employed.

Intercerebral injection is of special importance in cases where either the poison when distributed throughout the body is seized by the receptors or other less susceptible organs (tetanus in the rabbit), or where the receptors in the brain are not very numerous, so that only concentrated toxine can effect any serious injury (tetanus in the hen).

As regards the activity of toxines, there are two factors of fundamental importance—their *specific character*, and the *time of incubation.*

The specific character is one of the most prominent characteristics of true toxines. Although more or less thorough resistance to the action of crystalloid poisons is known, cantharidine, for instance, being relatively innocuous to the hedgehog and atropine to pigeons; yet in these cases there is only a weakening of the activity of the poison, and not an absolute resistance. Certain bacterial poisons, however, are completely harmless to refractory animals, while they act with the greatest energy upon susceptible animals.

But the most important point in this connection is that, in the case of refractory animals, the toxine is by no means destroyed, but that it circulates unchanged in their blood as a completely inert substance.

Thus arises the paradoxical phenomenon which we have described at some length above, that it is possible to kill a mouse with tetanus, by means of the blood of an apparently healthy hen which has previously been inoculated with large doses of tetanus poison. When the toxine cannot find corresponding receptors it is unable to make its attack. The toxophore group remains inactive, with the result that the toxine is a completely inert substance which the body regards as of so little importance that it does not even make speedy attempts to destroy it. This phenomenon, too, can readily be explained by the side-chain theory. According to EHRLICH, all nutritive substances, so far as they are not merely chemically changed by the secretions and their enzymes, enter into combination as haptines, and are thus brought within the power of the destructive and assimilative forces of the protoplasm. But, since the toxine does not enter into such combination, it also is not destroyed—not even treated in the same way as nutritive substances.

The specific nature of true toxines completely corresponds with that of the living bacteria.

It is also characteristic of most of the known toxines that, unlike most simple crystalloid poisons, they do not exert their activity instantaneously or after a very short time, but that their toxic effects only appear after a definite time—the *period of incubation.* In this respect, too, bacterial toxines behave exactly like their parent living cells. The period of incubation varies, not only with the nature of the toxine, but is also dependent on other factors, such as the amount of the dose, the body temperature, &c. There is a limit, however, to this dependency, particularly as regards the amount of dose. The period of incubation is not in any way inversely proportional to the quantity of toxine. For example, according to COURMONT and DOYON,[1] the period of incubation for guinea-pigs after injection of a lethal dose of diphtheria toxine is fifteen hours, and this cannot be reduced below twelve hours even by the introduction of enormous doses (90,000 times the lethal amount). A very interesting phenomenon in connection with this point is that in tetanus of the frog the incubation period can be delayed to any desired extent, by keeping the temperature low. Thus, if a frog into which the poison has been injected be kept at 8° to 10° C., it remains healthy, but at 30° C. tetanus begins after a definite time, and the frog dies. If, again, the warming be interrupted, the frog can be kept at 8° C. as long as desired, but after completion of the *remainder* of the incubation period at a higher temperature, tetanus symptoms appear (MORGENROTH).

In the case of some other toxines, however, notably *snake venom*, there is not this incubation period. These act with extreme rapidity.

KRAUS[2] found a true antitoxine-producing toxine in the filtrates of cultivations of Naskin's vibrio, which resembles the cholera vibrio. It killed rabbits without an incubation period when intravenously injected in doses of 0·5 to 1 c.c. There was an incubation period after subcutaneous injection.

Constitution of Toxines.—The characteristic of every toxine is that it is a haptine—in other words, that it contains a haptophore and a toxophore group.

Taking their constitution as thus fixed, most bacterial toxines

[1] Quoted by Deutsch and Feistmantel, *Die Impfstoffe und Sera*, Leipzig, 1903, 40.

[2] Kraus, "Ueb. ein acut. Wirkendes Bakterientoxin," *Centralbl. f. Bakt.*, xxxiv., 488, 1904.

are, according to EHRLICH'S terminology, *simple haptines of the first class.* Other toxines, however, have a more complicated structure. Thus, *ricine* and *abrine* possess, in addition to their toxic properties, the further characteristic of agglutinating the red corpuscles of the blood.

The question now arises whether this second property is to be ascribed to some peculiar active principle invariably present in preparations of ricine, or whether the ricine itself is endowed with this double power. One of the proofs that the toxic and agglutinating properties are separable was afforded by the fact, that digestion with pepsin-hydrochloric acid soon destroyed the toxicity, but did not affect the power of agglutination (JACOBY,[1] MICHAELIS and OPPENHEIMER[2]). It was thus possible that ricinus toxine and ricinus agglutinine might be quite distinct substances, analogous to tetanospasmine and tetanolysine, the two separate poisons of tetanus cultivations.

While, however, it could be proved that these were separate substances, which occurred in quite different proportions in different cultivations, and *produced two distinct anti-bodies*, similar experiments with ricine gave opposite results. The toxine and the agglutinine possess the same haptophore group, since they produce only *one antiricine* (JACOBY).

The structure of ricine, therefore, is complex. In additon to its haptophore group it possesses *two* ergophore groups, with toxic and agglutinating powers respectively; and it is thus a complex haptine of the first class. (For further particulars see *Ricine.*)

In the case of other toxines the constitution is still more complex and uncertain. Here, too, other activities (*e.g.*, hæmolytic), in addition to the toxic function, are frequently met with, notably in the case of eel's blood and snake poisons. In such instances, it still remains to be determined with certainty whether the disintegrating principles are *distinct substances.* This is probably the case, however, with snake poison, for example. And, if so, the further question arises whether the *lysines*, like the toxines, are haptines of the first class—*i.e.*, contain the haptophore and ergophore groups in *one* complex combination; or whether they do not rather approximate in constitution to the haptines of the second class, such as the hæmolysines of normal and immune sera. The latter contain

[1] Jacoby, "Ueber Ricinimmunität," *Hofm. Beitr. z. Chem. Physiol.*, i., 57, 1901.

[2] Michaelis and Oppenheimer, "Ueber Immunität geg. Eiweisskörper," *Engelmann's Arch.*, 1902, Suppl. H.

haptophore and ergophore groups which are *not* united in one complex grouping; but they have an *amboceptor* with two haptophore groups and a *complement*, which attaches itself to the *complementophile* group of the amboceptor after the latter has combined with the receptor of the cells, and thus brings about the hæmolytic action.

The latest results obtained with cobra hæmolysine lead to the conclusion that this poison actually has some such constitution —that it possesses an amboceptor which is seized upon and brought into action, not only by an *endo-complement* that occurs in the red blood-corpuscles, but also by *lecithin* (KYES and SACHS). We shall return to this in discussing snake poison.

Physiological Action of Toxines.—Although the primary characteristic of each of the bacterial toxines is its specific action upon the organism, as will be described more fully in the special part, they have also certain reactions in common, of which a brief mention should be made. They act primarily upon the general health, producing weakness, prostration, and eventually collapse. These phenomena are undoubtedly due, in the main, to a deleterious effect of the toxine on the *heart's action*, this effect being manifested in a reduction in the pressure of the blood, frequently accompanied by a diminution in the rate of pulsation and eventually by paralysis of the heart. According to BARDIER'S[1] comparative experiments, however, individual toxines differ in their action upon the heart.

Hypothermia, with or without preceding *fever*, is an almost invariable symptom. When injected into the *skin* and the subcutaneous tissue toxines frequently give rise to infiltrations, abscesses, and necroses. Loss of *hair* is frequently observed.

Internal organs, too—*e.g.*, the *intestinal tract*—are usually injured (*diarrhœa*, &c.). Nor does the *liver* remain unaffected, as has been demonstrated, notably by TEISSIER and GUINARD.[2] CLAUDE[3] has observed bleeding of the gall-bladder. According to PADOA,[4] the liver acts differently upon different toxines. Thus, while it combines with and lessens the virulence of the diphtheria toxine when introduced into the mesenteric vein, it allows typhus toxine to pass, which acquires a higher virulence

[1] Bardier, "Toxine et Cœur," *Soc. Biol.*, xlix., 311, 1897.

[2] Teissier and Guinard, "Effets des toxines microbiennes," *Arch. Méd. Expér.*, ix., 994, 1897.

[3] Claude, "Deux cas d'hémorrhagie de la vésic. biliaire, &c.," *Soc. Biol.*, 1896, 169; *Sem. Méd.*, 1896, 62.

[4] Padoa, "Ueber d. verschied. Wirkung. des Diphtherie- u. Typhustoxins," *Riform. Med.*, 1899, No. 26; *Malys Jb.*, 1899, 921.

when thus introduced into the system than when injected subcutaneously or into the veins. Degenerative changes also take place in the *kidneys*.

Alterations of the *blood* (*hæmoglobinæmia*) and vascular system are, in like manner, of frequent occurrence. Many toxines appear to have a solvent action upon the blood-corpuscles. According to KARFUNKEL,[1] they reduce the alkalinity of the blood, but this effect can be partially prevented by the application of artificial heat. Lastly, the *nervous system* and, in particular, its central organ are usually attacked, but in very different ways; this will be dealt with more fully in the special part.

CONSIGLIO[2] states that he has observed a peculiar characteristic of diphtheria toxine. He has found that in small doses it stimulates the process of fermentation, but in larger doses checks it; whilst, on the other hand, it has invariably a very injurious influence on the germination process of seeds.

Toxoids and Toxones.—According to the side-chain theory, we must regard toxines as bodies that possess two distinct stereo groups—the *haptophore* group and the *toxophore* group. Assuming, now, that under certain conditions the toxophore group may be so changed as to lose its characteristic activity while the haptophore group remains unaltered, new substances will be formed which still have the power of attaching themselves to receptors whether free (antitoxines) or combined (body cells), but without being poisonous. EHRLICH has investigated substances of this kind in the case of *diphtheria toxine*, and has established their great influence upon the toxicity of solutions of poisons and in the preparation of curative sera, with which we shall deal at length in the next chapter. These "*toxoids*," then, are *non-poisonous*, but are still *haptones* with specific powers of combination. When they are secondary decomposition products of true toxines they are termed "toxoids," in the narrower sense of the word; but there are also *primary* bacterial products, which are able to seize upon the same haptophore group as the toxine, but which have a different and much weaker toxophore group, capable of producing slight characteristic effects *of its own*, as has been demonstrated by EHRLICH and MADSEN in the case of diphtheria. These primary substances, which thus represent a *second secretion product* of the bacteria, are known as "*toxones*."

[1] Karfunkel, "Schwankungen des Blutalkaleszenzgehaltes nach Einverleibung von Toxinen, &c.," *Zeit. f. Hyg.*, xxxii., 149, 1899.

[2] Consiglio, "Azione di alcune tossine, &c.," *Arch. di Farm.*, vi., No. 3, 1898; *Malys Jb.*, 1898, 634.

Bruck[1] has shown that toxoids of tetanus poison which have become *quite* non-poisonous no longer induce the formation of antitoxine. According to his views, when side-chains are broken off there must be present in addition to the haptophore group a certain "combining ring" derived from the toxophore group.

Toxones are *not* absolutely innocuous, but eventually produce temporary symptoms of poisoning (*paralysis*, &c.), which, however, are absolutely different *in kind* from the effects of small doses of toxines.

Toxoids have been identified with certainty in the case of *diphtheria* (Ehrlich), of *tetanolysine* (*q.v.*) (Madsen) and of *staphylotoxine* (Neisser and Wechsberg, *q.v.*), and Jacoby (*loc. cit.*) has shown that in all probability they accompany *ricine*.

There is also, however, a considerable weight of evidence that some at least of the other bacterial poisons have the power of producing secondary toxoids, in the case of tetanus, for example, to which we shall return in the special part.

Van Calcar[2] succeeded in separating the toxones from the toxine by taking advantage of the fact that animal membranes stretched to a certain extent allow the toxine to pass, but not the toxones. Hence, toxones have a greater molecular volume than toxines. When the membrane was stretched to a greater extent the toxones also diffused through it.

This is the extent of our knowledge of toxoids and toxones. Since they, too, are specific haptines, they also produce antitoxines as Madsen and Dreyer[3] succeeded in demonstrating in the case of diphtheria toxones.

Madsen and Dreyer were also able to prove the existence of toxones which were poisonous to rabbits, but without action upon guinea-pigs. A poison that was without action upon guinea-pigs after neutralisation in the ratio of 200 : 200, did not become innocuous to rabbits until the ratio of the neutralising antitoxine reached 240 : 200. Ehrlich has given the name "*toxonoids*" to this variety of poison.

On Antitoxines in General.—Our definite knowledge of antitoxines is even smaller than our knowledge of toxines.

They occur in the fluids of the body, notably in the blood serum and milk of immunised animals. Slight traces are also

[1] Bruck, "Beitr. z. Theorie d. Immunität," *Zeit. f. Hyg.*, xlvi., 176, 1904.

[2] Van Calcar, "Ueb. die Constit. des Diphtheriegiftes," *Berl. klin. Woch.*, 1904, No. 39.

[3] Madsen and Dreyer, "Ueber Immun. mit den Toxonen d. Diphtheriegiftes, *Zeit. f. Hyg.*, xxxvii., 249, 1901.

of frequent occurrence in normal sera, as, for instance, *diphtheria antitoxine* in the serum of horses (in the case of about 30 per cent.). Other anti-bodies are also very frequently present in normal sera, notably anti-ferments, &c. They can be separated from the fluids of the body by precipitation methods similar to those employed for the concentration of toxines.

Blum[1] found that lymph glands that had undergone auto-digestion were antitoxic to tetanus poison, but not to diphtheria virus or cobra venom.

Precipitation with alum and ammonia (Aronson), with magnesium sulphate (Tizzoni), and with solid sodium or magnesium chlorides (Brieger and Boer), followed by a method of purification, yield dry preparations of antitoxines.

The nature of antitoxines is unknown. They are probably albuminous substances, but this has not been definitely proved. The fact that they offer considerable resistance to the action of trypsin is against this view, although they are very sensitive to the action of pepsin-hydrochloric acid. Experiments to obtain further proof that diphtheria is an albuminous substance, or to determine to which albuminous constituent of the blood it is related, have not as yet led to any conclusive result.

Antitoxines, like toxines, are sensitive to the action of heat, acids, &c., although, in general, they offer far greater resistance (*see Diphtheria*). Camus[2] found that anti-snake venom and anti-diphtheria poison could be heated for thirty minutes at 120° C. and for fifteen minutes at 140° C. without injury, provided they had been dried at a lower temperature, and then heated at 100° C. in a current of air.

According to Ehrlich antitoxines are *normal* constituents of the cells—broken-off receptors—and as such possess relatively little physiological or chemical activity. They are not invariably produced in the organs where the poison exerts its specific action, but are also developed in other groups of cells. This appears to be especially the case in tetanus of the rabbit (*q.v.*). They are *secreted* under the stimulus of the haptophore group. Ehrlich regards them as "*simple uniceptors*"—*i.e.*, as substances with only *one* haptophore group, which coincides with the corresponding group of the toxine. Hence, nothing is more erroneous than to ascribe to antitoxines an activity similar to that of the toxines—

[1] Blum, "Ueb. Antitoxinbildung bei der Autolyse," *Hofmeister's Beiträge*, v., 142, 1904.

[2] Camus, "Resistance aux tempér. élevées des vaccins dessêchés," *Soc. Biol.*, l., 235, 1898.

to represent them as "*substances akin to ferments.*" There is no support for the unthinking transference of this notion from the toxines, and its only result would be to weaken the conception that *toxines* are closely related to ferments. Toxines, and probably also some ferments, have a haptophore and at least one "*ergophore*" group. They can not only combine, but also attack. Antitoxines do not possess the latter characteristic; they can only combine, and so *ward off* the toxophore group from the threatened cell, but not injure it. WASSERMANN'S experiments on *pyocyaneus* poison and CALMETTE'S on snake poison prove that the toxine remains intact in a mixture of toxine and antitoxine, and that after destruction of the antitoxine the toxine can again manifest its activity (*vide infra*). Antitoxines, therefore, are not active substances, not "ferments." Antitoxines, as such, are physiologically completely inert, and are unable to produce any toxic effects.

This, of course, only holds good for antitoxines by *themselves* and not for the *sera* in which they are present. It is true that unlimited quantities of *horse serum* containing antitoxine can be injected into a *horse* without producing any by-reactions. But, on the other hand, albuminous substances foreign to the body are in a certain sense invariably *poisonous.* They, too, give rise to protective substances—the *precipitines* described by MYERS and others.[1] It is thus evident that unlimited quantities of horse serum cannot be injected into animals of different species. In fact, disturbances due to this cause have frequently been observed in the therapeutic use of diphtheria serum (*q.v.*). In such cases, however, the disturbances must be attributed to the serum rather than to the *antitoxine* itself.

There is, doubtless, a connection between the struggle of the organism to eliminate foreign albuminous matters and KNORR'S[2] observation that antitoxines injected with sera foreign to the body speedily disappear, whilst antitoxines introduced in the sera of the same species remain for a very long time in the organism.

Behaviour of Toxines towards Antitoxines.—We have already stated in the introduction that an essential characteristic in the definition of a toxine is that true toxines produce an antidote, an *antitoxine* in the body of the attacked organism. This fact,

[1] For further particulars and bibliography of precipitines *cf.* Michaelis and Oppenheimer, "Ueber Immunität geg. Eiweisskörper," *Engelmann's Arch.*, 1902, Suppl. H.

[2] Knorr, "Das Tetanusgift u. s. Bezieh. zum tier. Organismus," *Münch. med. Woch.*, 1898, 321, 362.

which was first discovered by EHRLICH[1] in his fundamental experiments with *ricine*, a vegetable poison closely akin to the bacterial toxines, has now become so firmly established that we even have to regard the formation of antitoxine as a radical property of the true toxine. It is not possible in this place to deal with the significance that this formation of antitoxine in the organism has for the disappearance of infectious diseases, and for the development of the state of acquired immunity, or with the way in which these phenomena have been used as supports of the monumental side-chain theory.

Here it is only necessary to describe the experimentally-determined relation between the *toxine* and its *antitoxine* as exactly as is possible in the present state of our knowledge, which, for the most part, we owe to the unwearying classical researches of EHRLICH.

According to the side-chain theory, the only poisons that can act as true toxines are those that possess a specific affinity for the definite cells. For the representation of this specific affinity, EHRLICH assumes that both sides, the toxine on the one hand, and the attacked cell on the other, each have in their protoplasm an atomic group which reciprocally coincide, and thus enter into combination, and bring the toxine within the immediate reach of the cells. *The first step in the action of the toxine is thus a concentration of the poison upon the cell by means of the reciprocal "haptophore" groups.* The cell is now through this concentration brought within the sphere of action of the toxine, and then follows as *the second phase the specific action of the poison upon the cell*—a function of the second specific the "*toxophore*" group.[2]

Toxines thus combine with the haptophore groups of cells, which are active as regards their "*side-chains.*" If now, as in artificial immunisation, such side-chains provided with haptophore groups are produced in excess, and separated in a free state in the fluids of the body, especially the blood serum, these haptophore groups retain their capacity of entering into combination with the corresponding haptophore groups of the toxine. Hence these broken-off side-chains represent *the specific antitoxine to the toxine.*

[1] Ehrlich, "Experimentelle Unters. über Immunitat," *Deutsch. med. Woch.*, 976, 1218, 1891; also "Zur Kenntnis der Antitoxinwirkung," *Fortsch. d. Med.*, 1897, 41.

[2] The theory only speaks of atomic groupings in a substance, and Ehrlich has never asserted that a toxine consists of two *substances*—a haptophore and a toxophore substance—as Danysz (*Ann. Past.*, 1899, 581) credits him with stating. Danysz, who confuses the process of plasmatolysis with toxine activity, has misunderstood Ehrlich.

This conception at once gives us two highly important points of view regarding the reciprocal relationship of the toxine to the antitoxine.

Its acceptance immediately renders untenable two possible modes by which the poison might be influenced by its specific anti-body—viz., by direct *destruction* of the poisonous substance in its entirety, as it might, for instance, be destroyed by, say, a strong acid ; and secondly, by the action of the antitoxine on the specifically injurious *toxophore* group of the poison, just as, for instance, the poisonous property of aniline is considerably reduced by the introduction of acetic acid into its toxic amino-group. Neither is reconcilable with the side-chain theory, for there can only be any influence in the sense that the antitoxine *saturates* the haptophore group of the toxine, and thus prevents the possibility of its bringing its toxophore group into action by seizing upon the cell, while in reality its toxic force *remains unchanged.*

While we have deduced this fundamental point of view as a consequence of the side-chain theory accepted by us as a *heuristic* principle, the actual development of the theory has naturally proceeded in a converse manner. Tedious experiments were first made to establish the correctness of these *facts* in order to use them as important supports of the *theory.* EHRLICH and BEHRING were at first of opinion that the *poisonous property* of the toxine was affected by the antitoxine, but subsequently came to the conclusion that it was a question of simple *combination.*

The facts that led to this now generally accepted conclusion were of different kinds.

At first it was the general view that the action of an antitoxine was only an indirect one, its function being to render the organism "proof" against the toxine.

This opinion was afterwards abandoned when it was found that toxine and antitoxine combined in accordance with definite arithmetical laws (law of multiples), with which we shall deal more fully later on. Then results of the greatest importance were obtained by a closer study of processes in which the intervention of the living organism could be absolutely excluded, and in which plainly visible reactions in the *test tube* were used as indicators of the influence of the antitoxine upon the toxine.

The earliest of these were EHRLICH'S[1] celebrated experiments upon the agglutinating action of *ricine* upon the red corpuscles of the blood, the results of which showed that there were fixed numerical relationships between ricine and antiricine, inasmuch

[1] Ehrlich, "Zur Kenntn. d. Antitoxinwirkg.," *Fortschr. d. Med.*, 1897, 41.

as that the action of definite doses of ricine upon the blood was invariably exactly neutralised by corresponding doses of anti-ricine. The action of other hæmolysines, such as snake poison (KANTHACK), *eel's blood* (KOSSEL), and tetanolysine (EHRLICH). &c., can be counteracted by similar means.

Direct relationships between toxine and antitoxine were thus established. But these might still depend upon a direct *destruction* of the poison by the antidote. The facts, however, showed that this was not the case, but that there was a simple combination of the two constituents.

In particular, the fact that it is possible, when this compound has only been formed a short time, to break it up in such a way that *the original toxic activity is restored*, has afforded the strongest support to the theory that there is a loose form of combination. CALMETTE was the first to demonstrate this fact with certainty in the case of the *animal* toxine, *snake poison*, the antitoxine of which is much more unstable than the toxine.

It has also been found by WASSERMANN that the antitoxine of *B. pyocyaneus* (*q.v.*) is much more readily destroyed than the toxine, so that we are justified in assuming that there is also a simple combination in the case of bacterial toxines.

The gist of these experiments was that a neutral mixture of toxine with antitoxine required a large proportion of the original toxic capacity on being heated, owing to the fact that the antitoxine, being the more readily attacked constituent of the loose combination, was destroyed by the higher temperature. The two components, however, must only have been mixed a short time, since otherwise it was no longer possible to separate the compound. A process of *diffusion* can also be used to separate the two components in the case of snake poison, the toxine of which is much more diffusible than the antitoxine (MARTIN and CHERRY) (*cf. Snake Poison*).

On the other hand, similar experiments with *diphtheria poison* have proved unsuccessful (DZIERZGOWSKI[1]). Now, here the conditions are quite different. In the first place, as EHRLICH has shown, the combination is *exceedingly stable*. But, apart from that, the *toxine* in this case is the more destructible, so that on heating the mixture it is not the toxine but the free *antitoxine* that ought to be regenerated. The reason why this does not happen is manifest *a priori;* for in the change that takes place in the toxine on heating the poison does not disappear, but is only trans-

[1] Dzierzgowski, "Zur Frage über die Beziehungen zwischen dem antidiphth. Heilserum u. d. Diphtherietoxin," *Arch. Internat. de Pharmacodyn.*, v., 1, 1898. *Cf.* Marenghi, "Ueber d. gegens. Wirkg. antidiphth. Serums und des Diphth.-Toxins," *Centralbl. f. Bakt.*, xxii., 520, 1897.

formed into *toxoids*, whilst, the combination not being broken up, no free antitoxine can be detected. Thus, the negative results of these experiments prove nothing, since they might have been predicted beforehand to be theoretically very probable.

This combination is a chemical reaction, and as such obeys the laws of chemical kinetics. Very considerable differences may be shown as regards *firmness of combination* and *speed of reaction*. Thus, diphtheria antitoxine has a far greater affinity for its toxine, and combines with it much more rapidly (five to ten seconds) than is the case with tetanus antitoxine and its toxine (EHRLICH).

The degree of attraction for reciprocal saturation and the speed of the reaction also depend, to a very considerable extent, upon the *temperature* (increasing with its rise) as well as upon the *concentration* (EHRLICH, KNORR[1]). Combination takes place much more rapidly in concentrated solutions.

We must defer consideration of the question of the *condition* of equilibrium between toxine and antitoxine.

The view that antitoxines do not combine with toxines in fixed numerical proportions, but that their action depends upon a protective influence upon the *cells*, has not yet been entirely abandoned, notwithstanding all proofs to the contrary. In particular, attempts have been made to base this conclusion on the alleged facts that when the dose of toxine is multiplied the same multiple of antitoxine is insufficient—*i.e.*, that its "*protective*" power fails with large doses of poison. This view has recently been advanced again—*e.g.*, by BOMSTEIN.

But EHRLICH's experiments on *ricine*, and the absolutely analogous results of CALMETTE with *snake poison*, of CAMUS, KOSSEL, &c., with *eel's blood poison*, and of many others with *erythrocytes*, lead us to exclude any intervention on the part of the organism, and are only to be explained on the assumption of a direct fixation of the poison by the antitoxine. And apart from this, the assertion that there is a discrepancy in the numerical proportions rests upon very insufficient data.

COBBETT and KANTHACK[2] were able to demonstrate that the multiples exactly corresponded with theory, provided a large multiple of the lethal dose was used at the very beginning of the experiment. They give a simple reason for concluding that when a quantity of poison approximating a single lethal dose is

[1] Knorr, "Die Entstehung des Tetanusantitoxins," *Fortschr. d. Med.*, 1897, 657.

[2] Cobbett and Kanthack, "Ueber das Schicksal d. Diphtherietoxins im Tierorganismus," *Centralbl. f. Bakt.*, xxiv., 129, 1898.

used at the beginning, it is extremely probable that symptoms of poisoning will appear on multiplying the respective doses. For it is quite possible, when a single lethal dose is neutralised, for a slight excess of poison to remain unnoticed in the mixture, since it may not even reach the minimum amount necessary to cause illness; but if now the relative quantities of poison and antitoxine be increased tenfold, this excess of poison is also multiplied by ten, and the poisonous action of the mixture is manifested. Ehrlich's theory cannot be upset by such proofs as these.

Taking all things into consideration, we are justified in assuming, on practical and theoretical grounds, that the *action of an antitoxine upon a toxine consists essentially of a reciprocal combination of two groups endowed with specific affinity.*

This at once leads to the fundamental conclusion that the reciprocal action of the two substances must follow the laws that hold good for the reciprocal saturation of two simple chemical substances possessing atomic groupings specifically adapted to each other—*i.e.*, that they must combine in fixed *quantitative proportions.* Just as the same amount of pure sodium hydroxide invariably requires the same amount of pure hydrochloric acid for neutralisation, so must the proportion between a definite dose of toxine and the amount of antitoxine that exactly "neutralises" it be an *absolute constant.* A given quantity of pure toxine must invariably require the same dose of pure antitoxine for its activity to be exactly neutralised, provided that the combination is *stable*, and does not lead to a *state of dissociated equilibrium*, a question with which we shall deal later.

The difficulty of establishing this extremely important fact is enormously increased by the circumstances. In the first place, neither toxines nor antitoxines are known in the free state. We are not dealing here with substances that can be isolated as chemical entities, and to which the balance can be applied in determining whether x grms. of diphtheria antitoxine invariably neutralise y grms. of diphtheria toxine. No; the only measurement that is applicable to these poisonous substances is the physiological one—the determination of the "*single lethal dose*," which we are compelled to adopt as the fundamental unit of measurement for toxines, or, in the case of *hæmolysines*, the measurement of the amount of solvent action.

This drawback, however, would not be so very serious if we could only establish a constant relationship between every toxine solution of definite strength and a given *antitoxine* solution, so

that eventually every "single lethal dose" of poison would correspond to a definite number of "*antitoxine units.*" For this purpose the strength of the solution might be readily calculated upon the known toxicity of 1 c.c. of a solution to be taken as unity (normal poison). Unfortunately, this is beyond the range of practicability. Almost every solution of toxine stands in a different proportion toward the amount of antitoxine required for its neutralisation, when the ratio of a "lethal dose" to the number of "antitoxine units" is calculated.

Here we are face to face with the most extraordinarily complicated conditions, and there still remain certain obscurities and difficulties to be explained, although the confusion has been for the most part cleared up by the painstaking researches of EHRLICH.[1] In the first place, the rapidity with which toxine and antitoxine combine depends not only on their nature but also on the concentration of the two components (*vide supra*). But, above all, it has been found that every bouillon of diphtheria virus contains, in addition to the specifically active toxine, varying proportions of other substances which possess the haptophore, though not the toxophore, group of the true toxines, and which, therefore, *make the same demand upon the antitoxine as the toxine itself*, although they have no influence upon the lethal dose—the toxic effect of the poison. Thus, in determining the unit of measurement,—the single lethal dose,—these substances escape observation, but their influence is immediately manifested when an attempt is made to determine the amount of a given antitoxine solution required to neutralise this single lethal dose.

If a pure solution of toxine would require a given number of c.c. of a standard solution of antitoxine, this number would be *increased* in proportion to the quantity of these non-poisonous, though antitoxine-consuming, substances in the *impure* toxine solution. Hence, the varying amount of these substances in *every* solution of toxine enormously increases the difficulty of establishing the absolute constancy of these proportions, as demanded by the side-chain theory; and these difficulties have not yet been completely overcome in every case.

In order to obtain a clear idea of the immediate factors in this question it is necessary for us to begin with the *physiological units of measurement* devised by BEHRING and EHRLICH for the study of the *action of antitoxines.* The numerical definitions fixed for *diphtheria virus* are as follows :—

[1] Ehrlich, "Die Wertbemessung des Diphtherieserums," *Klin. Jahrb.*, vi., 299, 1899; "Ueber die Constit. des Diphtheriegiftes," *Deutsch. med. Woch.*, 1898, 597.

The *single lethal dose* is defined by EHRLICH as that amount of toxine, expressed in c.c. of the poison solution or in grammes of the solid poison, that is just sufficient to kill a guinea-pig weighing 250 grms. (an animal about six weeks old) within four or five days. This dose is the physiological *toxic unit.*

The definition of *normal toxine*, as fixed by v. BEHRING, is a *solution of poison that contains 100 lethal doses in 1 c.c.* The term "normal toxine" is abbreviated by v. BEHRING into DTN, M_{250} (*Diphtherietoxin normal einfach, Meerschweinchen von 250 grms.—i.e., single normal diphtheria toxine, guinea-pigs of 250 grms.*).

Now the measurement of the strength of antitoxine solutions is based upon this arbitrary toxic unit. A "single" serum is one, 1 c.c. of which is capable of neutralising 1 c.c. of the normal toxine—*i.e.*, 100 toxic units. This amount (1 c.c. of the single serum) is the *antitoxine unit*, the so-called immunity unit, briefly designated I.E. (*Immunitäts-Einheit*)[1] and has thus been empirically established and retained (*vide infra*).

When, then, a serum is first prepared against a new toxine, it is invariably the rule in all the experiments made at the same time to express the relationship of the toxine to the antitoxine solution in terms of cubic centimetres. And since in the case of this fresh toxine the ratio of toxic activity to the number of c.c. used remains constant, there is also a constant relationship between the *toxic activity and the amount of antitoxine—i.e., every lethal dose invariably corresponds exactly with the same amount of antitoxine solution, expressed in cubic centimetres.*

If, however, this toxine be allowed to stand for some time and its relative value towards the serum be again determined, it will be found that the conditions of quantitative combination have materially changed in one respect. It is true that the ratio of toxine to antitoxine solution, expressed in c.c., will still remain constant—*i.e.*, that every c.c. of the toxine solution requires the same amount of antitoxine solution as the fresh toxine, but at the same time this quantity of toxine solution, expressed in c.c., has *considerably smaller toxic activity* than an equal amount of the fresh poison. If, on the other hand, a determination be made of the amount of antitoxine required to saturate *one toxic unit*, it will obviously be found that a con-

[1] Madsen ("Constitution du poison diphth.," *Ann. Past.*, xiii., 568, 1899) has introduced several other abbreviations. T = toxine unit; (T) the amount of toxine bouillon in c.c. containing T; I = immunity unit (in German abbreviated to I.E.); and (I) the amount of serum in c.c. that contains I. We shall occasionally use these abbreviations.

siderably greater quantity is necessary than was the case with the fresh toxine.

Hence, it follows that the toxine solution becomes weaker on keeping—*i.e.*, that the *toxophore group has become inactive* in part of the toxine; but since the toxine solution still requires the same number of c.c. of the serum for neutralisation as before, it is plain that the *haptophore groups have remained intact during this weakening process.* From this it follows that there must be present in this weakened toxine solution, substances which have become non-poisonous through loss of their toxophore group, although, owing to their possessing intact haptophore groups, they are as capable as before of entering into combination with antitoxine.

These substances are termed *toxoids*[1] by EHRLICH.

EHRLICH has also introduced two values of limitation which he terms L_0 (*limes*, "*Nil*") and L_+ (*limes*, "*Death*"). The numerical significance of these terms is as follows:—

L_0 is that quantity of the toxine solution under examination, expressed in toxic units (lethal doses), which, when mixed with an immunity unit, is completely neutralised thereby—so neutralised that *absolutely no symptom* of poisoning appears. This mixture of *one* immunity unit with the maximum of toxine solution that can be added without the production of any physiological toxic effect, is *physiologically neutral.*

It is not easy to determine the point L_0 beyond doubt, since it is difficult to determine with absolute certainty whether a toxine solution does or does not still exercise a slight action. Hence, EHRLICH has intoduced his second value: L_+ is that quantity of the toxine solution under examination expressed in toxic units (lethal doses) which, when added to an antitoxine unit, is still sufficient to kill a guinea-pig of 250 grms. in four or five days. This mixture then contains a *lethal dose* in the free state. This point can be determined with ease and certainty. The difference $L_0 - L_+$ is designated D by EHRLICH. In the case of pure poisons it must obviously equal *one lethal dose*, but in practice it is *invariably greater*—which is a point of great significance in the investigation of the constitution of toxines (*vide infra*).

These limitation values, then, apart from their practical use in *testing serum*, have been of extreme value in the investigation of the constitution of solutions of toxines such as diphtheria

[1] Fränkel (quoted by Ehrlich, *Deutsch. med. Woch.*, 1891, 978) and Aronson (*Berl. klin. Woch.*, 1893, 625) *inter alios* had already indicated the existence of such non-poisonous, though immunising, bacterial products.

virus. For by their aid EHRLICH has succeeded in determining the composition of toxine bouillon as regards the toxines and toxoids. He has discovered the existence of extremely complex relationships, and the final solution of the problems involved will doubtless still furnish work for the next generation.

The value of these limitation values in the determination of the amount of toxine and toxoids in solutions of poisons depends upon the following facts :—

The antitoxine unit is a measure based upon a *definite* toxine solution. The original cannot as yet be reproduced, and its value has only been preserved by EHRLICH having kept an originally standardised serum of 1,700 times the normal strength under special precautions (vacuum, darkness, ice, dryness), and using this serum after suitable dilution to standardise new toxine solutions, which then in turn can be used to test fresh sera. In the original normal toxine an antitoxine unit corresponded to 100 lethal doses ; hence, to neutralise an immunity unit 100 lethal doses of this normal toxine of BEHRING would be necessary—*i.e.*, L_0 *is exactly* 100. But this numerical relationship is not invariably necessary with all toxines, for there can also be some in the case of which a large proportion of the immunity unit would be fixed by non-poisonous haptines, *toxoids*, whose quantity is expressed in toxic units—*i.e.*, L_0 *then becomes less than* 100. This is not the case, however, with most *fresh* toxines, for with them L_0 is really 100, or, in other words, these fresh toxines have a constitution exactly like that of Behring's normal toxine.

But the case is different when we deal with *older* solutions of toxines, as we have already pointed out above. Here part of the toxine has been converted into toxoids—*i.e.*, L_0 has become *smaller*, and a smaller number of toxic units in an equal volume are required to reach the stage of neutrality. On the other hand, there are also toxines whose relative proportion in true toxine is greater than in the case of Behring's normal toxine, so that we are driven to the conclusion, which is difficult to follow, that even *fresh toxines* may contain not only poisonous *haptines*, true toxines, but also relatively non-poisonous substances, which EHRLICH has designated *toxones*, to distinguish them from the *toxoids*, which are only produced as secondary products. We thus arrive at the following definition of L_0 :—

Every solution of toxine, even in the fresh condition, contains, in addition to the true toxine, non-poisonous haptines, *toxones*, which usually stand in such relationship to the true toxines that, in the case of most *fresh* toxine preparations, L_0 is equal to 100.

In certain toxine solutions, however, there may be relatively more or less toxones, so that L_0, even in the case of fresh preparations, may sometimes be *greater or smaller than 100.* At the same time, secondary derivatives, *toxoids*, formed from the true toxine are produced in all toxine solutions on keeping, and these invariably lower the value of L_0.

We must reserve until afterwards a fuller discussion of the mode in which toxines are converted into toxoids, and, above all, of the quantitative relationships of this conversion. Here we have next to deal with the significance of the limitation value L_+. Whilst the toxoids *formed as secondary products* have a decided influence upon the amount of the L_0 dose, they are absolutely without influence upon the value L_+ (and, therefore, also upon D), as has been shown by EHRLICH.

Now there are, *a priori*, three conceivable kinds of toxoids :— Firstly, those that possess a greater affinity than the toxine for the antitoxine, which combine with it sooner, and are, under certain conditions, capable of dissolving existing combinations between toxine and antitoxine in their own favour. These are the *protoxoids.*

A second category consists of the *syntoxoids*, which have the same affinity as the toxine for the antitoxine, and thus have no influence upon the combination of toxine with antitoxine, just as their own combinations with the antitoxine are not affected by the toxine. Lastly, there remain the *epitoxoids*, which have a weaker affinity than the toxine for the antitoxine, and can be liberated again by the toxine from their combination with the antitoxine. Such *epitoxoids* occur, as EHRLICH was able to demonstrate, *not* as secondary products, but are already present in the *fresh* toxine solutions ; they are identical with the *toxones* mentioned above.

This consideration leads us to the following points of view as regards the influence of *toxoids* and *toxones* upon the value of L_+ or D :—

The toxoids formed as secondary products—viz., *pro-* and *syntoxoids*—have *no influence at all upon* L_+, as can very easily be demonstrated.

Assuming that we have a *neutral* mixture of antitoxine with toxine and protoxoid, we can represent this condition of equilibrium graphically by means of the equation—

90 Toxine-Antitoxine + 10 Protoxoid-Antitoxine
= Physiological Neutrality (L_0).

Additional quantities of the same poison are now added in order

to find L_+. This can cause no alteration in the compounds already formed between toxine and antitoxine or protoxoid and antitoxine, which might make them yet *combine with fresh doses* of toxine added, and so cause them to disappear as regards L_+—*i.e.*, as soon as the amount of poison added to the neutral mixture reaches *one* lethal dose, L_+ is reached, as is demanded by theory for pure poisons; this may be expressed graphically by the equation—

$$90\ \text{T-A} + 10\ \text{P-A} + 1\ \text{Toxine} = L_+.$$

Thus the presence of protoxoid cannot cause $L_0 - L_+$ (D) *to be greater than one lethal dose.*

The syntoxoids have just as little influence in raising the value of D. They, too, are not influenced in their combination by the addition of toxine, and L_+ is reached by the addition of new poison so soon as *one* lethal dose is added to L_0.

Thus all the secondary toxoids are without influence upon D.

We find quite a different state of things when we examine the behaviour of *toxones* in this respect.

Let us now leave out of consideration the toxoids, which have no bearing upon this question, and represent a neutral mixture of toxine and toxone with antitoxine (L_0) by the following equation :—

$$90\ \text{T-A} + 10\ \text{Toxone-A} = L_0.$$

We then add fresh quantities of poison. If, in the first place, we add a quantity containing exactly one toxic unit, we find that L_+ is by no means reached by that addition; for the *toxine liberates a toxone unit from its combination with the antitoxine;* and, instead of the free toxine added, we find a free *toxone*, as represented in the following equation :—

$$\begin{aligned} &90\ \text{T-A} + 10\ \text{Toxone-A} + 1\ \text{Toxine} \\ = &\ 91\ \text{T-A} + 9\ \text{Toxone-A} + 1\ \text{Toxone (free)} = L_0. \end{aligned}$$

And so the process continues until the *whole* of the toxones are *free.* Then, and not till then, the next toxic unit produces L_+.

$$\begin{aligned} 100\ \text{T-A} + 10\ \text{Toxones (free)} &= L_0. \\ 100\ \text{T-A} + 10\ \text{Toxones} + 1\ \text{Toxine} &= L_+. \end{aligned}$$

According to this equation, we should thus have to add, not *one*, but *eleven*, toxic units to L_0 before reaching L_+, D being thus equal to 11.

We see then that toxones have the property of raising the

difference D *above the amount "one" theoretically required by pure toxines.*

The relative amount of such toxones varies very considerably, and hence D is also a very variable value. EHRLICH found it to vary from 1·7 to 28 toxic units in the case of 11 toxine solutions.

The value D, *after reduction of the finally active one toxic unit* (D - 1), thus affords a measure of the quantity of toxones present in toxine solutions. The fact that these variations in the value of D occur even in fresh toxines, and do not alter as the poison becomes old, when L_0 decreases, shows that toxones are *not* secondary decomposition products of the toxine, but are primary bacterial products—*non-poisonous haptines.*

As regards other physiological characteristics, toxones are not absolutely inactive. Their action can be studied in what EHRLICH has termed the "*differential zones*"—*i.e.*, between L_0 and L_+, where, according to his view, free toxones are present. They produce slight toxic symptoms (which we shall discuss later on), which are essentially different from the effects of small non-lethal doses of toxines.

A special importance attaches itself to the discoveries of MADSEN, that it is possible to produce antitoxic *immunity* with the mixed poisons in the differential zones, which thus only contain toxones in the free state. We shall discuss this more fully in its proper place (see *Diphtheria Virus* in the special part).

EHRLICH next endeavoured, by means of elaborate, tedious, and difficult processes, to obtain a clearer view of the quantita tive relationships of the poisons and the numerical conditions of their decomposition. These relationships proved to be of an extremely complex character, and we do not wish to do more than briefly touch upon them here.

In the first place, EHRLICH fixed the following formula for any given poison :—

$$x \text{ Toxoid} + y \text{ Toxine} + z \text{ Toxone.}$$

The value of y must be found by a physiological method (determination of the lethal dose), and is then represented by α. z, the toxine value, is a function (F) of the value D - 1, which can also be numerically expressed. It is designated β by EHRLICH. Thus, the formula of every *known* solution of the poisons can be expressed as follows:—

$$x \text{ Toxoid} + \alpha \text{ Toxine} + F(\beta) \text{ Toxone.}$$

The formation of toxoids is illustrated by the following experiment :—

As already mentioned, the dose L_0 is equal to 100 in the case of most toxines in the *fresh* condition. Thus, EHRLICH found that one of his freshly-prepared diphtheria virus solutions was of such composition that one I.E. (Immunity Unit) neutralised 0·31 c.c. of the poison. Hence, it followed that the lethal dose of the poison solution had to be $\frac{0{\cdot}31}{100} = 0{\cdot}0031$ c.c., and this was found to be actually the case. Thus, with this poison, too, $L_0 = 100$. After three-quarters of a year the poison showed *the same neutralisation value in c.c., but the single lethal dose had risen to 0·009 c.c., so that* L_0 *was equal to about 33—i.e.*, that 33 toxic units (contained in 0·31 c.c.) corresponds to the dose L_0. The toxic value and L_0 dose then remained constant.

Other poisons decompose in such a way that L_0 is equal to 50, while others, again, become finally constant with an L_0 dose of 25, &c.

It would seem, then, that the toxines either decompose in such a manner that half of them becomes inactive, or that they undergo a *tripartite* change so as to consist of two parts of toxoid to one of unaltered toxine.

EHRLICH'S chief endeavour has been to establish the *absolute* value of an I.E. (Immunity Unit)—*i.e.*, to determine to how many *saturation units* the I.E. corresponds in poisons consisting of toxines, toxones, and toxoids, or, to express it quite crudely, how many *haptophore groups* correspond with the number in one I.E. He is strongly inclined to fix their number at 200, and he bases this conclusion on the following premises:—The value L_0 is usually equal to 100 in the case of fresh poisons, and these subsequently decompose in such a manner that their L_0 values stand in a very simple proportion to 100. From this he concludes that the absolute force of combination must also stand in some very simple relationship to the number 100. Now, no poison has yet been prepared, in spite of all attempts at purification, with a value L_0 exceeding 200, the highest observed being 160 in the case of a poison which was certainly not perfectly pure (MADSEN). Hence, EHRLICH concludes that every toxine bouillon must contain 200 saturation units—*i.e.*, that the I.E. is equivalent to 200 saturation units. *An absolutely pure toxine* (without toxones) would thus, in the fresh condition (*i.e.*, without toxoids), *show* an L_0 value of 200 and an L_+ value of 201.

In this case $x + y + z$, in the general formulæ given above, is equal to 200, and from this it is possible to calculate the quantity of toxones by the aid of the values α and β, where

α represents the number of toxic units and β the value D – 1. If z represent the amount of toxones, $200 - z$ represents the amount of toxines and toxoids, on the assumption of 200 units of combination. Thus, the formula for the value L_0 of every mixture of the poisons becomes

$$L_0 = (200 - z) \text{ Toxine-Toxoid} + z \text{ Toxone},$$

all of which enter into combination with the antitoxine. Hence, in order to liberate *one toxine* it is necessary to add $\frac{1}{200 - z}$, of which $\frac{1}{200 - z}\alpha$ represents the *proportion of toxine.* And this is, therefore, the amount of the poison solution, expressed in toxic units, which must be added to (I.E.) + L_0 to obtain a mixture in which the whole of the toxones are free, so that the addition of a single toxic unit produces L_+; the amount $\frac{z}{200 - z}\alpha$ is thus equivalent to D – 1 = β.

To find the amount of toxone in a poison bouillon, we have thus an equation containing one unknown value:

$$\beta = \frac{z}{200 - z}\alpha,$$

whence is derived

$$z = \frac{200\,\beta}{\alpha + \beta}.$$[1]

With the aid of this formula EHRLICH has calculated the proportion of toxones in the poisons examined by him, and has found that in the case of toxones, too, the values stand in a very simple relationship towards 100—*e.g.*, 100, 50, 25, or 33, 66, &c. Owing to these simple relationships it is possible, by determining the values of α and β, always on the basis of the assumption of 200 saturation units, to reproduce the immunity unit, which was previously only an empirical unit of measurement, since it is possible by this means to determine the proportion of toxine and toxone—*i.e.*, in the case of fresh poisons—which do not contain toxoids, to explain their whole constitution. Most of the poisons in the fresh condition appear to consist of 100 parts of toxine and 100 parts of toxone.

[1] $\beta(200 - z) = \alpha z$; $200\,\beta - \beta z = \alpha z$; $\alpha z + \beta z = 200\,\beta$; $z(\alpha + \beta) = 200\,\beta$; $z = \frac{200\,\beta}{\alpha + \beta}$.

The conversion of toxine into toxoids usually takes place by merely keeping the poisons. The L_0 value then generally becomes constant after some time, and new toxoids are no longer produced.[1] This rule, however, does not appear to be without exceptions; at least MADSEN,[2] whose work has completely confirmed EHRLICH'S results, has described a poison which appears to decrease continuously in its toxic activity. He found in the last determination that L_0 had already sunk to 10 and L_+ to 15, and hence considers it possible that the bouillon might eventually become completely non-toxic and contain nothing but toxoids.

A further interesting point is that the *toxones*, too, do not remain unchanged, as has been proved by EHRLICH and also by MADSEN. In their case it is the *haptophore* group that suffers, with the result that *toxonoids* are formed. This is made manifest by the *increase* in the value L_0, for when in a mixture consisting of 100 parts of toxine : 100 parts of toxone, part of the toxone that combines with the antitoxone changes as regards its haptophore group, so that it can no longer be identified by reason of its combining, it obviously follows that the proportion of toxine to the 200 saturation units is greater than 100. MADSEN regards the fact that L_0 values of, *e.g.*, 133 (which he found in one case) can occur even with fresh poisons, as evidence that the formation of toxonoids may probably be going on spontaneously the whole time during the production of the toxine.

Light, according to MADSEN, has an injurious effect upon both the haptophore and the toxophore groups. He found, on exposing a poison mixture to sunlight, that, although the toxicity diminished greatly, yet the values L_0 and L_+ showed a simultaneous increase. Eventually the specific character of the toxine completely disappeared, but the mixture still remained *poisonous*. It caused animals to die of cachexy, but none of the characteristic effects of diphtheria poison were found on section. Thus *toxic toxoids*, if the term be permissible, are formed under the influence of light.

Not content with all these tedious experiments to determine the constitution of diphtheria virus by repeated determinations of the L_0 and L_+ values, EHRLICH has obtained a further insight into their nature by the aid of a second and still more ingenious method.

If we grant the hypothesis that toxoids, toxines, and toxones have a different degree of affinity for the antitoxine, the postulate follows that they do not combine in equal proportions with a given quantity of antitoxine. This is already shown to be probable by a determination of the L_+ value, but it can only be proved by a direct quantitative estimation of the different

[1] This condition appears usually to be reached in a year. Hence, in testing sera, only such poisons are used as have been kept for a year, in considerable quantities (4 to 5 litres of bouillon), under a thick layer of toluene (Donitz, "Ber. üb. die Thätigkeit des kgl. Instituts f. serumforschung, &c.," reprint. *Klin. Jahrb.*, vii., 1899).

[2] Madsen, "Constitution du poison diphthérique, *Ann. Past.*, xiii., 568, 1899.

degrees of affinity. This has been done by EHRLICH and subsequently by MADSEN (*loc. cit.*) in the following manner:— If an immunity unit were added to 200 saturation units (*i.e.*, 100 toxic units in the case of fresh poisons), the mixture would be physiologically absolutely neutral (L_0). If now the quantity of antitoxine added to the same solution of mixed poison were to be reduced, a measured fraction of one immunity unit (200 units of combination) being added, the toxic characteristics would gradually appear again, since there would be an excess of free toxine. If the poison bouillon consisted of pure toxine, one toxic unit would be liberated by the reduction of the antitoxine to the extent of one unit of combination, two toxic units if the reduction amounted to two units of combination, and so on, until eventually the whole of the toxine had been liberated. If the poison bouillon contained, in addition to the toxine, only non-poisonous haptines of *equal* affinity, every diminution to the extent of one unit of combination would liberate a fraction of a toxic unit; but this phenomenon would occur quite regularly; so that if 10 toxic units were set free by a reduction to the extent of 20 units of combination, 50 would be liberated if the units of combination were reduced by 100. The result would be totally different, however, when substances of *different* affinity were present. Under such conditions the first effect of the reduction in the units of combination would be to liberate the haptines possessing the *smallest affinity* (*toxones*), next those with medium affinity (*toxines* and *syntoxoids*), and, last of all, those with the greatest degree of affinity (*protoxoids*). Or, to express it differently, if a given solution of the mixed poison were to be treated with *increasing* amounts of antitoxines, the protoxoids would be saturated *first*, then the toxines, and finally the toxones.

Now these theoretical requirements can be proved by experiment. If we proceed from 200 : 200 *no* toxine activity appears up to a certain stage, but only the quite distinct toxone activity mentioned above (*zone of free toxones*). But if we go beyond this limit the results are different according as the poison still contained only *toxines* (fresh poisons), or also syntoxoids and protoxoids in addition to toxines.

In the *first* more simple case *each reduction* by $\frac{1}{200}$ immunity unit (one unit of combination) then liberates *one lethal dose*, and this continues to the end. Usually this limit is at $\frac{100}{200}$; thus we have then—

$$\left.\begin{matrix} x \text{ c.c. of poison} \\ (100 \text{ lethal doses}) \end{matrix}\right\} + \frac{200}{200} \text{ I.E.} = 0$$

$$x \text{ c.c. of poison} + \frac{150}{200} = \text{toxone activity}$$

$$x \text{ c.c. of poison} + \frac{100}{200} = \text{toxone activity}$$

$$x \text{ c.c. of poison} + \frac{99}{200} = 1 \text{ toxine activity (1 lethal dose)}$$

$$x \text{ c.c. of poison} + \frac{70}{200} = 30 \text{ lethal doses}$$

$$x \text{ c.c. of poison} + \frac{10}{200} = 90 \text{ lethal doses,}$$

and so on.

The "*Spectrum*" (EHRLICH) of this simplest conceivable poison may be thus represented—

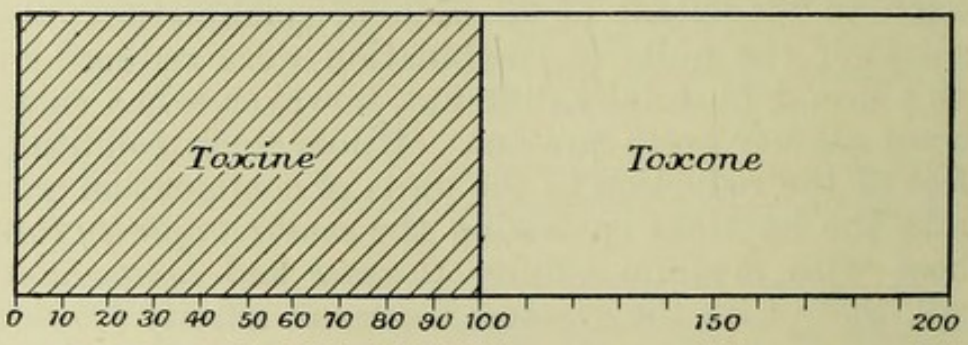

Fig. 1.—"Spectrum" of a Fresh Poison.

But the conditions would certainly never be as simple as these. For, in the first place, the toxines themselves are not uniform in their affinity (to which point we shall have occcasion to return), and, in the second place, they very soon form protoxoids, which alter the curve. For instance, take the following series of values :—

$$x \text{ poison} + \frac{200}{200} = 0$$

$$x \text{ poison} + \frac{180}{200} = \text{free toxone}$$

$$x \text{ poison} + \frac{160}{200} = \text{free toxone}$$

$$x \text{ poison} + \frac{159}{200} = 1 \text{ free toxine}$$

$$x \text{ poison} + \frac{100}{200} = 60 \text{ T. free}$$

$$x \text{ poison} + \frac{50}{200} = 100 \text{ T. free.}$$

And next the non-poisonous protoxoids :—

$$x \text{ poison} + \frac{59}{200} = 100 \text{ T. free}$$

$$x \text{ poison} + \frac{30}{200} = 100 \text{ T. free}$$

$$x \text{ poison} + \frac{1}{200} = 100 \text{ T. free.}$$

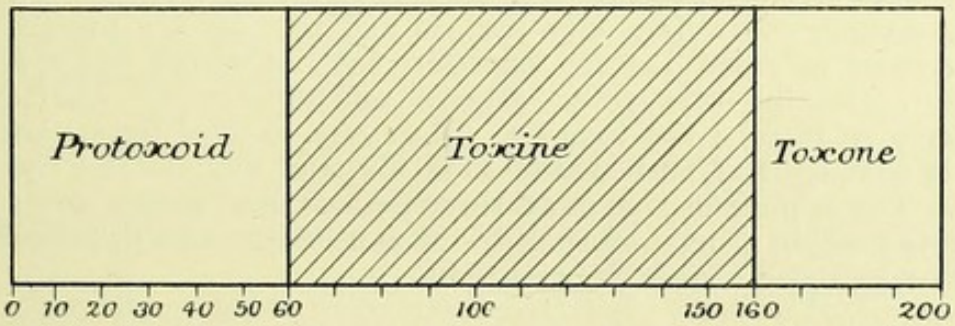

Fig. 2.—"Spectrum" of a Poison in the Protoxoid State.

A further complication is introduced by the formation of *syntoxoids* (*hemitoxine formation*).

Assuming that the toxine decomposes into equal parts of toxine and syntoxoid, the saturation takes place as follows :—

$$x \text{ c.c. of poison} + \frac{200}{200} = 0$$

$$x \text{ c.c. of poison} + \frac{160}{200} = \text{Toxone}$$

$$x \text{ c.c. of poison} + \frac{158}{200} = 1 \text{ T. free}$$

$$x \text{ c.c. of poison} + \frac{156}{200} = 2 \text{ T. free}$$

$$x \text{ c.c. of poison} + \frac{100}{200} = 30 \text{ T. free,}$$

and so on.

The "spectrum" (for the same poison), after the formation of syntoxoid, could then be represented thus—

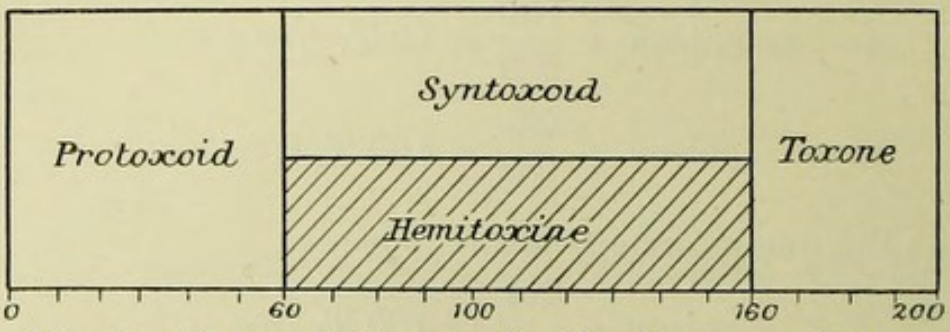

Fig. 3.—The same Poison in the Hemitoxine condition.

As a matter of fact, however, the diagrams are still much more complex. It is not my intention to deal specially with the individual "spectra" that EHRLICH and MADSEN have published. I only wish to give the principles of these methods, and can now content myself with a simple statement of the results that have been obtained in these analyses.

The quantitative decomposition of poisons on standing takes place in the following manner:—At first only toxines and toxones are present. The toxines consist of three distinct varieties, each of which has a different affinity for antitoxine—viz., *proto-*, *deutero-*, and *trito-toxines*—the last being most nearly related to the toxones. Moreover, each of these varieties of toxines is composed of two modifications—*α-* and *β-toxine*—in equal proportions. The *α-* modification of all three toxines decomposes very rapidly, losing its toxophore groups—formation of syntoxoids and development of the above-mentioned *hemitoxine condition.*

Then begins, at an early stage, the destruction of the toxophore group of the *β*-tritotoxine, which, however, never continues to the complete replacement of the toxine by toxoid. Small amounts of toxine invariably remain in this zone—*e.g.*, 3 : 7, 2 : 8, or 1 : 9 of toxoid—which can be recognised in the "spectra" by the fact that toxic action still takes place here, that, for example, in the case of the proportion 1 : 9, a reduction of the units of combination by 10 liberates 1 lethal dose.

Not until a later period does the *β*-prototoxine also disappear (development of *protoxoid zone*). Thus, there finally remains unchanged only the *β-deuterotoxine*, in addition to a small quantity of *β*-tritotoxine; and at this stage the decomposition usually stops, the poison remaining unaltered for a long period in this form.

There appear, however, to be occasional exceptions to this rule. Both EHRLICH and MADSEN have described "spectra" in which, even in the case of very fresh poisons, it is possible to detect the development of the *protoxoid zone*, although even the *α*-deuterotoxine is still intact, so that there is still a zone of unaltered toxine present. MADSEN (*loc. cit.*), however, considers that, taking into account the continued decrease in his pure poison mentioned above, we must conclude that the *β*-deuterotoxine, too, is not of uniform structure, but that it possesses more readily decomposable constituents which change into tritotoxine.

A very interesting confirmation of these extraordinarily complicated relationships is afforded by the fact that MADSEN (*loc. cit.*) frequently succeeded in again finding with sufficient

accuracy in these the quantities of toxones *calculated* by means of the formula $z = \frac{200\,\beta}{\alpha + \beta}$ as given above.

For example, in one instance the calculated toxone value was $z = 33{\cdot}33$, and he found that at $\frac{170}{200}$ all the animals remained alive, while they died at $\frac{160}{200}$, so that the toxone value must thus have been between 30 and 40.

It is also evident that a complete transformation of certain parts of the tritotoxine most nearly allied to the toxones must increase the toxone zone, for it is impossible to recognise tritotoxoids, even when they occur in a state of purity, in a place where it is no longer possible to detect such a small amount of toxine. This is due to their possessing only a slight affinity similar to that of the toxones, and thus escaping detection in the determination of the L_+ value, and also to their being confused with the toxones in making the diagrams. In this way we can explain an apparent increase in the amount of toxones compared with their quantity in the fresh poison, as has been found to be the case by MADSEN (*loc. cit.*, p. 819), while it is denied by EHRLICH.

It is not possible to fix an absolutely sharp limit between the different zones. There appear, rather, to be reactions taking place both between the toxones and toxines, and the toxines and protoxoids, which we must regard as being capable of having some slight influence upon the conditions of quantitative combination, unless we agree with MADSEN in attributing such uncertain reactions to the influence of varying conditions of concentration and temperature.

Toxines and Antitoxines in the Light of Physical Chemistry.—Modern physical chemistry, which has effected so profound a change in our views of the nature of chemical reactions, has of late also turned its attention to the study of physiological chemical processes. I have already shown elsewhere [1] how great an influence these conceptions and methods have had upon the theory of ferments. Quite recently attempts have been made to investigate also the processes that underlie the action of toxines and antitoxines, by means of the kinetic modes of representation of physical chemistry. And there is reason to hope that in this way it may be found possible to express mathematically, in numerical values, the conceptions that the genius of EHRLICH has given to us. As yet, however, these attempts are still in their earliest infancy.

[1] Oppenheimer, *Ferments and their Actions.* English edition. Griffin & Co., 1902.

The processes that take place during the combination of the toxines with the *receptors of the living cells* can obviously never be investigated by this method of examination. Hence, naturally, it has only been employed to throw light upon those processes in which we can watch their progress and result *in vitro—e.g.*, in *hæmolysis*. Here again we have to thank EHRLICH that we possess exact methods of measurement for these processes.

Thus, the first important research in this new field deals with *hæmolysis* under the influence of simple blood poisons, on the one hand, and of specific blood-solvent *haptines*, such as *tetanolysine*, on the other.

ARRHENIUS and MADSEN[1] investigated the course of hæmolysis under the influence of *ammonia*, *sodium hydroxide*, and *tetanolysine*.

The material used in the tests for hæmolysis was a 2·5 per cent. emulsion of the corpuscles of horse's blood washed thoroughly free from serum, and suspended according to requirement in physiological solutions of sodium chloride or cane sugar. The amount of hæmolysis was measured colorimetrically by comparison with solutions of horse's blood, solutions of the strength of 2·5 c.c. in 100 c.c. of distilled water being taken to represent 100, and a colour scale prepared by corresponding dilution.

For comparative determinations with constant amounts of blood (invariably 10 c.c. of the above emulsion), the only suitable interval is that relatively small one beneath whose lower limit hæmolysis just begins, and above whose upper limit it is complete.

The first result ascertained by this method is that hæmolysis increases very rapidly with the rise in the amount of toxine added,[2] so that, as a rough approximation, it is *proportional to the square of concentration of the toxine.*

Now the "*concentration*" of the solvent agent does not correspond absolutely with the amount added. Thus, in the case of ammonia and sodium hydroxide, a certain proportion *enters into combination* with the blood-corpuscles, and does not contribute to the concentration. With tetanolysine, however, this com-

[1] Arrhenius and Madsen, "Anwendg. d. physik. Ch. auf d. Stud. der Toxine u. Antitoxine," *Z. physik. Ch.*, xliv., 1, 1903.

[2] Arrhenius and Madsen give the collective name "*toxine*" to these blood-solvent agents, while they term the specific haptine "*lysine*." Unfortunately, this use of the term "toxine" is liable to create confusion, since it is not in accordance with the present happy limitation of the word to haptines.

bination is so *weak* that the numbers obtained do not need correction.

This explains why a lower limit is found in *which hæmolysis is entirely absent* with ammonia and sodium hydroxide, but not with tetanolysine.

Series of experiments were next instituted, in which variations were made in the percentage of blood-corpuscles on the one hand, and the amount of "toxine" (using the word in the above-mentioned sense) on the other hand. The simplest case is that in which the toxine is present in such excess that complete hæmolysis immediately ensues. If, in this case, we plot a curve, and place percentage amounts of the blood on the abscissæ, and the degrees of hæmolysis on the ordinates, the latter will naturally rise in a continuous line. But if the amount of toxine is smaller, only the initial part of the curve is a straight line—*i.e.*, so long as the quantity of blood is still so small that the hæmolysis is complete.

But when the concentration of the blood rises still further, there occurs after a short rise a point where the whole of the "toxine" (ammonia later than sodium hydroxide) has entered into combination, and the curve falls again. The shape of this maximum is sometimes a sharp point and sometimes a horizontal line, according to the difference in the stability of the compounds with the blood-corpuscles. These details of the action of different inorganic simple "toxines" do not concern us here.

It is, however, extremely important that this maximum is *almost entirely* absent in the case of tetanolysine—at least it cannot be identified with certainty. On the other hand, even this faintly indicated maximum occurs with a much smaller concentration of the blood than in the case of ammonia, &c. From this it follows that the *combination* of tetanolysine with the blood-corpuscles is much weaker than in the case of inorganic agents, but that, on the other hand, it takes place so slowly that part of the substance that may subsequently be fixed still shows activity, whereas the combinations with ammonia, &c., take place so rapidly that only the actual excess of "toxine" takes part in the action.

The proof of the fact that tetanolysine possesses so weak an affinity for the receptors of the erythrocytes might be employed as an argument against the general theory of specific combination.

We shall return to this question of weak combination in discussing the behaviour of tetanolysine towards antitetanolysine.

Speed of Reaction of Hæmolysis.—The measurement of the velocity of reaction is one of the most important means of

obtaining a closer insight into the nature of chemical reactions. ARRHENIUS and MADSEN measure the time within which the hæmolysis has reached a definite point.

For this purpose they cause an excess of "toxine" to act for a fixed time upon equal amounts of blood (which are taken as exactly 100). The hæmolysis is interrupted by cooling the mixture, which is then separated in a centrifugal machine, and finally the extent of hæmolysis is determined. Since the quantity of blood-corpuscles dissolved in the unit of time is the reciprocal of those remaining undissolved $(100 - x)$, the following equation is obtained :—

$$(1) \quad \frac{d\,x}{d\,t} = \mathrm{K}\,(100 - x)$$

whence

$$(2) \quad 1\,n\,\frac{100 - x_0}{100 - x_1} = \mathrm{K}\,(t_1 - t_0).$$

It has been shown that K is not a constant, but shows a rapid increase in the course of the experiment. This is due to the fact that the membranes of the blood-corpuscles at first offer resistance to the action of the toxine, but that this resistance becomes continually weaker with the destruction of the membrane.

At first no blood-corpuscles at all are attacked, and it is not until the weakest membranes give way that hæmolytic action is apparent. This power of resistance thus leads to the necessity of an "*induction period*" for hæmolysis, the explanation of which is evident in these cases.

Hence this method did not yield reliable results. Its authors, therefore, tried whether twice the amount of "toxine" in half the time had the same effect as half the amount in twice the time. It was found that, after making the necessary corrections for the alteration in volume, there was this *approximate* ratio, viz., that the *velocity of the reaction was proportional to the concentration of the toxine.* This held good in the case of *ammonia*, *sodium hydroxide*, and *tetanolysine.*

The quantity of unaltered blood-corpuscles can be expressed by the following equation (in which a represents the amount of toxine), at all events with low proportions (*i.e.*, where x is small) :—

$$\frac{d\,x}{d\,t} = \mathrm{K}\,a\,\sqrt{x},$$

after integration

$$\sqrt{x} = 2\,\mathrm{K}\,a\,t,$$

which, expressed in words, means that the quantity of blood hæmolysed is not only proportional to the square of the time of the reaction, but also to the square of the amount of toxine—a fact already experimentally determined, as mentioned above.

The acceleration in the velocity of the reaction with each *rise in temperature* of 10° C. amounts to 2·76 : 1 with ammonia and sodium hydroxide, and 3·04 : 1 with tetanolysine. The relative velocity of the reaction with ammonia compared with sodium hydroxide is 2·24 : 1. It is thus absolutely independent of the concentration of the OH-ions. Hence *the OH-ions are not the real active agents in the hæmolysis.*

Neutral salts check the action of their *corresponding bases.* The effect of the salt is approximately proportional to the cube root of its quantity. Ammonium salts, in particular, have a strong restrictive action. In the case of *tetanolysine* salts (though in larger quantities) have a stimulating influence. Normal blood serum and egg albumin check the action of "toxines," and particularly that of tetanolysine. Thus we have here a restriction of the effect of active substances by normal blood serum, which plays so great a part in the action of *ferments.* Here, too, without doubt, we have to deal with the occurrence of normal receptors as anti-bodies.

Relations between Toxine and Antitoxine.—The experiments described here were made by ARRHENIUS and MADSEN with tetanolysine, the method being an extension of Ehrlich's method of *incomplete saturation* described above. They treated a constant amount of toxine (2 c.c. of a 2 per cent. solution of tetanolysine) with increasing amounts of antitoxine (in a 0·0025 per cent. solution), and determined the toxicity of the mixtures —*i.e.*, the quantity that caused a definite amount of hydrolysis when added to 10 c.c. of a 2·5 per cent. emulsion of horse's blood. Allowance being made for the relative volumes, the toxicity can be expressed by the equation—

$$G = \frac{1}{x} \cdot \frac{10 + x}{10},$$

where x represents the observed amount of toxine and G the poison strength.

It was found *by experiment* that the required amount, x, *constantly* increased with the *quantity of antitoxine added*, and that G showed a corresponding decrease. There is thus no *reason for the assumption that the "poison spectrum" (vide supra) of tetanolysine assumes* a step-shaped form. On the contrary, the

ratios between toxine and antitoxine follow a *definite* curve *closely resembling* that which represents the relation between the decomposed part of a substance and the products of such decomposition. There is thus a *condition of equilibrium* between the free toxine and antitoxine, on the one hand, and the compound of the two, on the other, so that all three components are present. This deviates from EHRLICH's fundamental view in just the same way as we pointed out above in the case of the compound of lysine with the cell. For EHRLICH assumes that, in the case of diphtheria virus, there is a firm combination between toxine and antitoxine, so that only the actual excess of one component is active. We shall return to this point presently.

ARRHENIUS and MADSEN have strengthened the results of these observations by theoretical calculations of the values of G and x. G is obtained from the equation :—

$$\text{(i.)} \quad \frac{\text{Free Toxine}}{\text{Vol.}} \cdot \frac{\text{Free Antitoxine}}{\text{Vol.}} = \text{K}\left(\frac{\text{Toxine} - \text{Antitoxine}}{\text{Vol.}}\right)^2.$$

The amount of free and combined toxine can be calculated in a complicated way. The amount of toxine present in 1 c.c. of a 1 per cent. solution is taken as the unit of measurement. Now, the original mixture of toxine with 10 c.c. of blood (without antitoxine) contained 0·23 : 10·23 units per c.c. Suppose that, in an experiment with antitoxine, it is necessary to add x c.c. in order to obtain the same shade of colour—*i.e.*, to ensure that the same amount of *free* lysine is present. Then the amount of toxine that has combined with the antitoxine is equal to the difference between the amounts of added and free toxine $= x : (10 + x) - 0{\cdot}23 : 10{\cdot}23$, and obviously as great as the combined quantity of antitoxine. The quantity of antitoxine added (n) is distributed over 4 c.c. of the lysine solution, and hence each *unit* of toxine corresponds to $\frac{\text{N}}{4}$ c.c. of antitoxine. If, now, the ratio of antitoxine to toxine in c.c. be represented by p—*i.e.*, 1 c.c. of antitoxine solution saturating p c.c. of a 1 per cent. solution of lysine—it follows that

$$\frac{4}{n} \cdot \frac{x}{10 + x} \cdot p = \text{the amount of antitoxine per c.c.}$$

From this must be deducted the known quantity of antitoxine in order to obtain the actual amount of free toxine. Hence, by interpolation of these values into the first equation, there results :—

$$\text{(ii.)} \quad \frac{0{\cdot}23}{10{\cdot}23}\left[\frac{n}{4} \cdot \frac{x}{10+x} p - \left(\frac{x}{10+x} - \frac{0{\cdot}23}{10{\cdot}22}\right)\right] = \text{K}\left(\frac{x}{10+x} - \frac{0{\cdot}23}{10{\cdot}23}\right)^2.$$

K and p can be calculated approximately from the mean results of the saturation experiments, and the values thus obtained in 12 determinations being

$$\text{K} = 0{\cdot}115 \qquad p = 14{\cdot}55,$$

i.e., 1 c.c. of the 0·0025 per cent. antitoxine solution employed neutralises

14·55 c.c. of the arbitrary toxic unit, or this unit corresponds to 0·069 c.c. of antitoxin.

The values of G and x calculated from this equation are in very close agreement with the *observed* results.

When large quantities of antitoxine are used, a state of equilibrium between the toxine and antitoxine is only reached very slowly. At the same time the toxine becomes weaker, so that x becomes greater.

An analogous result was obtained in an experiment in which the toxine was replaced by ammonia, and the antitoxine by boric acid. Here, too, the neutralising effect of the boric acid was exerted to such an extent that the law held good :—

(Free Ammonia) (Free Boric Acid) = K (Combined Boric Acid)^2.

It follows, then, from these experiments that the same laws apply to tetanolysine and its saturation by its antitoxine as govern the *saturation of bases by means of weak acids.* Parts of the free components are invariably left uncombined.

This was now made the starting point for attacks on Ehrlich's theory of "*poison-spectra.*"

If we plot the curve of the neutralisation of ammonia by means of boric acid, we find that the first amount added neutralises 50 per cent. of ammonia, the next only an additional 16·7 per cent., and the third 8·3 per cent. more, and from this we could draw the conclusion that the first aliquot portion of the neutralising solution meets with a much more toxic ammonia than the later portions—*i.e.*, that ammonia consists of different toxic parts, which enter into combination with different degrees of affinity, these standing in simple relationship towards each other. This is obviously opposed to the views of Ehrlich on proto-, deutero-toxines, &c.

Hence, according to Arrhenius and Madsen, we must not employ as analogies for tetanolysine the conditions that govern the neutralisation of *strong* bases and acids, as Ehrlich has done in the case of diphtheria virus. In the case of strong acids and bases the amount of *free* components is *extraordinarily small*, and, practically, we have only to take into account the *compound* and the *excess* of one of the components. Measured by this standard, then, the neutralisation curve of diphtheria virus deviates, as Ehrlich has shown, *very considerably* from the simple curve of the neutralisation curve of acids and bases, and lends support to the view of the existence of different poisonous constituents. But if we take as the standard the *conditions of equilibrium* that occur in the case of *weak* affinity, conditions

such as we find with tetanolysine, these apparent differences of affinity can be explained by the *law of mass action*, without assuming the existence of different toxine zones. The presence of *one* toxine and *one* antitoxine, which by their reciprocal neutralisation produce different conditions of equilibrium, and can thus account for the numerical conditions of combination, are quite sufficient to explain why the *quantity* of antitoxine does not invariably correspond with the same amount of *antitoxic energy*.

If these considerations applied to other poisons, as well as to tetanolysine, they would modify a part, although not a very weighty part, of EHRLICH's views; but, at the same time, they would give us a further insight, based upon exact scientific methods, into these extremely important processes. Almost simultaneously with these attacks upon EHRLICH's views other voices were raised against practically the same parts of his theory.

Mention must be made, in particular, of the work of BORDET,[1] who, on theoretical grounds, has come to the conclusion that there is a kind of equilibrium between toxine and antitoxine.

Certainly these conclusions are not based upon exact physico-chemical measurements, but are purely speculative. According to them, the relations between toxine and antitoxine either present certain analogies with the processes of dyeing (*insorption*, &c.), which BORDET does not more closely characterise—for throughout he avoids proofs of identity—or complex compounds are formed containing one toxine molecule with several antitoxine molecules. The antitoxine is distributed over the whole quantity of toxine in such a way that one part of each toxine molecule appears to be neutralised and deprived of its toxicity. Regarded as a whole these speculations, which are not based upon new facts, are just as difficult, if not more so, to work into a theory as EHRLICH's "spectra," which at least explain all the facts on the assumption of *a single* although complicated hypothesis.

Moreover, BORDET's assumption of a "partially neutralised" toxine is absolutely incapable of being put clearly without falling back upon the old, now happily discarded, notion of the "destruction" of the toxicity.

Hence, BORDET's attack is surely not capable of overthrowing EHRLICH's theory of a plurality of poisons. Still less can EHRLICH's position be shaken by the different attacks of GRUBER, although their object was nothing less than the over-

[1] Bordet, "Sur le mode de l'action des antitoxines sur les toxines," *Ann. Past.*, xvii., 161, 1903 (reprint).

throw of the side-chain theory as a whole. These have been so completely answered and demolished by EHRLICH[1] that we can refrain from discussing them individually here.

At first sight the objections of ARRHENIUS and MADSEN to EHRLICH'S theory of the manifold nature of diphtheria virus appear to be much more important, since they are based on the unquestionable results of experiments. This is another instance, however, that in dealing with the theory of toxines there is nothing against which we must guard more than too hasty generalisations.

EHRLICH[2] maintains his position throughout in his reply to the criticisms of ARRHENIUS and MADSEN. He, of course, admits at once the correctness of the experimental proofs, though only in the case of *tetanolysine*, whose instability and slow combining power he himself had already recognised years ago. He refers to one experiment in which the antitoxine activity after two hours was forty times as great as immediately after the mixture had been made. But EHRLICH contends that the facts established in the case of this unstable, slowly-combining poison ought not to be transferred to the extremely active diphtheria virus, which combines rapidly (within a few minutes) with the antitoxine, and for which *alone* his "spectra" have been made.

In his reply he once more repeats, very forcibly, the reasons that led to the development of his conception of the complex structure of diphtheria virus, and cites in support of it numerous separate facts, some of which were previously unpublished.

It would take too long to deal with all his reasons again, since we should have to repeat nearly the whole of what we have said about toxoids and toxones. Hence we will only mention a few of them :—

The conclusion that there are toxoids of different degrees of affinity follows inevitably, from the fact that the toxicity shows a gradual decrease while the amount of antitoxine required for neutralisation remains constant. EHRLICH cites the simple example of the neutralisation of two distinct alkaloids—*e.g.*, quinine and codeine, which have a different affinity for hydrochloric acid—as evidence that we are here dealing with neutralisation limits, quite analogous to those which ARRHENIUS and MADSEN regarded as states of equilibrium between weak acids

[1] Ehrlich, "Toxin und Antitoxin," *Münch. med. Woch.*, 1903, No. 33-4 (reprint).

[2] Ehrlich, "Ueber d. Giftcomponenten des Diphtherietoxins," *Berl. klin. Woch.*, 1903, No. 35 (reprint).

and bases. If the alkaloids be mixed in the right proportion, the curve can assume an exactly similar form.

Even ARRHENIUS and MADSEN will admit the *existence of protoxoids*, but EHRLICH shows mathematically that there must also be other toxoids.

He further succeeded in demonstrating, by calculation of the L_+ value of a particular poison, that in this case the neutralisation of the toxine by the antitoxine exactly corresponded with the neutralisation of a strong base by a strong acid—*i.e.*, that its course had to be represented by a straight line. It was also possible to prove, in the case of this same poison, that the formation of tritotoxoids must have taken place in those parts of the toxine possessing the weakest affinity.

Again, it can be easily demonstrated that there can be *no* change in the degree of affinity during the conversion of toxine into toxoid. But since, now, a poison that has been allowed to stand *does* show differences in its affinity, it follows that these must have already been present in the *fresh* poison, in the toxine condition, and at the same time it is evident that this proves that there is a *plurality of poisons.*

EHRLICH thus firmly maintains his opinion that even in fresh *diphtheria virus* there are varieties of poison with different degrees of affinity, which subsequently undergo a partial conversion into toxoids with different degrees of affinity.

EHRLICH also firmly maintains the existence of *toxones* as primary decomposition products of the activity of the diphtheria bacillus, upon which doubt had been thrown by ARRHENIUS and MADSEN. In particular, the fact of the existence of a poison *without toxones* shows that we cannot here be dealing with a "*residue of unneutralised poison*," but with individual substances possessing only a slight affinity for antitoxine which are usually present. For if it were a question of conditions of equilibrium these would occur in the case of *all* poisons. Apart from this important extreme case *without* toxones, additional evidence against this view is furnished by the enormous variation in the relative amounts of toxones, which may range from 0 to 300 per cent.

A further argument in favour of definite existence of toxones is their frequent *diminution*, "*formation of toxonoids*," as well as their absolutely different physiological action (*vide supra*).

EHRLICH concludes from his arguments that diphtheria virus consists of at least three varieties of poison :—

1. The toxine.
2. The toxone, which kills rabbits suddenly, and guinea-pigs after symptoms of illness.
3. Toxonoids, causing illness in rabbits, but harmless to guinea-pigs.

He also maintains that *diphtheria toxine has so great affinity for its antitoxine that the curve of neutralisation of the pure toxine would form a straight line; the deviations can only be explained by the assumption that parts of it possess different degrees of affinity. These fractions of varying affinity are present in the original poison.*

The theoretioal deductions of EISENBERG[1] are very similar to the conclusions drawn by ARRHENIUS and MADSEN from their experimental results. He, too, is inclined to attribute the neutralisation of the poison and antidote to the production of a state of equilibrium, in which an excess of each of the two active components is present, in addition to the firmly-combined neutral compound. He claims, by means of this theory, to be able to obviate the difficulties in the way of accepting the view that *only* the compound and the excess of one component are present. His argument, however, also rests upon conditions that occur with *other poisons* than diphtheria, such as those of tetanus poison, hæmolytic complements, and, above all, *agglutinines.*

It is extremely probable that in the case of these substances loose combinations may occur with dissociated states of equilibrium. But in the case of diphtheria virus the conditions of combination appear to be the only important factors, which EISENBERG, too, admits to be possible, and that the quantity of components liberated is very slight, as it should be in the case of *firmly-combined* compounds. Thus EHRLICH's arguments are also a sufficient answer to *these* generalisations, which in fact do not apply to diphtheria virus.

Thus we cannot predict beforehand what the conditions will be in the case of other poisons. The question as to the influence of the several derivatives on the quantitative relation between toxine and antitoxine must be specially determined for each separate poison. Probably a whole scale of affinities will be found to exist, ranging from loosely combined, readily dissociated compounds, such as appear to be formed by tetanolysine to diphtheria virus. In this connection it may be mentioned that conditions of equilibrium also appear to occur in the case of *ricine*, in which when neutralisation is nearly complete free

[1] M. Eisenberg, "D. Bindungsverh. zwischen Toxin und Antitoxin," *Centralbl. f. Bakt.*, xxxiv., 259, 1903.

toxine and free antitoxine are both present. DANYSZ[1] has described this, and has based upon it absolutely untenable speculations similar to those of BORDET. We shall return to the facts when we are dealing with ricine. But we must never forget, as EHRLICH is fully justified in repeatedly insisting, that in these *weak* combinations a very important part is played by the time of reciprocal contact as well as by the concentration. Thus if too short a time be allowed for the reaction it is possible to draw an erroneous conclusion of a too high degree of concentration. Hence, when EISENBERG brings forward as a proof the separation of snake poison from its antitoxine at higher temperatures, it must not be forgotten that, according to MARTIN and CHERRY, this separation, which involves the secondary breaking down of the antitoxine, is only possible for a very short time after the admixture.

Lastly, a caution must be given against over-estimation of the influence of mass action as regards other haptines. Contrary to the views of EISENBERG, who concludes that conditions of unstable equilibrium are the rule with agglutinines and precipitines, v. DUNGERN[2] shows that such conditions are quite exceptional, and that, in general, the combination of the precipitine with the precipitable substance is complete and stable. In explanation of the quantitative ratios he concludes that there is a *plurality of precipitines*, which is quite analogous to the views of EHRLICH.

Heat Manifestation of the Action of Toxine upon Antitoxine.—With the aid of VAN T' HOFF's formula

$$\frac{d \log \text{nat K}}{dt} = \frac{W}{1{\cdot}99T^2}$$

ARRHENIUS and MADSEN were able to calculate from the alteration in the dissociation constant, K, the amount of heat liberated in the combination of 1 grm. molecule of toxine with 1 grm. molecule of antitoxine.

This value is equal to 6,600 cals. (with a possible error of 600 cals.). The evolution of heat is almost half as great as that liberated on neutralising a strong base with a strong acid.

Vigorous discussion as to the bearing of the law of mass action upon the relations between the toxine and antitoxine still continues. The more

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[1] Danysz, "Contrib. à l'étude des propr. des melanges des toxines avec leur antitox.," *Ann. Past.*, xvi., 331, 1902.

[2] v. Dungern, "Bindungsverh. bei d. Präcipitinreaktion," *Centralbl. f. Bakt.*, xxxiv., 355, 1903.

thoroughly the question has been studied the more it has been found that ARRHENIUS and MADSEN considerably over-rated the influence of mass action.

It was shown by VON DUNGERN in the case of diphtheria toxine and by HANS SACHS in that of tetanolysine that the fundamental condition theoretically essential for the view of ARRHENIUS and MADSEN—viz., the combination between the toxine and antitoxine should be reversible—was lacking, and that on the contrary the combination *gradually* became firm and irreversible. At the same time VON DUNGERN brought direct proof of the plurality of diphtheria poison by showing that a mixture of antitoxine and toxine could be non-poisonous after a single addition of the exactly sufficient quantity of toxine, but that when the same amount was added in two portions with an interval of twenty-four hours between them it was poisonous. In the latter case the antitoxine being present in excess is distributed between the toxine and the toxone, and when the second portion is added after twenty-four hours the combination between the toxone and antitoxine has become so stable that the toxine can no longer find sufficient free antitoxine.

The stability of the combination between toxine and antitoxine was shown by WASSERMANN and BRUCK in another way. They found that when they injected a physiologically neutral mixture of tetanus toxine and antitoxine, together with some adrenaline into an animal, the contraction of the vessels caused by the alkaloid led to a delay in the resorption of the antitoxine, whereas the toxine which is resorbed by the nerves (*vide Tetanus*) was immediately taken up, and poisoning resulted. But if they allowed the mixture to stand for two hours before the injection, the toxine had combined so firmly with the antitoxine that no resorption of the former took place and the animal remained well.

Lastly, it was found by MADSEN himself that it was only possible in the case of absolutely fresh mixtures of diphtheria toxine and antitoxine to effect a separation of the two components by means of their different rates of diffusion through gelatin, and that after the lapse of even a short time such separation could no longer be effected. This was also proved indirectly by an experiment of MORGENROTH, who found that fresh mixtures of diphtheria toxine and antitoxine were poisonous when injected intravenously into rabbits, but that after some time they were inert.

However, even if the complicated relationships that exist in the case of bacterial poisons cause the curve of saturation to take such a form that it can be shown to have some resemblance, externally at least, to the curve of equilibrium between substances of weak affinity, yet in the case of *Cobra venom* the conditions are such that there is apparently a pure toxine there. At all events, according to KYES, the curve of saturation between the toxine and antitoxine here *forms a straight line*, like that formed between a strong acid and a strong base.

The discussion has had the further very important result of raising the fundamental question whether there is any justification at all for applying the law of mass action to the reactions between colloidal substances. NERNST and others have given a vigorous denial to this. This has led in turn to a closer investigation of the properties of colloids, which has already established many important ratios between the reactions of toxine and antitoxine and those of the colloids, the consideration of which here would lead too far.

L. Michaelis, "Üb. die Gültigkeit des Massenwirkungsgesetzes bei der Reaction zwischen T. u. A.," *Biochem. Centralbl.*, iii. [1], 1904.

v. Dungern, "Beitr. z. Kenntn. der Bindungsverh. bei der Verein. v. Di.-Gift u. A.," *Deutsch. med. Woch.*, 1904, Nos. 8-9.

Wassermann and Bruck, "Üb. d. Wirkg. der A.," *Deutsch. med. Woch.*, 1904, No 21.

Madsen, "Constit. der poison dipht.," *Centralbl. f. Bakt.*, xxxiv., 630, 1903.

Morgenroth, "Üb. die Bindung von Di.-T. u. A.," *Berl. klin. Woch.*, 1904, 20; *Zeit. für Hyg.*, xlviii., 177, 1904.

H. Sachs, "Konstitution des Tetanusgiftes," *Berl. klin. Woch.*, 1904, 16.

Kyes, "Kobragift u. Antitoxin," *Berl. klin. Woch.*, 1904, 19.

Nernst, "Ueb. die Anwendbarkeit d. Gesetze d. chem. Gleichgewicht auf Gemische von T. u. A.," *Z. f. Electrochemie*, x., 1904.

H. Aron, "Ueb. organische Kolloide, *Biochem. Centralbl.*, iii. [15 and 16], 1905.

Endotoxines and Bacterial Proteins.—While the production and mode of action of *true toxines*, such as are formed by the bacilli of diphtheria and tetanus are fairly well known, the case is essentially different and more complicated with a large number of pathogenic organisms, of which we may take the bacteria of cholera and of typhus and *B. pyocyaneus* as the chief representatives. If a cholera cultivation of only a few days' growth be filtered through a bacterial filter, the filtrate is only toxic to a slight extent. Several c.c. are necessary to kill an animal by intraperitoneal injection, and even in these quantities the filtrates are not deadly to all animals. But if we take the residue of the filtration—*i.e.*, the filtered-off bacterial cells—and destroy these by means of weak disinfecting agents, such as chloroform, it will be found that these dead cells are highly toxic. A few milligrammes of them are sufficient when injected into the peritoneum to kill an animal almost instantaneously with severe symptoms of collapse. In this case, then, the conditions are the reverse of those that obtained with diphtheria bacilli, since at first but little of the poison passes into solution, whereas the dead cells of the bacteria are extremely toxic. If, instead of fresh bouillon cultivations, *old* ones that have stood for several weeks in an incubating oven are taken, it will be found that there is a considerable increase in the toxicity of the germ-free filtrates. Much smaller doses are sufficient to kill the animals used in the experiment. Yet even under these conditions the toxicity of these filtrates never attains the same degree, as is found with diphtheria and tetanus poison, of which even fractions of a milligramme may cause fatal results.

The explanation of these experimental results presents no difficulty. Obviously cholera bacilli, &c., secrete the greatest part of their poison within their vital substance. This part is only liberated when the bacteria undergo a process of destruction,

such as occurs when they are dissolved by the fluids in the bodies of animals, or such as take place spontaneously in old cultivations, where part of the cells of the bacteria are brought into solution by the alkaline and other products present in such cultivations; and to this extraction process must be attributed the fact that the filtrates from old cultivations are much more toxic than those from fresh cultivations.

If, now, we ask what position these poisons occupy as regards our definition: whether they are *true* toxines, against whose action the organism forms antitoxines, we have the following data to go upon:—Attempts to prepare a true antitoxine to the poisons contained in the cells of bacteria, the *endotoxines*, have as yet been unsuccessful. Hence, in the absence of further knowledge, we must assign an exceptional position to these poisons. Their sole distinguishing characteristic is their extreme toxic nature in experiments on animals. But as yet no convincing proof has been brought that they belong to the true toxines, and possess distinct haptophore and toxophore groups.

The case is different with the poisons that pass into the filtrate on filtration. A true antitoxine serum for these has been prepared—*e.g.*, by Ransom and by Roux and Metschnikoff for *cholera*, and by A. Wassermann for the poison of *B. pyocyaneus* (*q.v.*). This was done in the well-known usual manner, by previously treating the animals with increasing doses of the poisonous filtrates. By this means sera were obtained which were capable of neutralising with certainty many times the amount of the lethal dose of the poisonous filtrates.

Hence, according to the results of all experiments that have been made up to the present, the conditions in the case of these species of bacteria are such that the chief part of the poisonous substance adheres firmly to the cells of the micro-organisms, and does not pass into solution. Such substances are termed the *endotoxines*, and are comparable with the *endoenzymes* of yeast and of bacteria themselves.

Traces of a *true toxine* which passes into the filtrate also occur.

The further question now arises, whether we are justified in concluding from these experiments that the production of poison follows the same course under natural conditions—*i.e.*, in the organism.

It seems fairly certain that the answer is in the negative.

On the contrary, it is extremely probable that the slight traces of poison which we find in these cultivations, and which, as we have seen, increase somewhat in quantity on further extraction of old cultivations, are not those of the *primary* poison of the

micro-organisms—that which we see exerting its activity in the pathology of these infectious diseases in man. An extraction process, such as occurs spontaneously in old cultivations, is *by no means* one that has little effect upon the constitution of these unstable bodies. In such old cultivations great changes of reaction from acid to strongly alkaline occur abruptly; ammonium compounds and other chemical substances are produced, which we know tend to change and destroy bacterial poisons. Hence we must assume that even these traces of *cholera virus*, &c., that pass into solution, and *to which an antitoxine can be prepared*, do not represent the *primary* poisons of these micro-organisms, the poisons that they undoubtedly produce in the human organism, but are rather a secondary and more stable modification; and this conclusion is based on the fact that while, as WASSERMANN found in the case of the poison of *B. pyocyaneus*, we are certainly able to prepare an antitoxine to this dissolved poison, yet this antitoxine behaves quite differently to the antitoxines in *diphtheria*. For the latter neutralise the corresponding quantities of toxine in any given multiples of the dose, provided that their own quantities are increased in the same proportion. Thus, if 10 doses of diphtheria antitoxine neutralise 10 doses of toxine, 1,000 doses will neutralise 1,000 doses of toxine. But in the case of *pyocyaneus* poison this "law of multiples" only holds good within very narrow limits—up to about eight or ten times the lethal dose. Beyond that there is no neutralisation, and animals die in spite of large doses of antitoxine.

We must, therefore, conclude that it is altogether doubtful whether we have ever had the *primary true* toxine of cholera, &c., in our hands when using the culture media at our present disposal. It may be a question of suitable nutrient media, and further systematic research may be required before we get nearer to this important goal. The great influence of a suitable culture fluid upon the production of the true poison in artificial nutrient media is shown by the case of diphtheria virus, of which in the earlier experiments of ROUX and YERSIN from 30 to 36 c.c. were required to kill an animal with the typical acute symptoms, whereas a close systematic study of culture media and the choice of suitable cultivations have had the result of reducing the amount now required to 1 to 2 mgrms. Hence we must regard the question of cholera virus and similar poisons as still unsettled in many respects.

At the same time, we must not omit to call attention to the fact that so experienced a worker in practical research into the nature of cholera infection as R. PFEIFFER holds the view that

the striking symptoms of poisoning that appear in the spontaneous infection of man with cholera are also due to the absorption of poisons, *endotoxines*, set free through the solution of the cholera vibrio. In his opinion, endotoxines are thus the main effective poison in cholera and other infectious diseases, such as *typhus*, &c., that behave in an analogous manner. In support of this view, that in these infectious diseases we have only to deal with the bacteria as such, and the *poison contained in their cells*, and not, as in the case of diphtheria, &c., with a soluble poison *that can be separated* from the cells, but of whose presence in the human subject we cannot speak with certainty, as pointed out above; in support of this view we have the phenomena that occur in the disappearance of these diseases and in immunisation against these bacteria, and the substances that are then found in the serum. Thus we have seen that in cholera, &c., *only bactericidal* substances are present; but such exclusively *bactericidal* substances only occur, as WASSERMANN was able to show in the case of *B. pyocyaneus*, when there has been absorption of constituents of the *cell-substance* of the bacteria, while *toxines* invariably cause a simultaneous formation in the serum of *antitoxic* substances, and substances possessing specific bactericidal powers.

Bacterial Proteins.—When bacteria that produce soluble poisons are freed as completely as possible from those poisons, there will still remain substances that belong to the cell material. These substances, too, have a physiological action, producing inflammation, aseptic abscesses, and necroses at the point of application, as well as slight general symptoms, such as fever, faintness, headache, &c.

The same effects are also produced by the albuminous substances prepared by chemical methods from the cells of the bacteria, and termed *bacterial proteins*, as first proposed by BUCHNER. They are obtained by different methods, of which those chiefly employed are extraction with *superheated water* in autoclaves, simple *boiling* with water, and extraction with dilute *alkalies*. To these have recently been added those methods, due to KOCH and BUCHNER, in which the bacteria are first *triturated* either in the moist or dry condition, with, in some cases, the aid of hydraulic pressure, in order to obtain their contents.

In this way a long series of bacterial proteins has been obtained, which, although differing in some particulars, produce practically similar effects. We shall frequently meet with them in the special part, where, too, will be found references to the most important researches on this subject.

It is unnecessary to describe these proteins in detail here, for

it has been proved beyond doubt by the researches of Römer, Buchner, Schattenfroh, Klemperer,[1] and many others, that at least the albuminous substances isolated by long-continued extraction from unruptured micro-organisms are absolutely without specific action, and thus can be neglected in discussing the causes of disease *sui generis.*

But this only applies to the *albuminous substances* isolated from the cells in a state of ideal purity. To separate them, however, in such a state is only possible in the very rare cases when the bacteria produce *only* free soluble specific poisons, from which their cells can be completely separated, as has been done by Kossel in the case of diphtheria bacilli. There then remain behind proteins devoid of specific activity, exactly similar to those that can be obtained from the most innocuous bacteria, and, like other *foreign albuminous substances*, producing sterile abscesses, &c.

But usually it is impossible to effect a radical separation of these proteins, in the narrower sense of the word, from the poisons. The protein preparations from most bacteria are still contaminated with residual particles of the specific poisonous substances or their secondary products, especially *endotoxines* and their derivatives, so that even these protein preparations still produce characteristic symptoms of poisoning, as is the case with cholera, typhus, and tuberculosis (see Special Part). Here, then, the action of the pure protein cannot be demonstrated, but only theoretically inferred.

The actions of these poisons must be kept quite distinct from the *immunising processes* that are brought about by the cell materials of the bacteria, either in the uninjured cells or in chemical preparations—the problems of *bactericidal immunity* which have been completely elucidated by the researches of Pfeiffer and Wassermann as regards *cholera*, of Pfeiffer and Kolle in the case of *typhus*, and of Koch in the case of *tuberculosis.*

These processes have absolutely no connection with the toxic action of the cell proteins. Here we have to deal with the introduction of suitable receptors, which rouse into activity *bactericidal* protective forces—lysines, precipitines, and agglutinines; with processes that depend upon the complex *stereochemical* configuration of the protein molecule.

At present we can only assume with certainty the presence of such receptors in unaltered bacterial cells, which, like the

[1] For the bibliography see Klemperer, "Die Beziehg. verschied. B.-G. zur Immunität u. Heilung," *Z. f. klin. Med.*, xx., 165, 1892.

cholera vibrio, pneumococcus, &c., bring about those destructive processes *in toto*. On the other hand, it is fairly safe to affirm that vigorous extraction, involving the formation of *chemical* protein preparations, usually changes that sensitive atomic grouping to such an extent that injection is followed by no bactericidal reaction, or only a very faint one, but rather that these albuminous substances produce only the same reactions as every albuminous substance that is *foreign to the system—i.e.*, the formation of *precipitines*[1] which are certainly very closely allied to the *agglutinines*. Yet it is very probable that, in the case of somewhat more scantily represented examples, such as Koch's *tuberculin* (*q.v.*) and Buchner's *plasmines—e.g.*, of the cholera vibrio and tubercle bacillus—specific receptors are present, so that these preparations bring about bactericidal *immunising* processes.

SUMMARY.

1. A group of bacteria produces *true toxines* in the form of *free secretions*. After extraction of these soluble poisons there remains a residue of pure non-specific bacterial proteins. Type: *Diphtheria*.

2. Another large group appears to form only *endotoxines:* true toxines which are more or less firmly retained by the *living* cell, so that they are only secreted to a very small extent in an unaltered condition, and possibly not at all *outside the body*. When the cells die the toxines are *partly* liberated and partly retained, or are converted into secondary poisonous modifications no longer possessing the characteristics of toxines. Thus, in the case of this group the dead cells cannot be completely freed from other poisons; we cannot regard the results as being produced by pure protein alone. With this reservation, however, it is possible to detect the activity of proteins. Type: *Cholera, typhus, pneumococcus.*

3. A third group *possibly* forms no true toxines, even within the plasma. The cell plasma contains poisons of another kind, which obscure the effects of the action of the protein. Type: *Anthrax, tuberculosis.* It is possible that as our knowledge increases groups 2 and 3 may be united.

4. The pyogenic action of their proteins is common to all bacteria, and depends, in the main, upon their effects as albu-

[1] For further particulars about precipitines see Michaelis and Oppenheimer, "Immunität gegen Eiweisstoffe," *Engelmanns Arch.*, 1902, suppl. vol.

minous substances foreign to the system,—effects that can also be produced in an exactly similar manner by foreign albuminous substances of non-bacterial origin.

The formation of the specific precipitation ferments, the *precipitines*, after the introduction of any foreign albuminous substance into an organism, is a proof that every such foreign proteid is an injurious intruder which the organism endeavours to overcome. Just as, according to EHRLICH'S views, all food substances must find receptors in order to be assimilated, so must those receptors be made adaptable to foreign proteins when abnormally introduced—*i.e.*, by subcutaneous or intravenous injection—so as to seize upon them and render them innocuous.

In this process general reactions, fever, &c., are of frequent occurrence. And here, too, we have a means of elucidating the action of bacterial proteins apart from that of any specific toxic impurities that may be present.

SPECIAL PART.

I.—THE TRUE TOXINES.

DIPHTHERIA TOXINE.

Diphtheria toxine is the most important of all the bacterial poisons, both as regards its theoretical importance and especially on account of its relations towards artificial immunity and serum-therapy. It represents for us the exact fundamental type of the true *toxine;* it has been used in most of the investigations that have thrown light on the mode of action of toxines, their relations towards disease, and the formation of antitoxines. Diphtheria toxine is strictly a specific poison, producing in animals almost exactly the same symptoms of disease as are produced by infection with living diphtheria bacilli. The knowledge of the fact that the diphtheria bacilli themselves are not found distributed throughout the body, but only in the false membranes or point of inoculation long ago suggested the notion that the general symptoms were caused by soluble poison. Even in his first work, Löffler [1] indicated the existence of such a specific poison, and subsequently he isolated it by precipitation with alcohol. Then Roux and Yersin [2] succeeded in establishing the fact beyond dispute.

Roux and Yersin found that a cultivation of the diphtheria bacillus in calf's bouillon of seven days' growth when filtered through porcelain and proved to be completely clear and sterile, produced typical symptoms of poisoning, especially when injected into the peritoneum. The toxicity increased with the age of

[1] Löffler, "Unters. üb. d. Bedeutg. der Mikroorg. f. d. Entstehg. d. Diphth.," *Mitt. Kais. Ges.-Amt.*, ii., 1884. *Id.*, "Der gegenw. Stand der Frage nach der Entsteh. d. Diphth.," *Deutsch. med. Woch.*, 1890, 81.

[2] Roux and Yersin, "Contribution à l'étude de la diphtherie," *Ann. Past.*, iii., 273, 1889; iv., 385, 1890.

the cultivation. A culture forty-two days old killed a rabbit in five to six hours with toxic symptoms analogous to those of a severe attack of diphtheria, whilst six days was necessary with an equal dose (35 c.c.) of the same cultivation of seven days' growth. The poison was also characterised by the same phenomena and the same degree of infectiousness as in the case of inoculation with living cultures. The conclusions of ROUX and YERSIN were fully confirmed by KOLISKO and PALTAUF,[1] who produced the symptoms of poisoning by means of the filtrates of a bouillon cultivation of fourteen days' growth.

ROUX had already noticed that the poison was destroyed by heat, and concluded that it was akin to the enzymes. As such it was, in his opinion, a secretion product of the diphtheria bacilli excreted by them into the surrounding media. Yet this view was apparently quite out of keeping with the fact that young and vitally active cultivations produced relatively little toxine, whilst older cultivations gave richer yields.

Hence, GAMALEIA[2] concluded that diphtheria virus was not a secretion product of the bacilli but a constituent of their cell contents, which was not discharged by healthy bacilli, and that it was only when the cultivation became old and many of the bacilli died that the poison was extracted from their decomposed cells. This view, however, has frequently been convincingly opposed. H. KOSSEL,[3] in particular, proved that the toxine was a secretion product and not a decomposition product, by the fact that, on the one hand, he observed a very plentiful formation of poison, even in quite young cultivations (two days old) when grown by suitable methods, and that these began to grow weaker after as little as five days, whilst, on the other hand, he showed that but little toxine was present in the cells of the bacilli themselves.

He cultivated diphtheria bacilli upon as large a surface as possible, making the inoculations from the surface skin of a cultivation of one day's growth. After some days the bacilli formed a tough skin from which he poured off the bouillon. The cells of the bacilli were then repeatedly washed with distilled water and subjected to centrifugal force until the washings no longer gave the biuret reaction, after which they were extracted with water rendered faintly alkaline. This extract was only

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[1] Kolisko and Paltauf, "Zum Wesen des Croup und der Diphtherie," *Wien. klin. Woch.*, 1889, No. 2.

[2] Gamaleia, "Les poisons bactériens," *Arch. de Méd. Expér.*, 1892.

[3] H. Kossel, "Zur Kenntnis d. Diphtheriegiftes," *Centralbl. f. Bakt.*, xix., 977, 1898.

slightly poisonous. Aronson,[1] however, by trituration of the bacilli cells and extraction with ether-alcohol (4 : 1) followed by extraction with a 0·1 per cent. solution of ethylene diamine, obtained poisonous extracts from which he could precipitate the poison by means of alcohol. Again, according to Brieger and Boer,[2] the extracted cells have certainly a considerable degree of toxicity, but this manifests itself in quite a different manner to the toxine, producing no immunity, &c.

Diphtheria toxine is thus not a constituent of the cell contents, but a *metabolic product*. As regards the *preparation* of diphtheria poison, two things must be sharply differentiated—viz., on the one hand, the selection of methods to obtain the *largest possible yield* of poison, and, on the other hand, the experiments that have been made to isolate specific substances *from* the toxic cultivations and to investigate their nature, of course with the practical object also in view of obtaining as *pure* a poison as possible in a dry condition for the purposes of immunisation and serum-therapy. In preparing liquids rich in poison the methods must aim at the two cardinal points of, first, obtaining a solution containing as much poison as possible, and secondly, obtaining this poison as completely as possible in germ-free bouillon, either by sterilisation or separation from the bacilli.

As regards the production of the toxine, the choice of the *nutrient medium* is of primary importance. Roux and Yersin (*loc. cit.*) and Löffler (*loc. cit.*) cultivated their bacilli on an ordinary meat broth, and so naturally only obtained relatively weak solutions of the poison, especially in the early stages of growth. Subsequently, the addition of 2 per cent. of peptone became the general practice. According to H. Kossel (*loc. cit.*), the nature of this peptone is not a matter of indifference. He had employed with great advantage Dr. Aschmann's peptone from the Luxemburg Hygienic Laboratory. Chapoteaut's peptone has also been found an excellent material. It is also usual to add 0·5 per cent. of ordinary salt. von Dungern[3] has found it advantageous to add ascitic fluid or to cultivate the bacilli on the fluid itself.

Guinochet[4] asserts that diphtheria toxine is produced even in culture media devoid of albuminous substances. He was

[1] Aronson, "Zur Biologie und Chemie der Diphtheriegiftes," *Arch. f. Kinderheilkunde*, xxx., 23, 1900 (reprint).

[2] Brieger and Boer, "Ueb. d. Toxine d. Diphtherie, &c.," *Deutsch. med. Woch.*, 1896, 783.

[3] von Dungern, "Steigerung d. Giftproduktion d. Di.-B.," *Centralbl. f. Bakt.*, xix., 137, 1896.

[4] Guinochet, "Contribution à l'étude de la toxine des bacilles de la diphtherie," *Arch. de Méd. Expér.*, 1892, 487.

successful in producing it in urine rendered faintly alkaline, and his results were confirmed by USCHINSKY; to this we shall return shortly.

The choice of the culture medium is of great importance, particularly as regards the *reaction*. In ordinary bouillon the reaction is at first faintly alkaline, then becomes faintly acid, and then, finally, alkaline again. During the period of the acid reaction the production of poison is considerably checked or entirely suspended. Hence, means were sought to restrain this injurious acidification. Even the above-mentioned addition of 2 per cent. of peptone effects some improvement, but, by itself, is insufficient.

PARK and WILLIAMS[1] adopted the simple device of increasing the alkalinity of the 2 to 4 per cent. peptone bouillon by the addition of a measured quantity of soda solution. They exactly neutralised their fresh bouillon (with turmeric paper as indicator), and then added to each litre 7 c.c. of normal soda solution, the mixture still showing an acid reaction with phenol-phthalein. They found, in agreement with SPRONCK[2] and VAN TURENHOUT,[3] that the acidification was due to the presence of *glucose* and glycerin in flesh that was too fresh, and therefore concluded that horseflesh (which is rich in glycogen) should not be used; whilst SPRONCK advocated the employment of slightly decomposed flesh. By the artificial addition of glucose he was able to restrict the production of the toxine. This was in accord with the results obtained by BLUMENTHAL,[4] who also found that no toxine was formed in culture media containing sugar when the proportion of the latter reached more than 1 per cent. Moreover, he could obtain no toxine from solutions of pure egg albumin or pure peptone. On the other hand, SMITH[5] regards the presence of a slight amount of glucose (0·2 per cent.) as essential, and considers that it is only necessary to prevent the injurious acidification, in which respect he supports RUETE,[6] who effects this

[1] Park and Williams, "The production of diphtheria toxine," *J. Exper. Med.*, i., 164, 1896.

[2] Spronck, "Sur les conditions, dont dépend la production d. poisons dans les cultures diphtériques," *Ann. Past.*, ix., 759, 1895.

[3] Van Turenhout, "Over de bereiding van diphtheriegif," Utrecht, Abst., *Centralbl. f. Bakt.*, xviii., 295, 1895.

[4] Blumenthal, "Ueb. d. möglichk. d. Bildg. von Diphtherietoxin aus Eiweisskörpern und auf Zucker enthaltenden Nährboden," *Deutsch. med. Woch.*, 1897, No. 24.

[5] Smith, "The Relation of Dextrose to the Production of Toxine," *J. of Exper. Med.*, iv., 373, 1899.

[6] Ruete, "Ueb. Herstellg. d. Di.-Heilserums," *Münch. med. Woch.*, 1897, 213.

by the addition of fragments of marble to the cultivation. NICOLLE,[1] differing from SPRONCK, has recommended the use of quite fresh flesh. MARTIN[2] has obtained successful results by the use of a mixture in equal parts of extract of beef, or, better, veal, and of extract of pig's stomach.

He gives the following recipes:—

(i.) *Extract of Pig's Stomach.*—Five pigs' stomachs are finely minced or crushed, and the whole (*mucosa* and *muscularis*) mixed with 10 grms. of pure hydrochloric acid (20 per cent.) and 1,000 grms. of water to each 200 grms., and allowed to stand for twenty-four hours at 50° C. The mixture is then boiled and filtered through loosely-packed cotton wool, and the filtrate mixed with 0·2 per cent. of acetic acid, neutralised while still hot, filtered through paper, and heated to 120° C. in an autoclave. The last process can also be replaced by repeated heating to 100° C., careful removal of the albuminous scum and fat, and, finally, clarification by the addition of calcium chloride and sodium phosphate (formation of a precipitate of calcium phosphate which simultaneously carries down the albuminous turbidity).

(ii.) *Meat Extract.*—Perfectly fresh veal is allowed to stand for twenty hours at 35° C., after which it is treated with 1,000 grms. of water for each 500 grms. of material, and again left for twenty hours at the same temperature. It is then expressed, and the resulting liquid mixed with 5 grms. of sodium chloride and 20 grms. of peptone, neutralised, and, after the addition of 7 c.c. of normal soda solution, sterilised at 120° C.

A mixture of equal parts of the two extracts, heated to 70° C. and sterilised by filtration through Chamberland filters, forms an excellent medium which does not become acid and in which very active toxines are speedily formed, 0·1 c.c. being required for a lethal dose after thirty hours, and 0·002 c.c. after five to seven days.

MADSEN'S[3] method is to leave finely-minced lean veal, two to three days' old, in contact with twice its weight of water for fifteen hours, after which it is boiled and strained. The liquid is then mixed with 1 per cent. of Witte's peptone and 1 per cent. of sodium chloride, rendered faintly alkaline, boiled for forty-five minutes, filtered, and the filtrate transferred to Erlenmeyer flasks holding a litre, and sterilised for fifteen minutes at 120° C. in autoclaves. He determines the reaction with litmus paper, and also the "titer" of the bouillon—*i.e.*, the amount of normal soda solution required to give a distinct pink coloration with phenol-phthalein as indicator. The ratio of the two measurements is such that neutral solutions showing a "titer" of over 20 c.c. are unmistakably acid to litmus, while with 16 c.c. they are amphoteric, and below 10 c.c. are alkaline. According to his experience the age of the flesh has no material influence. The addition of calcium carbonate recommended by SPRONCK (*loc. cit.*) and VAN TURENHOUT (*loc. cit.*) keeps the reaction permanently alkaline, but at the same time does not materially promote the formation of toxine.

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[1] Nicolle, "Préparation de la toxine diphtérique," *Ann. Past.*, x., 333, 1896.

[2] Martin, "Production de la toxine diphtér," *ibid.*, xii., 26, 1898.

[3] Madsen, "Zur Biologie des Diphtherie bacillus," *Zeit. f. Hyg.*, xxvi., 157, 1897.

SPRONCK[1] subsequently abandoned his meat extract and cultivated his bacilli in a *decoction of yeast*, whereby he obtained very active solutions of poison. (After forty-eight hours the lethal dose was 0·05 c.c., and after five to six days 0·005 c.c.)

He boiled 1 part of commercial yeast with 20 parts of water for twenty minutes with continual stirring. He then allowed the mixture to settle, decanted the supernatant liquid, added ordinary salt and Witte's peptone (obtained directly from Rostock), neutralised it, and added normal soda solution in the proportion of 7 c.c. per litre. Lastly, he heated and filtered the liquid, and sterilised the filtrate at 120° C.

It is evident from these experiments that the production of toxines depends upon the alkalinity of the medium, and, as ROUX and YERSIN had already found, it increases with the increase in the alkalinity. MADSEN, however, found (*loc. cit.*) that the amount of toxine was not invariably proportional to the alkalinity.

A second method of increasing the yield of toxine is the introduction of *air*. ROUX and YERSIN found that a current of air promoted the production of poison, although, according to MARTIN (*loc. cit.*) and PARK,[2] this stimulation is not very pronounced, provided culture media, good in other respects, are employed; whilst MADSEN considers it directly injurious in such a case, on the ground that when there is a plentiful production of toxine the air destroys a greater amount of poison than is formed under its influence. SPRONCK, too, is inclined to believe that while the growth of the bacilli, and therefore the absolute production of toxine, is promoted by the admission of air, yet that the relative production of toxine by a given number of bacilli is thereby diminished rather than increased. VAN TURENHOUT (*loc. cit.*) considers that the admission of air accelerates the oxidation of the nitrogenous substances, and with it the cessation of the acid reaction, but that it also destroys poison. ARONSON[3] has very successfully replaced the introduction of air by growing the bacteria in *surface cultivations*, so that they offered as large an area as possible to the air. SCHIERBECK[4] recommends treatment with carbon dioxide.

[1] Spronck, "Préparation de la toxine diphtérique," *Ann. Past.*, xii., 701, 1898.

[2] Park, "The Preparation of Diphtheria Antitoxine," *Med. Record*, xlvii., 484, 1895.

[3] Aronson, "Immunisierungs- und Heilversuche bei der Diphtherie," *Wien. med. Woch.*, 1894, 1956.

[4] Schierbeck, "Ueb. d. Einfluss der CO_2 auf das Wachstum der Diphth.-B.," *Arch. f. Hyg.*, xxvii., 339, 1896.

Too much air and pure oxygen have a very injurious effect upon the poison, especially at the incubation temperature (Roux and Yersin, Martin, Madsen).

When cultivations are made in this way with every possible precaution to promote energetic toxic activity, very active toxines are usually obtained in a short time. Even after thirty to forty-eight hours they are unmistakably present. In seven to about thirty days the toxicity reaches its maximum, and then begins very slowly to decline, this being due to a simultaneous decrease of production in the cultivation as it grows old and to the commencement of the formation of toxoids. The transplantation of cultivations again restores their toxigenic power (Roux and Yersin). As a general rule, then, in testing primary poisons, cultivations are used that are not more than three weeks' old (Madsen, *loc. cit.*). For immunising purposes, however, much older poisons can frequently be used with advantage, since the toxoids they contain have also an immunising action.

The development of a bouillon of the greatest possible toxicity does not invariably correspond with that of a particularly virulent living cultivation; sometimes bacilli that are only slightly virulent may produce extremely active toxines (Martin, *loc. cit.*).

On the other hand, there are also varieties of diphtheria bacilli that possess neither virulence nor toxigenic power. Lubowski[1] has described an instance of this kind. A bacillus of human origin produced no active toxines whatsoever, whereas the serum of the child was strongly antitoxic. The inference is that the bacillus had been very toxigenic, but that it had completely lost its powers.

In general, the production of poison shows extraordinary variations, and its amount cannot be estimated beforehand, even in the case of a similar cultivation in the same nutrient medium. Madsen has vainly endeavoured to explain these variations; the kind of flasks and the sterilisation of the nutrient medium have no influence on the results.

We have reached such a stage in the preparation of the poison of diphtheria bacilli that for immunising purposes we now use only poisons 0·02 c.c. of which will kill with acute symptoms guinea-pigs of 250 grms. The essential conditions for the production of the virus are:—

1. A suitable cultivation which grows upon the surface—*i.e.*, develops on the bouillon in the form of a membrane;

2. Alkalinity of the bouillon;

[1] Lubowski, "Ueb. einen atoxischen- und avirulenten Diphtheriestamm," *Zeit. f. Hyg.*, xxxv., 87, 1900.

3. Presence of a suitable peptone, preferably Chapoteaut's, in the proportion of 2 per cent.;

4. A sufficient supply of air, for which reason the flask should only be filled to about a third of its capacity;

5. Not too short and not too long a period of growth at 37° C. This depends upon the culture, and tests must be made with each different variety to determine when the maximum production of poison has been attained. As a rule, it takes from about ten days to three weeks.

Lastly, means have also been sought to obtain a solution of poison which, while effecting rapid immunisation, would yet be but little poisonous, and so would not endanger the lives of the animals used for the experiments even when given in large doses. Brieger, Kitasato, and Wassermann[1] have found that diphtheria cultivations grown upon *thymus bouillon* lose their toxigenic capacity whilst their immunising power is but little affected. Here there is presumably a formation of immunising toxoids. Then, still more recently, Madsen has succeeded in effecting immunisation by means of the *toxones* of diphtheria, and we shall have more to say about this when we deal with them later on.

When liquids rich in poisons have thus been obtained, all that is necessary is to sterilise them. The methods employed for this purpose are the ordinary ones in general use: heat, addition of antiseptics, and filtration through bacterial filters.

Roux and Yersin found that solutions of the poison were rendered non-poisonous after a few minutes at 58° C., but that the dry poison could be heated for more than an hour at 98° C.

Fränkel[2] endeavoured to sterilise cultivations by heating them for an hour at 65° to 70° C., whilst Brieger and Fränkel[3] found that small quantities could be sterilised with certainty when heated from three to five hours at 50° C., but that the poison was speedily destroyed above 60° C.

Behring and Wernicke[4] added calcium chloride to obtain a precipitate of calcium phosphate in the cultivations, and sterilised the dried precipitate by heating it at 77° C., and this did not materially injure the toxine simultaneously carried down.

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[1] Brieger, Kitsato, and Wassermann, "Ueb. Immunität. u. Giftfestigung," *Zeit. f. Hyg.*, xii., 137, 1892.

[2] Fränkel, "Immunisierung Versuche bei Diphth.," *Berl. klin. Woch.*, 1890, 1133.

[3] Brieger and Fränkel, "Ueber Bakteriengifte," *ibid.*, 1890, 240.

[4] Behring and Wernicke, "Ueb. Immunisier. u. Heilung von Versuchstieren b. d. Diphth.," *Zeit. f. Hyg.*, xii., 10, 1892.

Of the chemical agents that destroy the living bacilli, and simultaneously have a preservative influence upon the poison solution, and are also added for the latter purpose *alone* after filtration, the following are employed :—

Iodine trichloride, ICl_3, by BEHRING and WERNICKE.

Tricresol, in a 0·3 per cent. solution, by ARONSON (*loc. cit.*).

Phenol, also in a 0·3 per cent. solution (SPRONCK, *loc. cit.*).

Toluene is used by EHRLICH, who keeps his poisons at 15° C. in the dark under a layer of that substance. According to ABBA,[1] diphtheria toxine can be preserved for two years if kept under toluene in the dark and in the cold.

The best and most suitable means of preparing and preserving diphtheria virus for practical immunising purposes is the following method, described by EHRLICH and WASSERMANN:—The bouillon cultures prepared in the manner described above are filtered through double filter paper, so that the coarse bacterial membranes are separated. The filtrate is then covered with a layer of toluene of the depth of about two fingers'-breadth, and the whole thoroughly shaken at frequent intervals during two days. All living micro-organisms will then be destroyed and the liquid will contain only the poison. When required for use the poison is invariably withdrawn by means of a pipette, inserted beneath the preservative layer of toluene. The poison must be kept in a cool place, and in particular protected from light by being placed in black flasks or flasks covered with paper. This is the method of preparing and preserving the poisons in use in the Prussian Control Station.

For more delicate biological and chemical investigations this rough filtration is insufficient, and in such cases the liquid must be completely freed from the cells of the bacilli by filtration through a proper bacterial filter, and then sterilised.

Attempts to Prepare Pure Diphtheria Toxine.—Attempts to isolate *diphtheria toxine* in a pure or, at least, concentrated condition were made even in the earliest period of the investigation of diphtheria bacilli.

LÖFFLER[2] found that evaporation or shaking with ether did not yield active poisons; but, on the other hand, by extracting inoculated meat broth with glycerin he obtained a slightly toxic substance which could be precipitated by means of alcohol. He termed this, even in this condition, an "enzyme."

[1] Abba, "Ueb. d. Dauer des toxisch. Vermögens beim D.-T. u. Antit.," *Centralbl. f. Bakt.*, xxiii., 934, 1898.

[2] Löffler, "Der gegenw. Stand d. Frage n. d. Entsteh. d. Diphtherie," *Deutsch. med. Woch.*, 1890, Nos. 5 and 6.

Subsequently ROUX and YERSIN, MADSEN, and others obtained, by means of precipitation with alcohol, saturation with ammonium sulphate, and precipitation with calcium phosphate, active dry preparations, which, of course, made no pretensions to purity. The fundamental idea underlying these experiments is that the diphtheria poison is carried down mechanically by the voluminous precipitate—*e.g.*, of calcium phosphate—produced in its solution.

BRIEGER was the first to undertake these investigations in a systematic and thorough manner. At first, in collaboration with C. FRÄNKEL,[1] he looked for ptomaines in the diphtheria cultures, but soon had to admit that there were absolutely no volatile bases present. On this account BRIEGER has considerably modified his views on the significance of *ptomaines*. He now obtained from the cultivations, not only of diphtheria bacilli, but also of many other bacteria, poisonous proteid substances which he termed toxalbumins.

The method by which BRIEGER and FRÄNKEL isolated their diphtheria toxalbumins is as follows :—

The *globulins* are first separated from the bouillon by saturating it with magnesium sulphate at 30° C. This gives a slight precipitate which is completely non-poisonous.

The poisonous proteids are next precipitated from the bouillon thus treated, or, equally well, from the fresh bouillon, by means of *ammonium sulphate* or *sodium sulphate*, or also by the addition of a large excess of alcohol. Since the ammonium sulphate precipitate must be redissolved and dialysed (not without loss), it is best to employ precipitation with alcohol.

The bouillon is evaporated to a third of its volume at 30° C., and treated with ten times its quantity of absolute alcohol, preferably with the addition of a few drops of acetic acid. After standing for twelve hours in an ice-chest the liquid is filtered, and the precipitate taken up with water and again precipitated with alcohol, this treatment being repeated six or eight times until the substances dissolve to a completely clear solution in water. Finally, the preparation is dialysed and dried *in vacuo* at 40° C.

This treatment yields a snow-white powder, the aqueous solution of which does not coagulate on boiling. It gives no precipitate with sodium sulphate, sodium chloride, magnesium sulphate, nitric acid, or lead acetate, but is precipitated by saturated solutions of carbon dioxide and by all the usual reagents for proteids. The substance is thus allied to the albumoses; it yields a *benzoyl* derivative, but not a phenyl-hydrazine compound. In the dry state it can be heated to 70° C. without injury. It is poisonous, a dose of 2·5 mgrms.

[1] Brieger and Fränkel, "Ueber Bakteriengifte," *Berl. klin. Woch.*, 1890, 241.

per kilo. causing certain death. Hence, it is not very poisonous, a part of the toxine apparently having been destroyed.

WASSERMANN and PROSKAUER[1] have modified this method. They evaporate the bouillon (which has been neutralised by the addition of 10 to 12 c.c. of normal soda solution, and sterilised by filtration through KITASATO'S filters, at a temperature of 27° to 30° C. *in vacuo* to a tenth of its volume. It is then dialysed in running water at a lower temperature, by which means the salts and *peptones* are removed, and the *globulins* (which are *not poisonous*) partially precipitated. The contents of the dialyser are filtered until clear—an important point—and then poured into ten times their volume of 60 to 70 per cent. alcohol acidified by the addition of a few drops of acetic acid, and allowed to stand for twenty-four hours. The resulting precipitate is separated by filtration and the filtrate allowed to fall drop by drop into *absolute alcohol*. The new precipitate that is formed is also filtered off, and both dissolved in a small quantity of water and precipitated by the addition of twice the amount of a saturated solution of ammonium sulphate. This precipitate is again dissolved, and the solution dialysed until it no longer gives the sulphate reaction, after which it is again introduced into absolute alcohol and the process repeated until the aqueous solution is perfectly clear. The preparations are then dried *in vacuo* at 37° C. They give all the reactions of albumoses. Only preparations precipitated by alcohol of, at least, 60 per cent. strength are poisonous—not the others, so that in this way it is possible to effect a further separation from the bulk of proteids simultaneously precipitated. These products were also obtained in the same manner from extracts made with glycerin and ordinary salt from the organs of human beings infected with diphtheria. All these preparations, however, were only *very slightly poisonous*. Ten mgrms. were required to kill a rabbit in three to four days, and with 3 mgrms. death did not take place until after eight weeks.

Products of greater toxicity were obtained by WASSERMANN and PROSKAUER by extracting the organs of poisoned rabbits with glycerin. This process yielded a white powder which, when injected in a dose of 0·2 mgrm. into the veins, killed a rabbit in six to fourteen days.

The separation of the true toxine from the associated proteid impurities, and therewith our knowledge that the active principle is not a protein is also due to BRIEGER,[2] who, in collaboration with BOER, has prepared the toxine in a fairly pure condition.

For this purpose, BRIEGER and BOER made use of the method of precipitating the toxine in the form of a double salt of zinc. The precipitation is practically quantitative, at all events in the case of diphtheria poison, but the difficulty of separating the toxine from the zinc is rendered the greater by the fact that hydrogen sulphide, which

[1] Wassermann and Proskauer, "Ueb. die von d. Diphtheriebacillen erzeugten Toxalbumine," *Deutsch. med. Woch.*, 1891, 585.

[2] Brieger and Boer, "Ueb. d. Toxine d. Diphth. u. d. Tetanus," *Deutsch. med. Woch.*, 1896, 783; *Zeit. f. Hyg.*, xxi., 259, 1896.

would otherwise have been a very suitable agent, destroys the toxine. After long and careful experiments they arrived at the following method of separation :—

The poison-bouillon (blood serum was used with good results as the culture medium) is treated with twice its volume of a 1 per cent. solution of zinc chloride, and the resulting precipitate thoroughly washed with water, and then vigorously shaken with a 3 to 6 per cent. solution of ammonium carbonate. Ammonium phosphate solution is next added until the whole of the precipitate passes into solution, and there is only a slight turbidity due to separated zinc phosphate. This is allowed to subside and then collected on a toughened filter and thoroughly washed with water. The filtrate is saturated with solid ammonium sulphate, the precipitate redissolved in water, and the solution precipitated with solid sodium sulphate, by which means the peptones are left in solution.

As thus prepared the toxine no longer gives the reaction of proteids. The zinc compound also shows no proteid reactions, and is optically inactive, but turns red when boiled with a solution of iron chloride.

Alcohol, ether, acetone, acids, and weak oxidising agents rapidly destroy the poison, while weak alkalies and reducing agents do not affect it. *Diphtheria toxine* can also be obtained, although, of course, only in very small quantities, from dialysed urine—*i.e.*, proteid-free culture medium (Guinochet, *loc. cit.*)—and from other similar nutrient liquids (Uschinsky[1]). The culture medium used by Uschinsky had the following composition :—Glycerin, 40 to 50 parts; sodium chloride, 5 to 7 parts; ammonium lactate, 10 parts; calcium chloride, 0·1 part; magnesium sulphate, 0·2 part; and potassium hydrogen phosphate, 1 part; in 1,000 parts of water. The poison did not give the usual proteid reactions.

Properties of Diphtheria Toxine.—The poison is not known in a state of chemical purity. Hence, all that can be stated about it relates to the preparations that contain it in admixture with other substances. Its most important properties have already been described by Roux and Yersin.

It is probably not a *proteid*, since the purest preparations (*vide supra*) do not give the proteid reactions. An attempt made by Arrhenius and Madsen[2] to determine the molecular weight from the speed of diffusion into gelatin has so far only led to the conclusion that toxines have at all events a much smaller molecular weight than antitoxines.

[1] Uschinsky, "Ueber Diphtherieculturen auf eiweissfreier Nährlösung, *Centralbl. f. Bakt.*, xxi., 146, 1897. *Id.*, "Les Poisons de la Diphtherie et du Cholera," *Arch. de Méd. Expér.*, 1893, 293.

[2] Arrhenius and Madsen, "On the molecular weight of Di.-T.," *Festschrift des Statens Serum Institut*, Copenhagen, 1902.

It is very sensitive to external influences. The true toxine is very rapidly destroyed by boiling and fairly rapidly at about 60° C., but the heated solution still retains a certain toxic power, and when injected into animals produces emaciation and paralysis as a secondary affection, and frequently causes death. The poison appears to undergo a similar change within the organism. ROUX and YERSIN have prepared poisonous substances with quite analogous properties from the organs and urine of children who had suffered from severe attacks of diphtheria. These produced toxic effects resembling those caused by *toxones* (*vide infra*).

The simultaneous action of oxygen and light injure diphtheria toxine very rapidly, but either of the factors alone has much less effect.

According to PIAZZA,[1] diffused daylight acts very slowly; a perceptible decrease can be observed after twenty-three days, but it does not become considerable until after ninety-six days. Direct sunlight acts very energetically when oxygen is admitted. Only the ends of the spectrum, the heat rays, and the actinic rays (red and violet) have any action, the centre (yellow) being completely inactive.

The toxine is also rendered inactive by *acids*. ROUX and YERSIN state that acidification of a solution of the poison with lactic or tartaric acids renders it nearly harmless; phenol, boric acid, and borax have a less injurious effect. Neutralisation partially restores the activity.

According to BRIEGER and BOER, it is extraordinarily sensitive to the action of oxidising agents, whereas *reducing agents* in slightly alkaline solution have but little effect. This explains why the slightly alkaline reducing fluids of the body form its best medium. DÉLÉARDE[2] asserts that it is rendered innocuous by *antipyrine*. It is also destroyed by other antiseptics when more concentrated, and by salicylaldehyde (SALKOWSKI[3]). Yeast weakens the toxine (NOBÉCOURT[4]).

It is extremely probable that, in general, the nature of the surrounding medium has an influence upon the condition and the action of the toxine, but it is far too sweeping, and is contra-

[1] Piazza, "Influenza della luce solare sulla tossina difter.," *Ann. d'Igiene Sperim.* [New Series], v., 521, 1895; Abst. *Centralbl. f. Bakt.*, xix., 914, 1896.

[2] Déléarde, "Rech. expér. sur les propriétés, &c., de l'antipyrine," *Arch. de. Méd. Exper.*, 1897, 786.

[3] Salkowski, "Ueb. d. Wirkg. d. Antiseptica auf Toxine," *Berl. klin. Woch.*, 1898, 545.

[4] Nobécourt, "Action des levures, &c.," *Soc. Biol.*, lii., 753, 1900.

dicted by the undoubted changes that can be detected in the poison itself, to attempt, like Danysz,[1] to attribute every alteration in the action to changes in the surrounding media. The other external characteristics are quite analogous to those belonging to all enzymes.

Diffusion through parchment can be plainly perceived. Thus, when 5 c.c. of the poisonous bouillon were diffused into 12 c.c. of water, there passed through, on the average, in twenty-four hours a lethal dose for guinea-pigs (Roux and Yersin). On the other hand, it does not diffuse through membranes formed from the organs of animals—*e.g.*, the œsophagus, large intestine, the gall bladder, and the small intestine (Chassin and Moussu[2]). Nor does it pass through collodion (Rodet and Guéchoff[3]).

It is completely insoluble in pure alcohol, which, however, slowly destroys it (Wassermann and Proskauer, *loc. cit.*), as also does acetone and ether (Brieger and Boer, *loc. cit.*). It is likewise destroyed by the digestive ferments in the stomach and intestine (Paltschikowski[4]).

Like the enzymes, it is carried down from its solutions by falling precipitates. On fractional precipitation with calcium chloride the resulting calcium phosphate precipitate simultaneously removes a large proportion of the toxine, the second precipitate being the most poisonous. The precipitation, however, is never quite complete, still less so in the case of aluminium phosphate. In particular, the characteristic poisons that produce the secondary paralysis remain for the most part in solution. This calcium phosphate precipitate can be heated to 70° C. without injury, and is not perceptibly damaged by being kept for twenty minutes at 100° C. When subcutaneously injected it slowly parts with its poison, besides producing inflammatory processes (separation of fibrin, false membranes), so that it appears to act exactly like the living bacilli. It is also precipitated by nucleo-histone and nucleic acid (Freund and Grosz[5]). Toxines almost free from ash can be obtained by dialysing the aqueous extract of this precipitate, or by precipitation with alcohol.

[1] Danysz, "Constitution des toxines," *Ann. Past.*, xiii., 581, 1899.

[2] Chassin and Moussu, "Influence de la dialyse, &c.," *Soc. Biol.*, lii., 694, 1900.

[3] Rodet and Guéchoff, *Soc. Biol.*, lii., 965, 1900.

[4] Paltschikowski, "Ueb. d. Veränderungen der diphtheritischen Toxine in dem Nahrungswegen," Abst. *Centralbl. f. Bakt.*, xxv., 1899. See also the General Part.

[5] Freund and Grosz, "Ueb. d. Bez. zw. Gerinnung u. d. Wirkg. d. Antitoxine," *Centralbl. f. inn. Med.*, 1895, 613, 637.

Special attention was given some time ago to the behaviour of solutions of diphtheria virus under the influence of *electric currents*. In the first place, SMIRNOW[1] and KRÜGER[2] allowed continuous currents of low intensity to act upon the toxines, and found that when the current was cautiously applied there was a slight development of acidity at the anode. When the degree of acidity was such that .1 c.c. of the liquid neutralised about 1·2 c.c. of normal soda solution the current was discontinued, and it was then found that the *toxic value* had been considerably lowered, but not the *immunisation value*. Perfectly analogous results were obtained by D'ARSONVAL and CHARRIN[3] in their experiments with high-tension alternating currents of great power. On these results were based theoretically untenable and very far-reaching speculations on the formation of "artificial therapeutic serum" without the aid of an animal, &c.; in addition to which the fantastic idea occurred to D'ARSONVAL and CHARRIN of treating infection in the body itself by means of such high-tension currents, which, as is well known, have no action at all upon man.

All these somewhat mystical conceptions, however, about the influence of electricity have been explained by MARMIER.[4] He has shown that during the electrolysis with continuous currents oxidising substances are formed, notably hypochlorite and free chlorine from sodium chloride invariably present, and that these have a secondary action on the toxophore group of the poison.

As regards alternating currents, MARMIER was able to demonstrate that, in spite of cooling with ice, such intense heating occurred that this alone was sufficient to explain the weakening of the poison. When he excluded this factor by means of ingenious devices, there was no perceptible action on the toxine either of diphtheria or tetanus.

Hence we have here to deal with *secondary* alterations under the influence of the electric current, and these evidently tend to a more rapid *formation of toxoid*, with accompanying destruction of the toxophore group.

Physiological Action of Diphtheria Toxine.—Diphtheria toxine is extremely poisonous to many animals. ROUX and YERSIN estimate the single lethal dose for a guinea-pig at about 0·05

[1] Smirnow, "Ueb. d. Behandlung d. Diphtherie," *Berl. klin. Woch.*, 1894, 683; 1895, 645, 675.

[2] Krüger, "Ueb. die chem. Wirkg. d. Electriz. auf toxische und immunis. Bakteriensubstanzen," *Deutsche med. Woch.*, 1895, 331.

[3] d'Arsonval and Charrin, "Action des Courants á haute frequence sur les toxines bactériennes," *Comptes Rend.*, cxxii., 280, 1896.

[4] Marmier, "Les toxines et l'électricité," *Ann. Past.*, 1896, 469.

mgrm., and for a rabbit about 0·1 mgrm. of the organic ash-free substance, containing only a fraction, and possibly only a very small one, of the pure toxine.

Calabrese and Zagari[1] assert that it has absolutely no action upon cold-blooded animals, but Courmont, Doyon, and Paviot[2] observed paralysis and emaciation in the case of warmed frogs. The action of the toxine corresponds in all essential points with the general effects produced by the living bacilli. Hence it is unnecessary to give an exact description of these symptoms here. We will content ourselves rather with a quite superficial survey, since the symptoms of diphtheria are described in detail in numerous text-books.

As regards the *general* action of diphtheria virus, the following points have been recorded:—

One of the most striking symptoms is the considerable enlargement of the blood-vessels, which occurs after a certain period of incubation (*vide infra*).

The *temperature* first rises and then sinks below normal (to 25° C.), this being due, according to Arloing and Laulanié,[3] to the decrease in the vital energy and the oxidising processes. With very large doses the hypothermia may persist (Courmont and Doyon[4]).

The whole metabolism of animals after poisoning with diphtheria toxine has been studied, with reference to the amounts of chlorine and nitrogen, by Pace[5] and others. One of the phenomena is an extensive decomposition of albumin.

Special interest attaches to the question whether diphtheria toxine has a *directly* injurious effect upon the heart's action or not. It is true that collapse of the heart eventually occurs, and that the heart remains arrested in diastole; but it is not yet decided whether this is not the secondary result of a primary *paralysis of the vasomotors.*

Krassnow[6] asserts that the main action of diphtheria toxine

[1] Calabrese and Zagari, "Ricerche sulla tossina ed antitossina difter.," *Giorn. Internaz. di Scienze Mediche*, 1895, No. 4, 19-21; *Baumgarten's Jb.*, 1895, 215.

[2] Courmont, Doyon, and Paviot, "Action de la toxine diphtérique sur le Systéme nerveux de la grenouille, &c.," *Soc. Biol.*, xlvii., 210, 1895.

[3] Arloing and Laulanié, "Ét. expér. sur les troubles imprimés . . . par les toxines diphth.," *Soc. Biol.*, xlvii., 433, 1895.

[4] Courmont and Doyon, "Marche de la température dans l'intoxication diphth.," *Arch. de Physiol.*, xxvii., 252, 1895.

[5] Pace, "Influenza della tossine difter.," *Il Policlinico*, 7; *Baumgarten's Jb.*, 1900, 180.

[6] Krassnow, "Zur Pharmakol des Di.-T.," *Wratsche-bnaja Gazetta*, 1904, 23; *Biochem. Centralbl.*, iii., No. 304, 1904.

is upon the respiratory centres, and that only in the final stages is the heart itself also affected.

ENRIQUEZ and HALLION[1] found that the pressure of the blood did not fall until after some time; this was confirmed by BECK and SLAPA,[2] who only observed a sinking in the pressure of the blood shortly before death.

Comprehensive investigations into the behaviour of the heart's action in cases of poisoning with diphtheria toxine have been made by ROMBERG, PÄSSLER, BRUHNS, and MÜLLER,[3] who conclude from the results that there is not a primary effect upon the heart's action, but that the injury to the vasomotors is the only *direct* action. The heart is only injured in consequence of a deficient supply of blood. STEJSKAL[4] has severely criticised these results and claims, on the strength of his assumed more accurate experiments, that diphtheria virus does cause direct injury to the heart.

He concludes that after a very short acceleration of the heart's action there occurs a short period of continuous weak action, which is followed by a period of acceleration, the final result being a decided decrease in the work done by the heart, attributable to injury of the heart rather than of the vasomotors.

These conclusions are supported by the researches of FENYVESSY[5] and SHARP,[6] who were also able to prove the direct effects of diphtheria virus upon the isolated heart of a frog.

MOLLARD and REGAUX[7] observed myocarditic alterations in the heart after death, which possibly are also to be regarded as evidence of direct injury.

EPPINGER[8] also observed serious changes, which he termed *myolysis*, in the muscle of the heart. From this he concluded

[1] Enriquez and Hallion, "Sur les effets physiol. de la toxine diphth.," *Arch. de Physiol.*, xxvii., 515, 1895.

[2] Beck and Slapa, "Ueb. den Einfl. des D.-G. auf den Kreislauf," *Wien. klin. Woch.*, 1895, 333.

[3] Romberg, Pässler, Bruhns, and Müller, "Unters. üb. d. allg. Pathol. der Kreislaufstörung bei akuten Infektionskrankh.," *Arch. f. klin. Med.*, lxiv., 652, 1899.

[4] Stejskal, "Kritisch-experim. Unt. üb. d. Herztod infolge von Diphth.-Toxin.," *Z. f. klin. Med.*, xliv., 367, 1902.

[5] Fenyvessy, "Ueb. d. Wirkg. d. D.-T. u. Antit. auf das Froschherz.," *Jahrb. f. Kinderh.*, new series, xliii., 216, 1896.

[6] Sharp, "The action of the products of the organism of diphtheria on the heart of a frog," *Journ. of Anat. and Physiol.*, xxxi., 199, 1897.

[7] Mollard and Regaux, "Lésions du myocarde dans l'intox. aigüe par la t. d.," *Ann. Past.*, xi., 97, 1897.

[8] Eppinger, "Die toxische Myolyse des Herzens bei Di.," *Deutsch. med. Woch.*, 1903, Nos. 15, 16.

that the toxine attacked the muscle directly, combined with it, and thus injured it.

As regards individual organs, the following facts are on record:—

Intestine.—Courmont, Doyon, Paviot[1] observed exudations and *enteritis membranacea* in the small intestine of dogs.

Liver.—Courmont, Doyon, and Paviot[2] found a *hepatitis parenchymatosa*, with *hyperæmia* and internal hæmorrhage, in dogs after intravenous injection. Baldassari[3] observed thick swellings and similar changes, such as occur in poisoning by *phosphorus* and *arsenic*. He records the occurrence of epithelial changes in the *kidneys*.

Local lesions of the *eye* similar to those that occur in diphtheria have been observed by Morax and Elmassian.[4] Gatti[5] has also found that direct contact of diphtheria toxine with the retina produced injuries which led to swelling of the tissue, with conservation of the nuclei and ganglionic cells.

But, above all, it also causes serious disturbances in the *central nervous system*, which we cannot describe in detail here. Investigations on this point have been made by Enriquez and Hallion,[6] Crocq fils,[7] Thomas,[8] Murawjew,[9] and others.

It is very questionable, however, whether these lesions of the nervous system are to be directly attributed to diphtheria toxine as such. So far as these changes have to do with the diphtheritic secondary paralysis, they must probably be attributed to the *toxones*. Whether the pure toxine can also produce secondary paralysis, or whether this is only a function of the toxones, cannot be asserted with certainty as yet, although the latter alternative is the more probable. The symptoms of

[1] Courmont, Doyon, and Paviot, "Lésions intestinales dans l'intoxic-diphth.," *Arch. de Phys.*, xxvii., 484, 1895.

[2] Courmont, Doyon, and Paviot, "Lésions hépatiques engendrés par la t. d.," *Arch. de Phys.*, xxvii., 687, 1895.

[3] Baldassari, "Ueb. d. Wirkg. d. D.-T. auf den Zellkern," *C. f. allg. Pathol.*, vii., 625, 1896.

[4] Morax and Elmassian, "Action de la toxine d. sur les muqueuses," *Ann. Past.*, xii., 210, 1898.

[5] Gatti, "L'azione di alcune tossine batteriche sopra gli elementi della retina," abst. by the author in *Biochem. Centralbl.*, i., No. 775, 1903.

[6] Enriquez and Hallion, "Myelite expér. par t. d.," *Soc. Biol.*, xlvi., 312, 1894.

[7] Crocq, "S. l'altérat. du. Syst. nerveux dans l. paralys. diphth.," *Arch. med. expér.*, vii., 503, 1895.

[8] Thomas, *Boston Med. and Surg. J.*, 1898, No. 4, *et seq.*

[9] Murawjew, "Des D.-T. u. Antit. in ihrer Wechselwirkg. auf das Nervensystem d. Meerschw.," *Fortschr. d. Med.*, 1898, 93.

general poisoning are completely analogous to those produced by the living bacilli, so that we are justified in assuming that the latter cause such deleterious effects *solely* by means of the toxine that they produce.

Guinea-pigs after poisoning with diphtheria toxine show, on *post-mortem* dissection, the typical appearance of the disease produced by the bacilli.

FLEXNER[1] has given a most careful description of the appearance on section after poisoning with diphtheria toxine: œdema, swollen glands, *congestion* and *hæmorrhage* of the suprarenal bodies; otherwise there is little that is characteristic even under the microscope. COUNCILMAN, MALLORY, and PEARCE[2] have also made a careful investigation of these symptoms.

ROUX and YERSIN were able to produce a typical pseudo membrane in guinea-pigs by inoculation of the trachea and vagina with diphtheria virus.

The *guinea-pig* is the *most susceptible* animal, but, according to EHRLICH, different varieties have different degrees of susceptibility. *Horses*, *goats*, and *sheep* are very susceptible. *Rabbits* are less susceptible, and *mice* still less, being almost immune.

After subcutaneous or intravenous inoculation the poison disappears from the circulation with extraordinary rapidity, being concentrated and fixed by the receptors of the tissue. This can be recognised by the fact that doses of antitoxine introduced *after* the injection of the poison lose all power of action with extreme rapidity as the difference in time increases.

DÖNITZ[3] found that a quantity of antitoxine just sufficient to neutralise seven times the lethal dose could no longer save the animal fifteen minutes after inoculation, and that after one and a half hours even very large doses were insufficient.

On the other hand, the toxic power of the blood after injection of diphtheria toxine has also been *directly* investigated. BOMSTEIN[4] inoculated rabbits with a dose double that required to kill a guinea-pig for each c.c. of the blood of the rabbit. After an hour there was still 0·5 thereof present, after three hours 0·25, and after twelve hours 0·12. The rate of decrease

[1] Flexner, "The pathology of toxalbumin intoxication," *Johns Hopkins Hosp. Record*, vi., 259, 1897 (reprint).

[2] Councilman, Mallory, and Pearce, "Diphtheria: A Study of Bacteriology, &c.," 1901. Quoted in detail by Vaughan and Novy, *The Cellular Toxines*, 1902, p. 75, *et seq.*

[3] Dönitz, "Ueb. d. Grenzen d. Wirksamkeit d. Diphtherieheilserums," *Arch. internat. d. Pharmacodyn.*, v., 425, 1899.

[4] Bomstein, "Ueb. d. Schicksal der Diphth.-T. in Tierorganismus," *Centralbl. f. Bakt.*, xxii., 785, 1898.

is thus continually smaller, and the last traces seem to disappear very slowly.

Similar results were obtained by CROLY.[1] He found the toxine still unchanged after five minutes, and that half, at most, had disappeared after two hours. The poison is then, also, not to be detected in the organs of the body, nor is it excreted by the *urine* or *intestinal fluid* (BOMSTEIN), although BRUNNER[2] claims to have detected it in the *juices of the muscle*, and SALTER[3] in sweat (?).

It does not act by way of the stomach or the intestines. It is destroyed, like all other toxines, by the digestive fluids, notably by the liquid of a *pancreatic fistula;* less readily by the gastric juice. In very large doses it shows a certain amount of activity (NENCKI, SIEBER, and SCHOUMANOWSKI[4]), as also when the mucous membrane is artificially injured (see also the General Part).

CONSIGLIO[5] claims to have observed a characteristic property of diphtheria toxine. He found that in small doses it had a stimulating effect upon fermentative processes, but a restrictive one in larger doses; whilst, on the other hand, it invariably had a very unfavourable influence upon the germination processes of seeds.

Toxoids and Toxones.—Nothing whatever is known of the properties of the *toxoids* that occur in old cultures (see General Part). We have not the slightest idea of the chemical process which induces the supposed inactivity of the toxophore group. Nor can we state whether they are physiologically indifferent in the free state, or whether they still possess a slight toxic capacity, although, according to numerical results—sharply differentiated values obtained during the alteration of the poison—the latter alternative is not very probable.

The only proof that other substances are present in weakened cultures is the fact recorded by BRIEGER and FRÄNKEL (*loc. cit.*) that they found in such cultivations a *non-poisonous* substance, which was somewhat soluble in dilute alcohol, and could be distinguished from the *toxine* by chemical tests—*e.g.*, by the formation of a phenyl-hydrazine compound. This sub-

[1] Croly, "Sur l. disparition de la tox. diphth. injectée dans le sang.," *Arch. internat. de Pharmacodynamie*, iii.

[2] Brunner, "Unt. üb. die Wirk. von Bakterien- u. Pflanzengiften," Abst. *Centralbl. f. Bakt.*, xxiv., 184, 1898.

[3] Salter, "The Elimination of Bacterial Toxines," *Lancet*, 1898, i., 152; *cf.* Walsh, *ibid.*, 362.

[4] Nencki, Sieber, and Schoumanowski, "Die Entgiftung der Toxine," *Centralbl. f. Bakt.*, xxiii., 840, 1898.

[5] Consiglio, "Azione di alcune tossine, &c.," *Arch. di Farm.*, vi., No. 3, 1898; *Malys Jb.*, 1898, 634.

stance was also found in abundance by WASSERMANN and PROSKAUER (*loc. cit.*) in weakened cultivations. It must be left an open question whether it has anything to do with the toxoids.

On the other hand, we know that free *toxones*, which form a second primary decomposition group of products, and can be studied beyond doubt in the transitional stage, do exert quite distinct toxic effects. EHRLICH[1] has observed œdema, and secondary paralytic affections. MADSEN[2] found that they produced *œdema*, but not *necroses* or *alopeciæ*. The secondary paralysis occurred in thirteen to thirty-three days, and, in the case of large doses, invariably ended in death. He also observed differences in the behaviour of different cultures. With very much weakened poisons, in particular (*i.e.*, those in which a large amount of toxones was present in the transition from L_0 to L_+), there was a more frequent occurrence of cases in which death was delayed. On the other hand, it is very remarkable that *rabbits* are killed with very acute symptoms by toxones (EHRLICH).

But the question of toxones is also of great theoretical interest. If toxones are really true haptines—*i.e.*, substances which possess haptophore groups identical with, or closely allied to, those of the true toxines—they must also be able to throw off receptors—*i.e.*, to produce immunity, if EHRLICH's side-chain is correct.

This very interesting question has been experimentally investigated by MADSEN and DREYER,[3] and answered in the affirmative. In the case of many poisons which were so far neutralised by antitoxine (*vide supra*) as to contain only toxones still in the free state, they succeeded in producing a fairly high degree of *immunity* in guinea-pigs, rabbits, goats, and horses. The serum of the animals thus treated contained fairly considerable, though very fluctuating, quantities of *antitoxine*, reaching nearly as much as 400 I.E. per c.c. in the case of a horse. They were then also immune against the action of large doses of the fully poisonous *toxine*, from which the conclusion may be drawn that there is a close relation between the haptophore groups of the toxones and those of the true toxine.

The rabbit occupied an exceptional position. At first the animal proved extremely susceptible. Mixtures which only produced the effect of toxones upon guinea-pigs still produced

[1] Ehrlich, "Zur Wertbemessung des Diphtherieheilserums," *Klin. Jahrb.*, vi.

[2] Madsen, "Constit. du Poison diphthérique," *Ann. Past.*, xiii., 568, 1899.

[3] Madsen and Dreyer, "Ueb. Immunisierung mit d. Toxonen d. Diphtheriegiftes," *Zeit. f. Hyg.*, xxxvii., 249, 1901.

acute symptoms in the rabbit. On the other hand, most of the animals experimented upon with such mixtures, which undoubtedly contained nothing but toxones still in the free state, perished later with secondary paralytic affections. One animal, however, remained alive and was able to receive without injury very large amounts of toxone, eventually even several multiples of a lethal dose of the toxine; but its serum never showed even *the slightest trace of an antitoxine constituent.*

No explanation of this remarkable phenomenon can yet be given, unless we are willing to accept the view that although the active immunisation had stimulated the formation of protective substances, these had not been thrown off in a free state into the circulation (" *sessile receptors* " in EHRLICH's terminology).

Owing to the weak affinity of diphtheria toxone it is fixed much more slowly by the receptors of the organism—*i.e.*, it does not disappear so rapidly from the circulatory system as the toxine. We have shown above that even fifteen minutes after the injection of seven times the lethal dose DÖNITZ was no longer able to save the animal by means of a quantity of antitoxine exactly neutralising the poison *in vitro.*

DREYER[1] has made corresponding experiments with the toxones.

After two hours the equivalent amount of antitoxine was invariably able to render completely harmless a dose of toxone which would otherwise have been undoubtedly fatal after twelve to eighteen days, both being introduced into the rabbits by intravenous injection. With an interval of five hours between the injections the first signs of paresis appeared; with ten hours' interval they invariably occurred, though not until after twenty to twenty-two days; with an interval of sixteen to twenty-four hours the antitoxine was *completely* powerless.

Similar results were obtained in the *therapeutic experiments* made by DREYER with larger quantities of antitoxine, both substances being subcutaneously injected into guinea-pigs. A five-fold dose introduced twenty-four hours after the injection of a dose of toxone which would be undoubtedly fatal after twelve to eighteen days, was sufficient to prevent death, though not invariably to prevent paresis.

Of seven animals that received after forty-eight hours a dose of antitoxine neutralising 5,000 to 10,000 times the amount of toxone injected, one had no paresis, while the others had only slight attacks ending in a cure after eighteen to twenty-five days.

[1] Dreyer, "Ueber die Grenzen d. Wirkung d. Diphtherieheilserums gegenüber d. Toxonen," *Zeit. f. Hyg.*, xxxvii., 267, 1901.

After an interval of 4 × 24 hours, and with a dose of antitoxine neutralising 21,000 times the amount of toxone, there were two fatal cases of paresis, while one recovered after eighteen to twenty-one days. With an interval of 5 × 24 hours and the same dose of antitoxine (neutralising 21,000 times the amount of toxone) there were three cases of paresis, one of which ended in death after sixteen to twenty-nine days, while the other two recovered. The toxone is thus fixed relatively very slowly, and can still be influenced for a long time by the antitoxine, and probably also *detached* much more readily than the toxine from an already formed combination with the receptor.

Diphtheria Antitoxine.—Diphtheria toxine produces in the body of an animal the corresponding antidote, the antitoxine. The antitoxine, which is thus a normal, but complementary, product of the body, occurs therefore in the fluids of the tissues and in the secretions of individuals that have recovered from diphtheria, or in artificially immunised animals. We cannot deal here with the mode of production of antitoxine and its relationship to artificial immunity and serum therapy; only what is known of diphtheria antitoxine, as such, can be described here.

The two main sources for the artificial preparation of antitoxine are *blood serum* and the *milk* of immunised animals. The more highly the animal is actively immunised, the richer these fluids in antitoxine, and the greater the prospect of obtaining it from them in a highly concentrated, if not pure, condition.

The antitoxine is present in considerable quantities only in the serum of artificially immunised animals, although, naturally, it has been found to a less extent in the blood of those convalescent from diphtheria.

It is, however, particularly interesting that even the normal serum of many species of animals contains diphtheria antitoxine, and that thus haptophore side-chains are normally present in the circulatory system. A. Wassermann[1] was the first to detect antitoxine in 85 per cent. of the adults and 60 per cent. of the children examined. Antitoxine has also been frequently found in horse serum—*e.g.*, by Dieudonné[2] and Cobbett.[3]

[1] A. Wassermann, "Ueb. d. pers. Prophylaxe gegen Diphth.," *Zeit. f. Hyg.*, xix., 408, 1895.

[2] Dieudonné, "Ueb. Diphtheriegift neutralis. Wirkung d. Serumglobuline," *Arb. a. d. Kaiserl. Ges.-Amt.*, xiii., 293, 1897.

[3] Cobbett, "Enthält das normale Pferdeserum Antitoxin?" *Centralbl. f. Bakt.*, xxvi., 458, 1899. See also *Lancet*, 1899, ii., 332.

As an explanation of the fact that children at the breast are seldom attacked by diphtheria great importance attaches to the discovery of Fischl and v. Wunschheim[1] supplementing the work of Wassermann, that the serum of healthy suckling babies already contains fairly considerable quantities of diphtheria antitoxine; they detected it in 83 per cent. of all the children examined. The correspondence with the numbers observed by A. Wassermann indicates that here there must be a transference of antitoxine from the mother to the baby by means of the placental blood and the milk.

As in the case of the toxine we must here distinguish between two aims—those that have in view only the preservation and concentration of the antitoxine for practical purposes with or without the production of a solid product, and, on the other hand, the attempts that are made in careful investigations to obtain an insight into the constitution of the antitoxine itself, by determining its ratio to the different proteids in its mother liquids and by isolating as completely as possible the active principle.

Preservation of the Serum.—The first necessity is to preserve the serum so that it retains its full amount of antitoxine, while decomposition is prevented. The serum will keep for a fairly long time if protected from the action of air, light, or bacterial contamination. Since it is a good nutrient medium for bacteria it must be prepared and kept with strict aseptic precautions. In addition to this an antiseptic agent, such as phenol in solutions up to 0·5 per cent. in strength, is usually added.

According to di Martini[2] sterilisation by filtration is unsuitable, since a considerable proportion of the antitoxine is kept back by the Chamberland filter. This is categorically denied by Dzierzgowski.[3] Cobbett[4] has thoroughly investigated this point, so extremely important from a practical point of view, and asserts that such retention frequently occurs, especially with the Berkefeld or Martin filter, and may be very considerable, particularly when the pores of the filter begin to be clogged. Hence the filtration ought not to be forced. When it does not take place easily without the application of high pressure from

[1] Fischl and v. Wunschheim, "Ueb. Schutzkörper im Blute der Neugeborenen," *Prager med. Woch.*, 1895, No. 45, *et seq.*

[2] di Martini, "Sul comportamento del siero antidifterico filtrato," *Rif. Med.*, 1896, No. 266; Abst. *Centralbl. f. Bakt.*, xxiv., 861, 1898.

[3] Dzierzgowski, "Z. Frage: Ueb. die Verluste des Diphth.-Heils. bei der Filtration," *Centralbl. f. Bakt.*, xxi., 233, 1897.

[4] Cobbett, "Der Einfluss der Filtration auf das Diphth.-Antitoxin," *Centralbl. f. Bakt.*, xxiv., 386, 1898.

outside it is very scanty. The antitoxine is retained by a gelatine filter (BRODIE [1]).

The antitoxine is rapidly *destroyed* when heated to the boiling point. Even a temperature of 60° to 70° C. is injurious (VAN DE VELDE [2]). The dry antitoxine, however, can resist a temperature of 110° C. for half an hour, and of 140° C. for fifteen minutes (CAMUS [3]). It can resist lower temperatures to some extent. SPRONCK [4] has heated it to 59° C. for sterilisation without materially weakening its antitoxine power. Temperatures up to 36° C. have no influence upon it (PALMIRSKI and ORLOWSKI [5]), although, according to MÜLLER,[6] they are very injurious when continued for longer periods (two months).

It appears to be unaffected by very low temperatures (BUJWID [7]). Direct sunlight and plentiful introduction of air (long continued shaking) are injurious (PALMIRSKI and ORLOWSKI, *loc. cit.*). MÜLLER (*loc. cit.*) found that yellow and red light were harmless, even after being allowed to act for months, but that blue and green light were very injurious, as was also daylight after long continued action, whilst MARENGHI [8] found it had no effect if the exposure were short. According to MÜLLER *all* gases have a very injurious action, and hence he recommends that the tubes should be filled as full as possible so as to exclude any considerable amount of gas. It is thus best to keep them in the *dark*, upon *ice*, and closed in such a way as to *exclude air* and to protect them from contamination. Solid preparations of antitoxine should also be protected from moisture by means of *phosphoric anhydride in vacuo*, as recommended by EHRLICH; under such conditions they remain unaltered for years.

[1] Brodie, *J. of Pathol.*, 1897, 460, quoted by Martin and Cherry, "The Antagonism between Toxines and Antitoxines," *Proc. Roy. Soc.*, lxiii., 420, 1898.

[2] Van de Velde, "Beitr. z. Kenntnis der antitox. Kraft d. antidiphth. Serums," *Centralbl. f. Bakt.*, xxii., 527.

[3] Camus, "Resistance aux temp. élévées des vaccins desséchées," *Soc. Biol.*, l., 235, 1898.

[4] Spronck, "Chauffage du serum antidiphthérique," *Ann. Past.*, xii., 695, 1898.

[5] Palmirski and Orlowski, *Medycyna*, xxiii.; Abst. in *Centralbl. f. Bakt.*, xix., 916.

[6] Müller, "Ueber d. Resistenz d. Diphtherieheilserums gegenüber verschiedenen Einflüssen," *Centralbl. f. Bakt.*, xxiv., 251, 1898.

[7] Bujwid, "Ueb. e. Methode d. Concentrat. d. diphth. Heils. mittelst Ausfrieren," *Centralbl. f. Bakt.*, xxii., 287, 1897.

[8] Marenghi, "Ueb. die gegenseitige Wirkung des antidiphth. Serums und des Diphth.-Toxins," *Centralbl. f. Bakt.*, xxii., 520, 1897.

Spronck[1] asserts that antidiphtheritic serum that has been kept for twenty minutes at a temperature of 58° C. no longer possesses the property of producing the unpleasant erythema resembling urticaria, which is frequently the case with serum that has not been thus heated.

It appears to be destroyed in the digestive tract. Dzierzgowski[2] found that immunisation could not be effected by introduction of the antitoxine *per os*. Only in the case of rabbits could a slight absorption be observed after its introduction into the *empty* stomach.

Hydrochloric acid has a particularly injurious action, while neutralised pepsin and the pancreas and gall are fairly innocuous to it. Yet it is not absorbed by way of the intestine.

There is no doubt, however, but that it must be gradually destroyed under the influence of the digestive ferments, for its resistance to the action of trypsin is not absolute. At all events, Pick (*loc. cit.*) found that the antitoxine was destroyed to a large extent (almost two-thirds), even after nine days' exposure to the digestive action of trypsin.

It also disappears rapidly from the blood after subcutaneous injection, as was shown by Passini,[3] whose explanation is that a partial combination takes place within the tissues.

Bomstein[4] has studied the question of the survival of the antitoxine. After a single introduction of a quantity of the antidiphtheritic serum it rapidly disappears from the blood.

It is not known what becomes of it, but it appears to be destroyed, for it is only during the first days that slight traces are present in the urine; nor can it be detected with certainty in the organs.

An interesting observation recorded by Murawjew[5] is that the antiserum is far from being inert, and that it produces somewhat serious injuries in the cells of the spinal cord of guinea-pigs. He, therefore, gives a warning against the use of too large doses at once.

This, however, is doubtless to be attributed to the serum of the foreign species of animal, and not to the antitoxine, since normal serum also pro-

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[1] Spronck, "Chauffage du Sérum antidiphth.," *Ann. Past.*, xii., 697, 1898.

[2] Dzierzgowski, "Die Bezieh. d. Verdauungsfermente zum Antidiphtherieserum," *Arch. d. Sciences Biol.*, vii., 337; *Maly's Jb.*, 1899, 957.

[3] Passini, "Vers. über die Dauer d. antidiphth. Schutzimpfung," *Wien. klin. Woch.*, 1896, 1111.

[4] Bomstein, "Z. Frage der passiven Immunität. bei Diphtherie," *Centralbl. f. Bakt.*, xxii., 587, 1897.

[5] Murawjew, "Das Diphtherietoxin u. Antitoxin in ihrer Wechselwirkung auf das Nervensystem d. Meerschweinchens," *Fortschr. d. Med.*, 1898, 93.

duces similar injuries, although the results of my experiments contradict the conclusion of LINOSSIER and LEMOINE[1] that even minute quantities of normal horse-serum produce serious illness (persistent albuminuria) in rabbits.

Concentration of the Antitoxine.—Soon after the first active antitoxine sera had been prepared, experiments were begun with the object of isolating the antitoxine.

At first these were concerned with the purely practical idea of preparing solid substances which had not lost the specific property of the antitoxine. For this purpose the usual method employed was simply to precipitate the antitoxine and the proteids of the serum simultaneously—*e.g.*, by means of concentrated *ammonium sulphate*, and to dry these precipitates at a low temperature *in vacuo*. In this way fairly active, dry residual preparations of the antitoxine were obtained, and these, when shaken with very weak alkalies, yielded solutions of the antitoxine ranging up to several hundred times the strength of the original serum.

ARONSON,[2] for example, proceeded as follows:—One hundred c.c. of the serum were diluted with 100 c.c. of water, and treated with 70 c.c of a 10 per cent. solution of aluminium sulphate. Ammonia was then added in such quantity that the solution still remained slightly acid, and the resulting precipitate was washed with 150 to 200 c.c. of cold water. It contained as much as 95 per cent. of antitoxine. Similar precipitates were produced by zinc sulphate and potassium ferrocyanide, and by iron chloride and ammonia. These precipitates were repeatedly shaken for long periods in a shaking apparatus with a hundredth part (calculated on the original serum) of very dilute alkali solutions, which just turned red litmus blue.

BUJWID[3] freezes the serum, with the result that the water first separates as ice, leaving behind a turbid solution very rich in toxine. The crystals can then either be separated by "centrifuging" or slowly thawed, in which process two layers are formed, so that the upper layer, containing pure water, can be withdrawn. Results quite analogous to these were simultaneously obtained by ERNST, COOLIDGE, and COOK.[4]

BRIEGER and BOER[5] found that precipitates of calcium phos-

[1] Linossier and Lemoine, "Action nephrotoxique des inj. d. sérums normaux," *Soc. Biol.*, lv., 515, 1903.

[2] Aronson, "Weit. Unters. über Diphtherie," *Berl. klin. Woch.*, 1894, 425.

[3] Bujwid, "Ueb. e. Methode z. Concentr. d. Diphtherieheils. mittelst Ausfrieren," *Centralbl. f. Bakt.*, xxii., 287.

[4] Ernst, Coolidge, and Cook, "The Effect of Freezing upon the Antidiphtheritic Serum," *J. Boston Med. Soc.*, ii., 166; *Baumgarten's Jb.*, 1898, 242.

[5] Brieger and Boer, "Ueber Antitoxine und Toxine," *Zeit. f. Hyg.*, xxi., 259.

phate or of the hydroxides of the heavy metals could not be used as means of isolating diphtheria antitoxine. Moreover, the method employed by TISSONI for the isolation of his tetanus antitoxine (*q.v.*)—viz., salting out with solid magnesium sulphate at 30° C.—gave a yield of at most 50 per cent. On the other hand, BRIEGER and BOER have obtained quantitative yields by the following method :—

Ten c.c. of the immune serum are mixed with 10 c.c. of distilled water, and the mixture treated with 4 grms. of dry potassium chloride, and 4 to 5 grms. of finely-powdered sodium chloride, and left for eighteen to twenty hours in an incubating oven, after which the precipitate is dissolved in water and dialysed. Frequently the precipitate agglomerates into nearly a solid mass on stirring with water, and no antitoxine is dissolved. In such cases it can only be extracted with weak alkalies. A more exact explanation of this anomaly cannot be given. After the residue has been dissolved, the solution is treated with an equal volume of finely-divided magnesium sulphate, and again left for two to three hours in the incubating oven, the result being that the antitoxine is quantitatively precipitated. In this way they obtained 0·2 grm. of active dry substance from 10 c.c. of the serum.

BRIEGER and BOER have also endeavoured to concentrate the antitoxine by precipitation with the salts of heavy metals. Zinc salts proved to be the most suitable.

The serum was diluted with five times its volume of water and treated with twice the quantity of a 1 per cent. solution of zinc sulphate or zinc chloride, and the precipitate washed with water. It was then dissolved in a $\frac{1}{400}$ N. solution of alkali hydroxide, and the zinc precipitated with carbon dioxide. These experiments showed that in the precipitation with zinc sulphate the antitoxine was precipitated with the zinc, while the zinc chloride remained in solution. The portion containing the antitoxine was dried in a desiccator, and it was found that the zinc albuminate was somewhat soluble in water, while the zinc antitoxine was insoluble. The latter was, therefore, again dissolved in alkali solution and the zinc once more precipitated with carbonic acid. It was not possible to effect by this means a complete separation of the antitoxine from the zinc.

BRIEGER and EHRLICH[1] were the first to obtain active dry preparations from the milk of immunised animals by means of ammonium sulphate. They obtained one preparation which contained 14 per cent. of ammonium sulphate and was 400 to 600 times as active as the original milk.

WASSERMANN[2] has modified this method to some extent, proceeding as follows :—

[1] Brieger and Boer, "Beitr. z Kenntn. d. Milch immuner Tiere," *Zeit. f. Hyg.*, xiii., 336, 1893.

[2] Wassermann, "Ueb. Concentrierung v. Antitoxin aus Milch, *Zeit. f. Hyg.*, xviii., 236, 1894.

The milk collected with aseptic precautions is treated with about 20 c.c. of N.-hydrochloric acid per litre, coagulated as rapidly as possible by means of rennet enzyme, and filtered from the separated paracasein. The whey is then thoroughly shaken with chloroform and allowed to stand, after which the supernatant liquid is decanted from the layer of chloroform charged with fat and bacterial cells. Each 5 litres of the whey is next treated with a calculated amount (determined by a preliminary experiment) of 30 to 33 per cent. ammonium sulphate solution, and the precipitate dried on porous earthenware at 35° C. *in vacuo*, separated as completely as possible from solid ammonium sulphate and dissolved in water to form a solution of the desired concentration.

The experiments to obtain antitoxine preparations from the liquid serum have hitherto proved nearly worthless from the practical point of view, since at present it is preferred to immunise animals to such an extent that the serum of the animal itself contains so many antitoxic units per c.c. as to render concentration superfluous.

On the other hand, the scientific side of the question has been followed with equal interest, and attempts have been made to isolate the antitoxine as such as completely as possible and to determine whether it is to be considered as an individual substance, and with which proteid of the serum it combines. In addition to this, experiments have been made to discover points of difference between ordinary serum and that containing antitoxine.

Szontagh and Wellmann[1] made a comparative examination of normal and antitoxic horse-serum. In neither did they detect any *nucleo-albumin*, while the proportions of *globulin* and of ash and chlorine, and the specific gravity were the same in each. The only difference observed was that the antitoxic serum contained, on the average, about 0·25 per cent. more nitrogen than the normal serum; they rightly attribute, however, no special importance to this difference. On the other hand, they claim to have found that the therapeutic serum shows a decrease in the lowering of freezing point and in the electrical conductivity, which are apparently proportional to the amount of antitoxine.

Freund and Sternberg[2] have endeavoured to isolate the antitoxine in as pure a state as possible by the following method:—

The therapeutic serum was treated with alum and dialysed. No precipitate was produced in the dialysate by zinc salts. Nor was the

[1] Szontagh and Wellmann, "Vergleichende chem. Unters. üb. d. normale Pferdeserum und das Diphtherieheilserum," *Deutsch. med. Woch.*, 1898, 421.

[2] Freund and Sternberg, "Ueber Darstellung des Heilkörpers aus d. Diphtherieheilserum," *Zeit. f. Hyg.*, xxxi., 429, 1899.

antitoxine precipitated together with the zinc on the addition of alkali carbonate or phosphate, though it was carried down with the *zinc hydroxide* on the addition of an alkali hydroxide. From this precipitate it could be extracted by means of an excess of very dilute alkali, of only sufficient strength to just colour phenolphthalein. A solution of baryta could not be used since it destroyed the antitoxine. Unsatisfactory results were also obtained with iron salts. The solution was then saturated at the ordinary temperature with magnesium sulphate, which precipitated the antitoxine quantitatively. After further extractions with alkali and dialysis the final product was dried *in vacuo*.

Still better results were obtained by the following method:—The serum was first treated with a third of its volume of a 5 per cent. solution of alum. This precipitated the *albumins*, leaving the whole of the antitoxine in solution. The solution was then filtered and dialysed. The *globulins*, and with them the antitoxine, were precipitated from the dialysate by semi-saturation with ammonium sulphate, and the precipitate washed with a semi-saturated solution of ammonium sulphate. The precipitate was then redissolved in water and the solution dialysed, concentrated *in vacuo*, and filtered.

In this way they obtained from 500 c.c. of serum 9 grms. of a dry preparation in the form of a reddish-brown gelatinous mass. This dissolved slowly in water (4·7 grms. in 16 c.c.) yielding a syrupy reddish-brown fluid, which readily became turbid and could only be filtered slowly. The filtrate contained the same proportion of antitoxine as the unfiltered preparation. The addition of phenol was not injurious. The preparation behaved like a globulin.

Astros and Rietsch[1] claim to have separated the antitoxine from the serum almost quantitatively.

They diluted the therapeutic serum with four parts of water, and then added sufficient sodium chloride and potassium chloride to give a 20 per cent. solution, after which 0·5 per cent. of phenol was added, and the liquid left for twenty-four hours at 33° C.

The theoretically important experiments of Pick may possibly be also of practical importance. He showed that by saturating the liquid with ammonium sulphate, to the extent of about a third, a part of the globulins without any therapeutic value was precipitated, whilst the active anti-bodies were separated when the degree of saturation reached 38 to 46 per cent. By this means he effected a tenfold to fifteenfold concentration. We will deal presently with the significance of his work.

Equally important, from the practical point of view, are the experiments of Pröscher,[2] who claims to have freed the antitoxines from proteid admixtures by digestion with trypsin.

[1] Astros and Rietsch, "Essais d'Extraction de l'antitox. diphth.," *Soc. Biol.*, lii., 337, 1900.

[2] Pröscher, German Patent, F. 13,756, June 6, 1902.

Especial interest attaches to the question whether the antitoxine circulates as such in the blood, and, if so, whether it is a proteid or not, or whether it enters into combination with a definite proteid of the blood. This also involves the question whether its precipitation together with proteid substances is simply a mechanical carrying down, or whether the precipitation is due to chemical reactions of a specific nature.

It is undoubtedly very probable, *a priori*, that the antitoxine is a *proteid*, since according to the theory it is a constituent of the cell protoplasm. There is also absolutely no ground, as in the case of toxines, for regarding the antitoxine as an agency of *enzymic character*. On the contrary, it has absolutely no action by itself, either *toxic* or *fermentative*, and only enters into combination with the toxic ferment of bacteria, and it is a quite unwarranted generalisation to transfer the enzymic character of toxines to their antitoxines without further proof. The antitoxine lacks, to retain the graphic mode of representation, every specific group with the exception of the *haptophore group*, and thus has neither a *toxophore* nor a *zymophore* group; it is indeed a haptine, but one that possesses only *one*, the haptophore, group. In point of fact, there are many reasons against the antitoxine being of an enzymic character. The only real argument in support of that view is the fact that it becomes inactive at 100° C., and that is surely not a sufficient reason. Against this, and in support of the view that it is a specific *proteid*, must be set the fact that it is not simply carried down mechanically, like the enzymes, but that it shows well-marked *precipitation reactions*. As has already been shown by Brieger and Boer (*loc. cit.*), the antitoxine is only carried down, together with zinc carbonate, when it has previously been precipitated by means of zinc sulphate, and not when *zinc chloride* has been used for the purpose. In like manner Freund and Sternberg were able to demonstrate that the antitoxine was not precipitated simultaneously with the slight precipitates produced by alum in the serum, although these carried down all albumins.

It would appear, then, that the antitoxine either actually enters into combination with one of the proteids of the blood, or we must assume that the particular proteid of which the antitoxine consists approximates so closely in its reactions to a proteid of the serum that it is very difficult to distinguish between them.

Quite recently, however, Pröscher (*loc. cit.*) has stated that he has prepared a diphtheria antitoxine which no longer shows

any proteid reactions at all. The further development of this question will be awaited with the greatest interest. The other question as to which of the serum proteids the antitoxine is combined with has been decided in favour of the *globulins*.

Belfanti and Carbone[1] were the first to discover that the antitoxine was carried down with the *globulins* when they were precipitated by means of ammonium or magnesium sulphate, but not by acetic acid. The antitoxine was also found by Smirnow[2] in the globulin precipitate thrown down by magnesium sulphate.

On the other hand, Dieudonné[3] found that the globulins which he had precipitated by means of acetic acid and carbon dioxide were completely nactive. On dialysis, the separated globulins exhibited but little protective power and that was mainly due to the filtrate. If, however, magnesium sulphate were used for the precipitation the whole of the antitoxine remained in the precipitate. A very similar result was obtained in the case of the globulins of *normal* serum, which also had a slight antitoxic power.

Again, Hiss and Atkinson,[4] and also Atkinson,[5] found that the antitoxine could be quantitatively precipitated by magnesium sulphate, and that the immune serum yielded a more copious precipitate than normal serum.

Ide and Lemaire[6] assert that the antitoxine is precipitated by saturating its solution with ammonium sulphate to the extent of 28 to 44 per cent.

These apparent contradictions as to the rôle of the globulins have been satisfactorily explained by Seng.[7] He was able to show (as was also done simultaneously by Marcus[8]) that there were two kinds of globulins in the therapeutic serum—viz., *insoluble* globulins, which could be precipitated by acetic acid, carbon dioxide, dilution with water, and dialysis, and a second category, the *soluble* globulins, which could only be precipitated

[1] Belfanti and Carbone, "Contributo alla Conoscenza dell'antitossina difterica," *Arch. per le Scienze Med.*, xxii., No. 2; abst. in *Centralbl. f. Bakt.*, xxiii., 1898.

[2] Smirnow, "Note sur la détermin. du pouvoir antitoxique du sérum antidiphthérique," *Arch. d. Science Biolog. de St. Petersbourg*, iv., No. 3, 1895.

[3] Dieudonné, "Ueb. Diphtheriegift neutralisier. Wirkg. der Serumglobuline," *Arb. a. d. Kaiserl. Ges.-Amt.*, xiii., 293, 1897.

[4] Hiss and Atkinson, "Serumglobulin and Diphtheria Antitoxine," *J. of Exper. Med.*, v., 47, 1901.

[5] Atkinson, "The fractional precipitation of the globulins and albumins of normal horse serum and diphtheric antitoxic serum," *J. of Exper. Med.*, v., 67, 1901.

[6] Ide and Lemaire, "Et. s. l. repartition de l'antitoxine diphth., &c.," *Arch. Internat. d. Pharmacodyn.*, vi., 477.

[7] Seng, "Ueb. d. qual. u. quant. Verhältnisse d. Eiweisskörper im Diphtherieheilserum," *Zeit. f. Hyg.*, xxxi., 513, 1899.

[8] Marcus, "Ueb. in Wasser lösl. Serumglobulin," *Z. f. phys. Ch.*, xxviii., 559, 1899.

by the other reagents for globulins, especially ammonium and magnesium sulphates. The antitoxine combined exclusively with the latter class, and thus remained in the filtrate during the precipitation by dialysis, carbon dioxide, &c. This explains the contradictory results in the work of BELFANTI and CARBONE and of DIEUDONNÉ.

SENG proceeded as follows in the isolation of his "*soluble globulins*":—

The albumins (which do not combine with any antitoxine) are first separated by means of a 5 per cent. solution of alum. The filtrate is dialysed, and this causes the precipitation of slight quantities of the *insoluble globulins*, which are also free from antitoxine and amount to $\frac{1}{11}$ to $\frac{1}{23}$ of the total quantity of globulins present. This precipitate is collected and washed with water, and the filtrate ought not to become turbid on the dilution with a large volume of water, or give any reaction for sulphuric acid. The globulin is then precipitated by magnesium sulphate at 30° C., or by semi-saturation with ammonium sulphate, and the precipitate redissolved and subjected to further treatment by the usual methods (*vide supra*). It contains somewhat more ash, notably aluminium salts, than the globulins precipitated directly from the serum by means of ammonium sulphate.

The quantity of these soluble globulins, as compared with those of the normal serum, appears to have been somewhat increased at the cost of the insoluble globulins, but this cannot be demonstrated with certainty.

SENG has also studied the very important question whether chemical differences can be observed between the soluble globulins of the therapeutic serum, as thus obtained, and those of normal serum separated in the same way. As was to be expected, he has not succeeded in coming to a definite conclusion, but it appears as though at least two important constants—viz., the *specific rotation* and the *temperature of coagulation*—are higher in the case of the antitoxine globulin than in that of normal globulin. SENG himself remarks, very justifiably, that the physiological bearing of all these factors is far too great to allow of distinct differences being observed between the sera of different animals; it would be necessary to examine the serum of one animal *before*, *during*, and *after* immunisation. Some definite result might then possibly be obtained.

Meanwhile this question has received further thorough investigation in a series of researches carried out by Hofmeister's pupils.

It was found that *three distinct kinds* of globulins could be separated from the blood serum by fractional precipitation with ammonium sulphate. In the first place, *fibrinoglobulin* is precipitated when the saturation with ammonium sulphate reached 21·5 per cent. by volume; then, according to FULD and SPIRO,[1] Hof-

[1] Fuld and Spiro, "Ueb. labende u. labhemmende Wirkung d. Blutes," *Z. f. phys. Ch.*, xxxi., 132, 1900.

meister's so-called *euglobulins* are precipitated when the degree of saturation reaches 28 to 36 per cent.; whilst the *pseudoglobulins* are deposited at a saturation of 36 to 44 per cent. by volume. The *pseudoglobulins*, which dissolve in water forming a clear solution, correspond to the "*soluble globulins*" of MARCUS and SENG.

PICK[1] was able to prove that neither the fibrinoglobulin, nor those proteids which were precipitated on the further addition of a saturated solution of ammonium sulphate (up to 36 per cent.), possessed any antitoxic capacity when separated from the antidiphtheric serum of a horse.

Only that portion which is precipitated at a greater degree of saturation (from 38 per cent. upwards) contains antitoxine.

The aqueous solution of this substance gave only a slight turbidity when treated with a saturated solution of ammonium sulphate to the extent of 36 per cent., whereas at 38 per cent. there was a dense deposit containing a third to a fourth of the total quantity of antitoxine; at 42 per cent. the precipitate contained as much as five-sixths; and at 46 per cent. the whole amount of antitoxine. The filtrate, which now contained only serum albumin, had absolutely no protective power.

Hence, in the case of the immune serum of the *horse*, the antitoxine is combined with the *pseudoglobulin*. On the other hand, in the *immune serum of the goat*, it is combined with the *euglobulin*, as is also the case with goats' milk in which the protective substances are precipitated when the degree of saturation with ammonium sulphate reaches 27 to 30 per cent.

We may again point out here that the *euglobulins* are insoluble in water, and are therefore precipitated on dialysis; this explains the great losses experienced by WASSERMANN and BRIEGER and COHN in their attempts to concentrate the antitoxine in goats' milk by precipitation with ammonium sulphate and subsequent dialysis.

TETANUS TOXINE.

The specific spasm-producing poison formed in cultivations by Nicolaier's tetanus bacilli is not the only toxine of these micro-organisms. They produce, in addition to tetanus poison in the narrower sense, also another true toxine, *tetanolysine*, which has a solvent action on the blood-corpuscles, and with which we will deal presently, and possibly also other poisons not of the nature of toxines. Here our interest centres chiefly about the characteristic convulsion-producing poison, *tetanospasmine*. It is unquestionably a true toxine with haptophore and toxophore

[1] Pick, "Z. Kenntnis d. Immunkörper," *Hofm. Beitr. z. chem. Phys.*, i., 1902 (reprint).

groups, and is closely related to *diphtheria toxine* in its main characteristics, and, like the latter, is very valuable for our theoretical conceptions.

The specific convulsion-producing poison of tetanus was isolated at an early period from the cultivations by the same methods as were employed for diphtheria poison. Almost simultaneously KITASATO and WEYL[1] and BRIEGER and FRÄNKEL (*loc. cit.*) separated from cultivations their toxalbumin which they recognised as the specific poison, soluble in water. FABER[2] was the first to obtain, by filtration of not quite pure cultivations, an active poison, which he found even at that stage to possess the essential properties of tetanus toxine.

The results of further investigations into *tetanus toxine* were then published by TIZZONI and CATTANI,[3] KITASATO,[4] and by VAILLARD[5] and his co-workers.

The bacteria, when grown anaërobically on ordinary bouillon and on blood serum, produce very poisonous toxines. Particularly active solutions of poison were obtained by VAILLARD and VINCENT, when they first allowed a cultivation to grow for eighteen days on a culture medium and then filtered it. Fresh inoculation of this culture medium was fruitless, but by adding a certain amount of fresh nutrient liquid, a new growth of very active toxines was obtained. VAILLARD subsequently employed a slightly alkaline bouillon containing some *peptone;* KITASATO recommended that the culture medium should be freshly prepared on each occasion. FERMI and PERNOSSI[6] found that *agar cultivations* were the most poisonous, especially so when grown in an *atmosphere of nitrogen.*

BRIEGER and COHN[7] found that the toxicity of the cultivations could be raised by adding to the culture media precipitates obtained by means of alcohol from old typhus cultivations or

[1] Kitasato and Weyl, "Zur Kenntnis der Anäerobien," *Zeit. f. Hyg.*, viii., 404, 1890.

[2] Faber, "Die Pathogenese des Tetanus," *Berl. klin. Woch.*, 1890, 717.

[3] Tizzoni and Cattani, "Sur le poison du tétanos," *Arch. Ital. d. Biol.*, xiv., 101, 1890.

[4] Kitasato, "Exper. Unters. über das Tetanusgift," *Zeit. f. Hyg.*, x., 287, 1891.

[5] Vaillard, "Sur l'immunité contre le tétanos," *Soc. Biol.*, xliii., 147, 1891; Vaillard and Vincent, "Contrib. a l'étude du tétanos," *Ann. Past.*, v., 1, 1891; Vaillard and Rouget, do., *ibid.*, 385; Vaillard, "Sur quelques points concernant l'immunité contre le tétanos," *Ann. Past.*, vi., 224, 1892.

[6] Fermi and Pernossi, "Ueber das Tetanusgift," *Zeit. f. Hyg.*, xvi., 385, 1894.

[7] Brieger and Cohn, "Unters. üb. d. Tetanusgift," *Zeit. f. Hyg.*, xv., 1, 1893.

from decomposed flesh. Wladimiroff[1] cultivated the bacilli in Erlenmeyer flasks in an atmosphere of hydrogen, and after seven days added to the culture medium 0·5 per cent. of phenol. He introduced the hydrogen through tubes, which passed through two openings in an india-rubber stopper, and the ends of these tubes were subsequently closed by fusion. Debrand[2] asserts that tetanus bacilli grow very well in symbiosis with *B. subtilis*, and produce powerful toxines. In the case of these bacilli also it has been found that there is not invariably a parallelism between the amount of growth and the toxicity.

Ruppell and Ransom[3] found that the freezing point of the bouillon was somewhat lowered on the production of toxine, which indicates a fresh formation of molecules; and, as the cultivation becomes weaker, the freezing point ought to be slightly raised again.

Uschinsky[4] cultivated tetanus bacilli on a proteid-free medium of the following composition :—

Water,	1000·0
Glycerin,	30 to 40
Sodium chloride,	5 ,, 7
Calcium ,,	0·1
Magnesium sulphate,	0·2 to 0·4
Dipotassium hydrogen phosphate,	2 ,, 2·5
Ammonium lactate,	6 ,, 7
Sodium aspartate,	3·4

preferably with the addition of 1 to 2 per cent. of grape sugar. The air was excluded by means of liquid paraffin.

The usual means of sterilisation are employed, especially filtration through a Chamberland filter, and heating to 60° C.

Tetanus toxine is extraordinarily sensitive to physical and chemical influences. According to Behring and Knorr[5] it frequently loses very rapidly a considerable proportion of its toxic power on keeping; its toxicity is frequently reduced to a hundredth part of the original amount in a few days.

The atmospheric *oxygen*, in particular, has a very injurious

[1] Wladimiroff, "Antitoxinerzeug. d. Tet.-Giftes," *Zeit. f. Hyg.*, xv., 405, 1893.

[2] Debrand, "Sur un nouveau procédé de culture du bacille du tétanos," *Ann. Past.*, xiv., 757, 1900.

[3] Ruppel and Ransom, "Ueb. Molekularverhältnisse von Tetanusgiftlösungen," *Z. f. phys. Ch.*, xxvii., 109, 1899.

[4] Uschinsky, "Ueber eine eiweissfreie Nährlösung f. pathog. Bakt.," *Centralbl. f. Bakt.*, xiv., 316, 1893.

[5] Behring and Knorr, "Ueb. den Immunisierungswert des Tetanusheilserums," *Zeit. f. Hyg.*, xiii., 407, 1893.

effect upon the toxine, so that when slowly filtered, for example, it is very rapidly weakened; and this is especially the case with an alkaline solution and in the presence of *light*, which, by itself, is not very injurious. KITASATO found that sunlight destroyed the poison in fifteen to eighteen hours. FERMI and PERNOSSI give the time as eight hours, and assert that in the dry condition or in benzene it is not affected by sunlight.

A current of 0·5 amp. is stated by FERMI and PERNOSSI to destroy it in two hours, but alternating currents of high tension have no influence if heating be prevented (MARMIER [1]).

It is very sensitive to the action of heat. According to VAILLARD it is destroyed, for the most part, even at 65° C., but not completely at 80° C. KITASATO asserts that it is destroyed at 60° C. in twenty minutes, at 55° C. in one and a half hours, and gradually destroyed at 35° to 37° C. In particular, the addition of sodium chloride in the proportion of more than 5 per cent. causes the temperature of incubation to be rapidly injurious. In the *dry* condition the toxine is destroyed in thirty minutes at 150° C.; in amyl alcohol and benzene it can be heated for an hour at 80° C. (FERMI and PERNOSSI). According to MORAX and MARIE [2] it resists a temperature of 154° C. for about fifteen minutes. Alcohol destroys it (TIZZONI and CATTANI). Phenol in the proportion of 0·6 per cent. is not injurious, and chloroform has little or no influence.

Gases, such as carbon dioxide, carbon monoxide, hydrogen, and hydrogen sulphide, were found by FERMI and PERNOSSI to be without influence.

According to ROUX and VAILLARD [3] oxidising substances, such as dilute potassium permanganate solution, are specially injurious, as is also carbon dioxide under pressure.

Other acids are also injurious, tartaric acid to a very slight extent, whilst dilute lactic acid has a favourable influence (BRIEGER [4]). A comprehensive research on the influence of the most diverse substances has been made by FERMI and PERNOSSI (*loc. cit.*).

Iodine trichloride has also an extremely injurious influence. The action of a dilute (1 : 500) solution of iodine is very characteristic. Even when only present in small quantities it

[1] Marmier, "Les toxines et l'électricité," *Ann. Past.*, x., 469, 1896.

[2] Morax and Marie, "Action de la Chaleur sêche sur la tox. tet.," *Ann. Past.*, xvi., 418, 1902.

[3] Roux and Vaillard, "Contrib. à l'étude du tétanos," *Ann. Past.*, vii., 65, 1893.

[4] Brieger, "Weitere Erfahrungen über Bakteriengifte," *Zeit. f. Hyg.*, xix., 101, 1895.

speedily renders the toxine non-poisonous, but leaves its immunising properties uninjured. Very similar results were obtained by EHRLICH in experiments with *carbon bisulphide*. It can hardly be doubted that there is here a rapid destruction of the toxophore group with a survival of the haptophore group, or in other words a *formation of toxoid*. Thymus extract appears to have a similar result upon the growth, according to BRIEGER, KITASATO, and WASSERMANN (*loc. cit.*).

It appears to offer great resistance to putrefaction; at all events, SYMANSKI[1] found tetanus poison still present in decomposing cadaveric remains after forty-eight days. There is, however, some reason for doubting whether this was true tetanus poison.

Concentration of the Toxine.—Experiments with the object of isolating the active principle from tetanus cultivations were made at an early period.

BRIEGER and FRÄNKEL prepared a "toxalbumin" by the method described by them. VAILLARD obtained by evaporation of the solution of poison *in vacuo* a brown residue, which was insoluble in alcohol and could be slowly dialysed.

TIZZONI and CATTANI either simply dried the cultivations and then dialysed them, or treated them with ammonium sulphate, extracted the precipitate with water, and then used dialysis. Subsequent evaporation *in vacuo* then left solid toxines.

BRIEGER and COHN[2] first treated the cultures with ammonium sulphate. The precipitate was dissolved in water and part of the proteids separated by means of very small quantities of basic lead acetate and ammonia. The liquid was filtered from the lead precipitate and salts, amino-acids and peptones removed by dialysis. In this way they obtained a slightly lævorotatory solution of the toxine, which was free from sulphur and gave no proteid reactions. They also prepared it from a proteid-free culture medium similar to that of USCHINSKY (*vide supra*).

Then BRIEGER[3] found that the poison could not be precipitated by means of ammonium sulphate from very toxic solutions which no longer contained albumoses. He endeavoured to effect a further purification by precipitation with uranium acetate and decomposition with metaphosphoric acid or lead acetate and obtained preparations that no longer gave the biuret reaction.

[1] Symanski, "Sitzungsbericht," *Deutsch. med. Woch.*, 1901, 318.

[2] Brieger and Cohn, "Unters. üb. d. Tetanusgift," *Zeit. f. Hyg.*, xv., 1, 1893.

[3] Brieger, "Weit. Erfahrung. üb. Bakteriengifte," *Zeit. f. Hyg.*, xix., 101, 1895.

Brieger and Boer (*loc. cit.*) also made further experiments to obtain purer preparations by means of the *zinc method* described in the section on diphtheria toxine, or they used ammonium sulphate for the precipitation, dissolved the precipitate and reprecipitated the toxine with an equal volume of a 0·5 per cent. solution of mercuric chloride. They then collected the precipitate on a filter, thoroughly washed it, and treated it with ammonium carbonate as previously described. Hayashi[1] by a somewhat modified method (precipitation first with ammonium sulphate and then with zinc chloride) has obtained preparations which, in his opinion, no longer contained non-poisonous albumoses. Hence, he concludes that since these preparations are invariably precipitated by ammonium sulphate and give the biuret and Millon's reactions, *tetanus* poison itself must be an albumose.

Owing to the very poisonous effects which tetanus toxine, even in the smallest doses, has upon certain species of animals, special stress has been laid upon the analogy that exists between that toxine and the ferments. In point of fact there are many reasons in support of that view. Vaillard definitely terms it a ferment, and other authors, such as Tizzoni, Brieger, &c., incline to this view, which as a matter of fact has only been opposed, and that not very strongly, by Fermi in his various published researches. In my opinion we are justified in concluding that there is a considerable analogy between the *toxines* and also some other haptines and the ferments, and I have advocated this view in several places.[2]

There is no doubt that rennet is a haptine, but, as regards the other ferments, there are no data for settling their precise affinities. But there is absolutely no justification for the assumption that tetanus toxine acts like a ferment in the sense of affecting the enzymic decomposition of substances, for tetanus toxine is only akin to a ferment in the *mode*, and not in the *scope*, of its action. It does not effect the changes in starch, cane sugar, &c., brought about by enzymes. It is true that Vaillard found a gelatin-liquefying enzyme in virulent cultivations, but many micro-organisms, pathogenic as well as non-pathogenic, produce similar proteolytic and other enzymes.

Toxoids and Toxones.—The question of the presence of such

[1] Hayashi, "Ueber die chemische Natur des Tetanustoxin," *Arch. f. exp. Pathol.*, xlvii., 9, and *Chem. Centralbl.*, i., 411, 1901.

[2] Oppenheimer, "Toxine u. Schützstoffe," *Biol. Centralbl.*, 1899, 799; *id.*, *Ferments and their Actions*, London, 1901; *id.*, "Zur Theorie der Fermentprozesse," *Münch. med. Woch.*, 1901.

non-poisonous haptines has as yet hardly been systematically investigated. The haptophore group appears to undergo a simultaneous change during the extraordinarily rapid decrease in strength that occurs in fresh poisons; but at the same time it has not yet been proved beyond doubt that there can be a diminution in the toxic activity while the neutralisation value remains constant. All observers, however, have recorded differences between the *direct* and the *indirect* toxic value (*i.e.*, that required to neutralise a corresponding quantity of antitoxine), which point to the existence of non-poisonous haptines.

Behring,[1] in particular, has called attention to this fact. He found that in the case of fresh poisons the direct toxic value was the same as the indirect value (expressed in terms of $\frac{1}{1000}$ of an antitoxine unit). Hence he named these "*equivalent poisons.*" On the other hand, in older cultivations he invariably found the direct toxic value was smaller—*i.e.*, toxoids had obviously been formed, and had neutralised part of the antitoxine, so that it was necessary to add fewer toxic units to reach L_0 than in the case of fresh poisons containing only toxine. Such poisons containing toxoids are well adapted for immunising purposes, as has been stated by Brieger, and is easily explicable. The toxoids appear to have different toxic effects upon different animals; at all events, the poison scale approximates very closely to that of the equivalent poisons (*vide supra*) in the case of certain species.

The existence of *toxones* appears probable in the light of the experiments of Knorr,[2] who found, for example, that on approximate neutralisation with antitoxine (and therefore in the "differential zones") an added quantity of poison had a considerably smaller toxic value than its corresponding direct toxic value, just as in the case of diphtheria poison far more than a lethal dose is required to convert the value L_0 into L_+. He himself, however, appears to reject this conclusion, and to account for the fact in a different way.

Physiological Action of Tetanus Toxine.—Tetanospasmine varies very greatly in its action upon different species of animals, especially with subcutaneous injection.

In the case of very susceptible animals, its toxic effect is astonishingly great when it is introduced subcutaneously in the usual way. Vaillard prepared a solution, 0·001 c.c. of which killed a guinea-pig. This dose contained about 0·000025 grm. of

[1] Behring, "Ueber Tetanusgiftmodifikationen," *Fortschr. d. Med.*, xvii., 501, 1899.

[2] Knorr, "Die Entstehung des Tetanus-Antitoxins," *Fortschr. d. Med.*, 1897, 657.

organic substance—*i.e.*, only a very small trace of pure toxine. The organic matter required for a mouse was only 0·000000025 grm. According to BRIEGER and COHN (*loc. cit.*), the lethal dose of a far from pure toxine was about 0·0000005 grm. for a mouse, and 0·00023 grm. for a man, but occasionally still more poisonous preparations have been obtained. The individual variations are also very great. According to BEHRING and KNORR, the *certain* lethal dose for a mouse is about six times as great as that which is just below that required to kill. Still greater, however, are the variations in the toxicity due to the susceptibility of the different species of animals.

KNORR,[1] in his paper on the subject, gives a scale of susceptibility to tetanus poison. He finds the *horse* to be the most susceptible animal. If we take the dose that kills 1 grm. of horse as unity, the amounts of poison required by the following animals are as follows :—

1 grm.	of	guinea-pig, . .	2	times as much.
1 ,,		goat, ...	4	,,
1 ,,		mouse, . . .	13	,,
1 ,,		rabbit, . . .	2,000	,,
1 ,,		hen, ...	200,000	,,

BEHRING[2] gives the following scale for *fresh poisons*, which show no difference between the direct and indirect toxic value (*equivalent poisons*) :—

One lethal dose for 1 grm. of mouse (+ Ms) kills

12	grms. of	horse.
6	,,	guinea-pig.
0·2	grm. of	goat.
$\frac{1}{150}$	,,	rabbit.
$\frac{1}{1000}$	,,	goose.
$\frac{1}{30000}$	,,	hen.

The numbers thus agree fairly well. In the case of old poisons, in which, according to BEHRING, the direct toxic value (+ Ms) is much smaller than the indirect value (+ ms)—*i.e.*, the relative value for the quantity which, after neutralisation of $\frac{1}{1000}$ antitoxine unit, is still lethal ($\frac{1}{1000}$ antitoxine units = 40,000 Ms), are quite irregular. In like manner the ratios between the smallest dose that causes illness and the smallest fatal dose vary very greatly with the species of animal.

[1] Knorr, "Das Tetanusgift und seine Beziehg. zum tier. Organismus," *Münch. med. Woch.*, 1898, 321, 362.

[2] Behring, "Ueber Tetanusgiftmodifikationen," *Fortschr. d. Med.*, xvii., 501, 1899.

The toxic effect is generally considerably increased when the poison is introduced by *subdural* or *intercerebral* injection. Thus, for example, the otherwise comparatively refractory *hen* can be poisoned fairly readily by the intercerebral injection of tetanus poison. We will deal with the probable cause of this on a later page.

The phenomena on *intravenous* injection are the normal ones, but the *incubation period* is somewhat longer. When introduced into the stomach tetanus poison is practically non-poisonous. Ransom[1] concluded that it passed unchanged through the intestines, and claimed to have found it again in the fæces. Carrière, however, and numerous other investigators were unable to detect any toxine in the excreta after the introduction of large doses of toxine *per os*. Carrière[2] accordingly made experiments to determine where tetanus toxine becomes *innocuous*. He found that the poison was attacked even by the *saliva diastase*, that *pepsin* was less injurious, that *trypsin* had a considerable action upon it, and that *bile* in large quantity completely destroyed it. He could not detect any influence of the *intestinal mucous membrane* and the intestinal bacteria upon the poison, although Fermi and Pernossi had found it to be very pronounced. Nencki and Schoumow-Simanowski[3] assert that the digestive fluids render it completely innocuous, and notably the bile in conjunction with the fluid from a pancreatic fistula, whilst trypsin alone has less effect, being less injurious than pepsin. Vincenzi,[4] on the other hand, states that *normal* bile has hardly any destructive influence, but that the bile of animals infected with tetanus has a slight effect under certain conditions; thus, for example, death inevitably follows in three to four days when the bile is active. On the other hand, the poison is somewhat weakened by the *oxydase* of the leucocytes, and, according to Sieber,[5] is completely destroyed by the oxydase of the spleen.

It is absorbed with great rapidity on subcutaneous injection.

[1] Ransom, "Das Schicksal d. Tetanusgiftes nach seiner intestinal Einverleibung," *Deutsch. med. Woch.*, 1898, 117.

[2] Carrière, "Toxines et digestion," *Ann. Past.*, xiii., 435, 1899 (gives a bibliography of the subject); *cf.* the General Part.

[3] Nencki and Schoumow-Simanowski, "Ueber die Entgiftung der Toxine durch die Verdauungsäfte," *Centralbl. f. Bakt.*, xxiv., 84; *cf.* Dzierzgowski and Sieber, *Archiv. des Sciences Biol. de St. Petersb.*, viii.

[4] Vincenzi, "Ueb. antitoxische Eigenschaften der Galle tetanisierter Tiere," *Deutsch. med. Woch.*, 1898, 534.

[5] Sieber, "Ueb. d. Entgiftung der Toxine durch die Superoxyde, &c.," *Z. f. phys. Ch.*, xxxii., 573, 1901.

It has been established by numerous experiments that a rat into whose tail the toxine has been injected can no longer be saved by amputation of the tail even after two to three hours.

At the same time tetanus poison shows an unmistakable *period of incubation.* COURMONT and DOYON [1] state that, in the case of guinea-pigs, the symptoms appear at the earliest after twelve hours, and in mice after six to eight hours. With medium doses the latent period for mice is two to three days; for guinea-pigs, two days; rabbits, two to four days; the ass, four days; and for the horse five days. In the case of man it fluctuates between one day and sixty days. The action is much more rapid after subdural injection (BLUMENTHAL and JACOB [2]), or *intercerebral* poisoning (ROUX and BORREL [3]), as well as after direct injection into the spinal cord (MEYER and RANSOM [4]).

According to a table given by MEYER and RANSOM the periods of incubation after subcutaneous injection are as follows:—

Mouse,	8 to 12 hours.
Guinea-pig,	13 ,, 18 ,,
Rabbit	18 ,, 36 ,,
Cat,	28 ,, 70 ,,
Dog,	36 ,, 48 ,,
Man,	4 days.
Ass,	4 ,,
Horse,	5 ,,

Hence it increases with the size of the body, and thus stands in relationship to the slowness with which the poison is diffused (*vide infra*).

The incubation period becomes smaller with the increase of the dose, but not proportionally so, and still occurs even after the largest doses. In the case of mice it *never* falls below eight hours. Thus, in one series of experiments, the incubation periods for mice were as follows:—

Lethal Doses	Hours.
13,	36
100,	24
333,	20
1,300,	14
3,600,	12

[1] Courmont and Doyon, *vide supra.*

[2] Blumenthal and Jacob, "Zur Serumtherapie des Tetanus," *Berl. klin. Woch.*, 1898, 1079.

[3] Roux and Borrel, "Tétanos cérébral," *Ann. Past.*, xii., 1898.

[4] Meyer and Ransom, "Unters. üb. d. Tetanus," *Arch. exp. Path.*, xlvi., 369, 1903.

Tetanus poison is therefore rapidly absorbed without at once manifesting its activity. Yet, even a short time after inoculation, free toxine can no longer be detected in the organism of susceptible animals. On the contrary, it has, for the most part, entered into combination with the receptors. Only in the blood, and, to a less extent, in the lymph, can toxine be detected after the injection (Ransom [1]), and in the case of the lymph for an hour afterwards at most.

With these exceptions it cannot be found in any of the organs or secretions (Marie [2]). Brusschettini [3] alone claims to have detected it in the kidneys, whence, according to him, it is excreted. In like manner it has been found by various observers in the *urine*. Brunner [4] detected it in the urine of animals used in experiments, but not in that of infected men, and his results were confirmed by Behring.[5] Kartulis [6] could only find it in the urine when very large doses had been given. It is usually vainly sought in the urine of men suffering from tetanus, though Vulpins [7] found the urine left in the bladder after death so poisonous that 2 c.c. rapidly infected a guinea-pig.

In the case of refractory animals the toxine circulates for a long time in the blood, as we have described in the General Part, without entering into combination with the organs, until eventually it slowly disappears.

In addition to its effect upon the central nervous system, which is the absolutely dominating symptom of tetanus poisoning, and with which we deal at length below, tetanospasmine also produces certain general symptoms, which can only be briefly mentioned, since it can be no part of our task to give a complete description of the pathology of tetanus.

The *pressure of the blood* remains unchanged, as has been found by Hans Meyer and Halsey,[8] contrary to the results of former investigators, and in this respect the symptoms differ from those of strychnine poisoning which is otherwise so similar.

[1] Ransom, "Die Lymphe nach intravenöser Inj. von T.-T.," *Z. f. phys. Ch.*, xxix., 349, 1900.

[2] Marie, "Rech. s. l. toxine tetanique," *Ann. Past.*, xi., 591, 1897.

[3] Brusschettini, quoting from Brunner, *loc. cit.*

[4] Brunner, "Klin. und exp. Stud. üb. Tet.," *Beitr. z. klin. Chirurg.*, ix. to xii., 1892-4.

[5] Behring, "Die Blutserumtherapie," iii., 54, Leipzig, 1892.

[6] Kartulis," Unters. über das Verhalten des Tet.-Giftes im Körper," *Diss.*, Berlin, 1892 (Dec.)

[7] Vulpins, "Ueb. einen Fall von Wundstarrkrampf m. Tiervers.," *Deutsch. med. Woch.*, 1893, 992.

[8] Hans Meyer and Halsey, "Tetanusstudien," *Festschr. f. Jaffé*, Brunswick, 1901.

Eventually, infected animals show a marked reduction in temperature and severe inanition before death (HARNACK and HOCHHEIM [1]).

Effect upon the Central Nervous System.—With the exception of these few pronounced general symptoms, tetanus and also the completely identical poisoning with tetanospasmine are characterised in the case of most animals by the predominance of symptoms due to an affection of the central nervous system. It would appear then that the receptors which combine with the poison and thus initiate the disease only occur in the nervous system. There are only a few facts that have been urged against the view that this is the only point of attack for the poison. Thus VAILLARD, using very small doses of toxine, claims to have observed local poisoning of the neighbouring *muscular tissue.* The *rabbit* also occupies an exceptional position, since under certain conditions the poison can be fixed in other ways to an overwhelming extent, so that the animal dies of "*tetanus sine tetano*" (DÖNITZ). This is a particularly striking instance of the observation that has frequently been made, that the poison can combine with receptors situated in organs of little vital importance, so that the fixation of the poison does not lead to any predominating symptoms of illness. We shall return to this phenomenon presently.

The direct symptoms, the combination of the poison with the substance of the central nervous system, were first observed by SHAKESPEARE [2] and VERHOOGEN and BAERT,[3] who poisoned animals by the subdural injection of material from the central nervous system of tetanised animals.

BESREDKA [4] in repeating this experiment left the normal brain substance of a guinea-pig in contact with a large quantity of toxine for a considerable time (up to six days) in an ice chest, and then removed the excess of free toxine by careful washing. He was able to produce tetanus in mice by means of this substance.

PASQUINI [5] also found the central nervous system to be poisonous.

[1] Harnack and Hochheim, "Ueb. Wirkg. d. Briegerschen Tet.-G.," *Z. f. klin. Med.*, xxv., 46, 1894.

[2] Shakespeare, "Preliminary report on exper. researches concerning the infectious nature of traumatic tetanus," *Centralbl. f. Bakt.*, ii., 541, 1887.

[3] Verhoogen and Baert, "Premières recherches sur la nature du tétanos," *Baumg. Jahresber.*, 1890, 198.

[4] Besredka, "De la fixation de la tox. tét. par le cerveau," *Ann. Past.*, xvii., 138, 1903.

[5] Pasquini, "Sulla presenza del veneno tetanico negli organi," *Rif. Med.*, 1902, 22, 23; Abst. in *Centralbl. f. Bakt.*, xxxi., 117.

Goldscheider and Flatau,[1] Joukowsky,[2] and others have succeeded in demonstrating directly under the microscope the action of tetanus toxine upon the ganglionic cells, especially those of the anterior cornua. Particularly vigorous discussion has taken place on the question whether the nervous system is the only point of attack for the poison; and also whether it is only the *central* nervous system that is attacked.

The question appears to have been decided with certainty by the investigations of Gumprecht[3] in favour of the view that the central nervous system, and above all the spinal cord, is *exclusively* attacked by the poison, and that the symptoms elsewhere are to be attributed to these primary ones. Courmont and Doyon[4] conclude that the whole of the sensory *neuron* is liable to the primary attack.

The statement that the poison attacks primarily *only* the central nervous system is apparently not easy to reconcile with the fact of "*local tetanus.*" The two can only be brought into agreement by the assumption that there is a direct transmission of the poison *in the nerves* from the point of infection to the spinal cord. This conclusion was first drawn by Brusschettini[5] who was himself able to detect the poison in the nervous system, whereas other blood-free organs, including the muscles at the point of infection did not contain it.

This view has recently received support from the particularly comprehensive experiments of Hans Meyer and Ransom.[6] Their results were as follows:—After subcutaneous inoculation with tetanus toxine the poison could be detected in the nerve. This most important result was simultaneously confirmed by Marie and Morax[7] who found the poison in the sciatic nerve of one infected leg, and also in the other after the occurrence of general tetanus; it was also found, in particular, in the masseteric nerve but not in the optic nerve, which is indeed an integral portion of the brain.

[1] Goldscheider and Flatau, "Ueber die Ziele der modernen Nervenzellenforschung," *Deutsch. med. Woch.*, 1898, 165.

[2] Joukowsky, "De l'influence de la toxine tétan. sur le system nerveux," *Ann. Past.*, xiv., 464, 1900.

[3] Gumprecht, "Zur pathol. d. Tetanus," *Deutsch. med. Woch.*, 1894, 546; *Id.*, "Vers. über d. physiol. Wirkg. des Tetanusgiftes," *Pflügers Arch.*, lix., 105 (Bibliography), 1895.

[4] Courmont and Doyon, "Le Tétanos," Paris, *Baillière*, 1899.

[5] Brusschettini, *Rif. Med.*, 1892, quoted from Brunner.

[6] Hans Meyer and Ransom, "Unters. üb. d. Tetanus," *Arch. exp. Path.*, xlix., 369, 1903.

[7] Marie and Morax, "Rech. sur l'absorption de la tox. tét.," *Ann. Past.*, xvi., 818, 1902; xvii., 335, 1903.

The motor, sensory, and sympathetic nerves are equally suitable for the attack, according to MORAX and MARIE, although according to MEYER and RANSOM the normal axis cylinder is the exclusive conveyor of the poison, which reaches it *only* by way of the nerve end plates on the muscle and not through the blood. The poison is only distributed in a centripetal direction. MEYER and RANSOM also found that, in addition to severance of the nerves, *injection of antitoxine* into them prevented the poison from reaching the spinal cord. This happened with certainty in local (subcutaneous) poisoning, and sometimes also after intravenous injection. The centres belonging to the protected nerves remain free, while the animal suffers from general tetanus. Even in the *spinal cord* itself severance can prevent the further distribution of the poison. In the case of animals thus treated no poisoning of the brain occurred; they lay for three weeks in a continuous tetanic state and eventually died apparently from exhaustion. The direct transmission of the poison by way of the nerves appears the more probable from the fact that when directly injected into the nerves its action is much more intense (about ten times as much). In these experiments any quantities of poison which might possibly have passed into the lymph glands or blood on injection, were in each case neutralised by large quantities of antitoxine. The period of incubation is considerably shortened, and if the injection is made directly into the lumbar cord it is reduced to a few hours, as had already been shown by the experiments of ROUX and BORREL[1] on tetanus in the head. The variations in the length of the incubation periods (*vide supra*) are explained by the slow transmission through the nerves.

It follows from these experiments that the poison is first transmitted *exclusively by the nerves themselves* to the centres of the spinal cord, and that the specific symptoms are produced *by these.* If the poison attacks only the sensory centres, there results a simple *tetanus dolorosus without convulsions.* It also results from this strict differentiation that the poison does *not* reach the spinal cord by way of the blood or lymph channels. Under normal conditions the poison only reaches the motor ganglia by way of the motor neuron, and produces there a condition of *over-excitability* towards the usually latent stimuli which proceed from the sensory neurons. This is not the place to go further into the theory of tetanus itself.

The usual point of attack of tetanus poison, and so of the disease, is thus the *spinal cord.* But it is not exclusively the

[1] Roux and Borrel, "Tétanos Cérebral," *Ann. Past.*, xii., 225, 1898.

spinal cord that offers suitable haptophore groups for tetanus toxine. ROUX and BORREL (*loc. cit.*), for example, were able to prove that, on subdural introduction of the poison, the poison combined exclusively with the *brain cells* and produced cerebral tetanus. And, lastly, the experiments of DÖNITZ[1] on rabbits and of MIYAMOTO[2] show that, under certain conditions, the poison can be fixed so rapidly by haptophore cells, other than those of the central nervous system, that the animal dies without spasms from a "*tetanus sine tetano.*" In the case of rabbits this fixation of the poison in the less vitally important organs, which withdraw it from the central nervous system, undoubtedly partially accounts for their smaller susceptibility. At any rate, ROUX and BORREL were able to poison *rabbits* much more readily by intercerebral injection, in which the poison comes into direct contact with the central nervous system, than by subcutaneous injection, which was not the case with *guinea-pigs.* Even the *hen*, so little susceptible in other respects, can be poisoned by intercerebral injection. Under certain conditions, which are still imperfectly known, many poisons (MIYAMOTO employed a very old preparation) appear to lose their predominating affinity for the central nervous system. It is surely not unjustifiable to assume, provisionally, that in this case substances have been formed from the original toxine which are undoubtedly still poisonous, but have lost their characteristic action upon the central nervous system, there being possibly a formation of *toxoids* of a special kind. On the other hand, in the case of refractory animals—*e.g.*, alligators—and also in that of *hens*, which are not completely refractory, METSCHNIKOFF (*vide infra*) observed a slight formation of antitoxine without preliminary symptoms of illness, which surely points to a combination with distributed individual receptors.

The conclusion drawn from the study of the symptoms of the disease—viz., that the tetanus poison combines with the substance of the central nervous system, could also be confirmed experimentally.

It was also shown, simultaneously, by WASSERMANN and TAKAKI[3] and by RANSOM,[4] that an emulsion of the fresh brain

[1] Dönitz, "Ueber das Tetanusantitoxin," *Deutsch. med. Woch.*, 1897, 428.

[2] Miyamoto, "Beiträge zur Tetanusvergiftung," *Deutsch. med. Woch.*, 1900, 479.

[3] Wassermann and Takaki, "Ueber tetanusantitoxische Eigenschaften des Centralnervensystems," *Berl. klin. Woch.*, 1898, 5; Wassermann, "Weiterere Mitt. über Seitenkettenimmunität," *ibid.*, 209.

[4] Ransom, quoted by Behring, *Deutsch. med. Woch.*, 1898, 68.

of a guinea-pig could combine with and render non-poisonous a certain quantity of tetanus toxine. It is true that this power of combination is not very considerable; according to PALTAUF[1] 1 c.c. of the emulsion can combine with 100 lethal doses at most—a point which must not be overlooked in attempting to come to a correct conclusion about this question.

It is the *cells* of the brain that enter into combination, as was shown by BLUMENTHAL[2] and MILCHNER,[3] who found that the liquid was free from toxine after being subjected to centrifugal force.

RANSOM,[4] too, was able to show that on injection of tetanus poison into the subarachnoid space the nerve substance combines with more poison than can be accounted for by the blood that it contains, but that the combination is not absolute, for a part of the toxine passes into the circulatory system even in this method of injection. On the other hand, MEYER, like BLUMENTHAL and JACOB[5] before him and SCHULZE,[6] found the cerebro-spinal fluid to be *free* from toxine.

WASSERMANN regarded the results of this experiment as a confirmation of the side-chain theory. The receptors that are capable of combining with the poison in the brain substance, and also in the living brain, are the same that circulate in the blood as free receptors, so that WASSERMANN is right in forming his conception of *side-chain immunity* from these experiments.

Objections have been raised on various sides, and notably by the school of METSCHNIKOFF[7] and by BEHRING and KITASHIMA,[8] against the conclusions drawn from WASSERMANN'S experiments. It would take us too far from our theme to deal thoroughly with the whole subject in dispute; and so I will content myself with

[1] Paltauf, "Diskussion zu dem Vortrag von Grueber," *Wien. klin. Woch.*, 1901, 51.

[2] Blumenthal, "Ueber die Veränderungen des Tetanusgiftes im Tierkörper," *Deutsch. med. Woch.*, 1898, 185.

[3] Milchner, "Nachweis der chemischen Bindung von T.-G. durch Nervensubstanz," *Berl. klin. Woch.*, 1898, 369.

[4] Ransom, "Die Injektion von Tetanustoxin bezw. Antitoxin in den subarachnoidalen Raum," *Z. f. phys. Chem.*, xxxi., 282, 1900-01.

[5] Blumenthal and Jacob, "Zur Serumtherapie des Tetanus," *Berl. klin. Woch.*, 1898, 1079.

[6] Schultze, "Spinalpunktion u. Einspritz. v. Antitoxinserum bei Tet. traumaticus," *Mitt. Grenzgeb. d. Med. u. Chir.*, v., 169, 1900.

[7] Metschnikoff, *Immunität*, German ed. by Meyer, Jena, 1902. Also, Metschnikoff, "Influence de l'organisme sur les toxines," *Ann. Past.*, xi., 801, 1897; xii., 81, 1898. Marie, "Proprieté antitétanique des centres nerveux.," *ibid.*, xii., 91, 1898. Besredka, "De la fixation de la toxine tét.," *ibid.*, xvii., 139, 1903.

[8] Behring, *Allg. Ther. d. Infekt.-Kr.*, i., 1033.

calling attention to the work of MARX,[1] who has resurveyed the whole ground.

MARX has found, by over 200 experiments on mice, that the antitoxic action of the brain is of such a nature that it is merely supplemented by the addition of serum, and that the action of the brain does not, as was found by KITASHIMA (*loc. cit.*) in experiments with very large amounts of toxine, *destroy* the action of the antitoxine.

MARX thus confirms WASSERMANN'S view that the combined receptors of the brain act qualitatively and quantitatively in a completely analogous manner to the free receptors that are present in the antitoxine serum.

And this affords a fresh support of EHRLICH'S view that the poison can only combine with suitable receptors, and that these receptors, when broken off, have a neutralising effect upon the poison, although they promote its action when acting in conjunction with living cells.

It is impossible to see the force of the objection first brought against these experiments—viz., that the brain of animals, such as hens, &c., that are only slightly susceptible, has *no protective action*—for it is self-evident to anyone who bases his conclusions on the fundamental axioms of Ehrlich's theory, that only such nerve substance as actually combines with the tetanus toxine—*i.e.*, possesses suitable haptophore groups—can exert a protective influence ; and such haptophore groups are evidently only present in the brains, &c., of these animals, which indeed are not refractory, not *naturally* immune to tetanus. It is thus one of the most striking results of Ehrlich's theory that it at once throws a clear light upon the formerly so inscrutable problem of natural immunity. Both infection and the relative formation of antibodies can only take place where there is a reciprocal combination of haptophore groups. Where these are lacking there can be absolutely no infection or neutralisation of the poison.

Indeed, the central nervous system is not the exclusive place where the antitoxine can be produced. As we have mentioned above, receptors that can combine with the poison also occur in other places where the fixation of the poison does not lead to any injurious effects—*e.g.*, in the connective tissue, &c. But *wherever receptors are present, there, too, can the formation of antitoxine take place.* The production of antitoxine is a function of the haptophore group, and has absolutely nothing to do with the *action of the poison* as such. Thus, according to EHRLICH

[1] Marx, "Die Tet.-G. neutralis. Eigensch. d. Gehirns," *Zeit. f. Hyg.*, xl., 231, 1902.

and WASSERMANN, the central nervous system has a protective influence, not because it offers a point of attack for the toxophore group, but because it contains *receptors*, which may also be present in other tissues.

Against this view METSCHNIKOFF raised the objection that even the brain of *frogs* has absolutely no protective influence. Although frogs are absolutely insusceptible to tetanus in the cold, yet when warmed above 20° C. they are extremely susceptible, as is also the case to a less extent with other amphibia and reptiles, and also with marmots (BILLINGER[1]), which do not die during hibernation (*i.e.*, when their temperature is lower), but only after they wake from their sleep. Bats, too, when kept cold, exhibit considerable resistance so long as they sleep (MEYER and HALSEY, *loc. cit.*). Now the brain of the frog does not combine with, or show any protective influence against, tetanus toxine, and no antitoxine ever appears in the body of the frog when poisoned with tetanus. Although this assertion is doubtless correct, and although the process of the breaking off and free movement of the haptophore groups has as yet been but little explained, yet the interpretation given by METSCHNIKOFF of this phenomenon—viz., that the central nervous system of the frog does not combine in the slightest degree with tetanus toxine—is certainly not correct. MORGENROTH,[2] in a very interesting research, was able to demonstrate that tetanus poison, even in the cold, does unquestionably combine, although slowly, with the central nervous system of the frog, but that the *toxophore* group is inactive. On raising the temperature, however, it immediately begins to act.

The combination of the toxine with the central nervous system takes place very rapidly after its injection into the circulatory system. DECROLY and RONSSE[3] showed that in the case of rabbits the blood was not poisonous even *one minute* after the intravenous injection of a lethal dose of tetanus poison. Such is the rapidity with which the toxine disappears from the blood.[4]

[1] Billinger, "Winterschlaf und Infektion," *Wien. klin. Woch.*, 1896, 769.

[2] Morgenroth, "Zur Kenntnis des Tetanus des Frosches," *Arch. Internat. d. Pharmacodynamie*, viii., 255, 1900 (reprint).

[3] Decroly and Ronsse, "Pouvoir toxique et antitoxique du Sang, &c.," *Arch. Internat. de Pharmacodyn.*, vi., 211, 1899.

[4] Dönitz (*vide supra*), of course, assumes that in the case of *rabbits* receptors are also present in other organs, and that this accounts for the extraordinarily rapid disappearance of the toxine. In the case of guinea-pigs and mice it circulates longer in the blood. I have already had frequent opportunities of calling attention to the importance of such distributed receptors.

Now, very important consequences follow from this extraordinarily great affinity of the toxine for the nervous system. Thus it is easy to render tetanus poison harmless by means of antibodies before it enters the nerve tracks, but much more difficult to break up the compound when once formed, to separate the toxine from the receptor, to *cure* the tetanus. With every hour the combination becomes more stable, and the activity of the serum less. It has been shown by Dönitz [1] how rapidly this injurious result takes place. The same quantity of antitoxine, which, when simultaneously injected, protected a rabbit from a quadruple lethal dose, was absolutely unavailing when injected four minutes after the toxine (intravenous injection being used in each case). After one hour as much as 40 times the amount of antitoxine was necessary. After five hours even huge doses, such as 600 times the single effective dose, were useless. Morgenroth, to his disappointment, found that the serum failed in its action in an exactly analogous manner in the case of frogs, although more slowly. In the therapeutic treatment of men it has frequently been observed that after the occurrence of the characteristic symptoms of tetanus—*i.e.*, after the lapse of the incubation period—even huge doses of immune serum are usually of no avail.

I have only dealt with this question to this extent, and the further discussion of it does not concern us here, because, on these facts, taken in conjunction with the occurrence of a longer or shorter *incubation period* (*vide supra*), attempts have been made to base conclusions as to the alterations in tetanus poison even in the human system.

Thus certain authors (*e.g.*, Courmont and Doyon [2]) have assumed that the poison that eventually produces the outbreak of tetanus is *not* the primary toxine of the cultivations. The latter is asserted to act only as a ferment, which, under suitable conditions, decomposes the protoplasm of the attacked cell with the formation of the *true* tetanus poison, which can then produce its poisonous effects without a period of incubation. In reply to the arguments urged by these authors in support of their view, the following remarks may be made:—As regards the *period of*

[1] Dönitz, "Ueber das Tetanusantitoxin," *Deutsch. med. Woch.*, 1897, 428.

[2] Courmont and Doyon, *inter alios.*—(*a*) "Mecanisme de production des contractures du tétanos," *Arch. de Phys.*, 1893, 64; (*b*) "La substance toxique qui engendre le tétanos," *Sem. Med.*, 1893, 122; (*c*) "Du tét de la grenouille, *ibid.*, 1893, 302; (*d*) "De la produc. d. t. chez la poule," *ibid.*, 1893, 486. See also their papers already quoted, and their work *Le Tetanos*, Paris, 1899. (For other investigations *see* Blumenthal, *loc. cit.*)

incubation it is assumed that the true poison is produced during this period, and that until then there is no action. This complicated view is not necessary, for the mode of action of the poison is at least as satisfactorily accounted for by the slow action of the toxophore groups and by the different degrees of slowness in the distribution of the poison in the nerve tracks as found by MEYER and RANSOM (*vide supra*). A further point against their view is that the period of incubation becomes shorter in proportion to the amount of toxine introduced into the animal, although, as is the case with every toxine, there is a limit to this reduction of the incubation period by larger doses.

Again, the complicated behaviour of the incubation period in the case of the frog, which has been brought forward as a special argument in support of this view can be explained more simply.

MORGENROTH was able to prove that on warming the animal there was at first only a very rapid and firm combination of the toxine, but no poisoning. If the frog, after being kept at 32° C. for twenty-four hours, was then again placed in the ice chest, it remained permanently well, although if again exposed to a temperature of 32° C. it died after an incubation period that was *shortened by twenty-four hours*, notwithstanding the injection of enormous quantities of antitoxine. If, as COURMONT and DOYON assume, a secondary change in the poison were produced by the warming, the frog would not remain permanently well even in the ice chest. The heat has here *only* promoted the formation of an extremely stable combination, and this requires further warming before it causes death by the action of the toxophore group. The fact that *after* combination has taken place the antitoxine is more or less completely *ineffective* has been put forward as of special significance in support of the theory of the secondary poison, but in my opinion this is far from being the case. The predominance of the antitoxine circulating in the blood over the toxine, which it prevents from combining with the central nervous system is solely due to the fact that it does so circulate in the blood and thus immediately *seizes upon* the toxine when introduced into the circulatory system and mechanically wards it off from the threatened tissues, and not to its possessing any greater *affinity* for the toxine. It has but little power of breaking up the combination between the nerve cell and the toxine when once formed, and it does so only by mass action when very large doses of antitoxine have been used. It is thus obvious that the antitoxine must be ineffective if introduced some time after the poisoning, and much more so when only applied after the appearance of the symptoms, and

there is absolutely no need for us to assume the existence of a new poison without affinity for the antitoxine. Nor can the theory derive any greater support from the fact that the brain of animals that have died of tetanus can still combine with the poison.

But if these considerations do not altogether explain the want of influence of the antitoxine upon the toxine after the lapse of a certain time, the discovery by MEYER and RANSOM (*loc. cit.*) of the fact that tetanus antitoxine *cannot follow the poison in its passage along the nerve tracks* affords all that is still needed in explanation of this phenomenon. This fully accounts for the want of activity of the antitoxine, and there is no longer any need to call to our aid the hypothesis of a secondary poison. This hypothesis has not, however, as yet been thereby definitely disproved.

An experimental proof brought forward by COURMONT and DOYON in support of their view was the fact that the transfusion of blood from a dog suffering from tetanus into another *immediately* produced symptoms of tetanus in the latter. KRAUS,[1] too, succeeded in rapidly poisoning other mice with the blood serum from a mouse infected with tetanus, as had previously been done by Nissen.[2] Here then, in their opinion, there was manifested the activity of the secondary poison. It was next claimed that this poison had been detected in animal organs.

BLUMENTHAL[3] prepared from the organs of an animal that had died of tetanus, a poison which, in a dose of 0·35 c.c., caused death with tetanic symptoms in seventeen minutes without an incubation period, and was not rendered ineffective by the antitoxine. BUSCHKE and OERGEL[4] have also succeeded in preparing a poison that acted instantaneously from the liver, spleen, and spinal cord of an animal that had died of tetanus, whilst TAUBER[5] obtained a similar preparation in smaller quantity from the spinal cord, brain, and liver. Such reputed dis-

[1] Kraus, "Beitrag zur Klinik des Tetanus," *Z. f. klin. Med.*, xxxvii., 247, 1899.

[2] Nissen, "Ueb. den Nachweis von Toxin im Blut, &c.," *Deutsch. med. Woch.*, 1891, 775.

[3] Blumenthal, "Weit. Beitr. z. Kenntn. des Tetanusgiftes," *Z. f. klin. Med.*, xxxii., 325, 1897; *Id.*, "Ueber die Veränderungen des T.-G. im Tierkörper," *Deutsch. med. Woch.*, 1893, 149.

[4] Buschke and Oergel, "Beitrag zur Kenntnis des Tetanus," *Deutsch. med. Woch.*, 1893, 149.

[5] Tauber, "Ein Beitr. z. Kenntnis d. Tetanus," *Wien. klin. Woch.*, 1898, 747.

coveries of poison in the organs of dead animals must be received with great scepticism; it is impossible to know with absolute certainty what has actually been extracted and what kind of poisonous substances may have been formed during the process of disease with its alterations in the protoplasm—substances that may have absolutely no connection with tetanus toxine, and against which the antitoxine is powerless. Even BLUMENTHAL himself, as also more recently COURMONT and DOYON, now cite these discoveries with great caution, and leave it an open question how far this poison from the organs corresponds with the actual tetanus poison. BLUMENTHAL regards it as a combination of the true poison with the cell substance. This cannot be proved, however, by such means. To recapitulate, COURMONT's fermentation theory of tetanus toxine has not as yet been proved, and is as yet superfluous. Whether, notwithstanding, it is not correct has yet to be decided. But it is advisable not to obscure this extremely difficult field by additional hypotheses for which definite support is lacking.

Tetanus Antitoxine.— The antitoxine of tetanus behaves towards its toxine in a manner essentially analogous to that of *diphtheria—i.e.*, its antitoxic power can be shown to stand in certain definite relationships to the toxine. Both enter into combination with their toxines, and subsequent separation is impossible. The combination does not take place so rapidly, however, in the case of tetanus toxine, and hence, according to DÖNITZ,[1] the test should not be applied until after forty-five minutes. Moreover, the degree of saturation depends upon the concentration; the greater this is, the more rapid and complete the saturation, so that solutions of as nearly as possible equal concentration must be taken for the comparison. As regards the quantitative relationships the conditions are approximately the same as in the case of diphtheria. In this case, however, they have not been so fully elucidated, which is to be attributed, in the main, to the extraordinary instability of the toxine. The compound also appears to be much less stable, and sooner to attain conditions of equilibrium (on this point see the General Part). The serum can be preserved for a long time by the addition of 1 per cent. of chloroform or 0·6 per cent. of phenol if kept cold and in the dark. Alcohol and distilled water are harmless to it (BEHRING[2]).

ROUX and VAILLARD (*loc. cit.*) were able to dry the cow's

[1] Dönitz, *Bericht üb. d. Thätigkeit des kgl. Instituts f. Serumforsch., &c.* Reprint from the *Klin. Jahrb.*, 1899, vii.

[2] Behring, *Die Blutserumtherapie*, iii., Leipzig, 1892.

serum in a vacuum without loss, and when it was required for use dissolved the residue in six times its quantity of water.

The *milk* of immunised animals also contains antitoxine. According to Brieger and Cohn[1] it can be concentrated from the milk in the following manner:—

The milk is first coagulated by means of rennet in an analogous manner to that used in the preparation of diphtheria antitoxine. The filtered whey is shaken with chloroform, allowed to stand, decanted, and then saturated with ammonium sulphate to the extent of 32 per cent. The precipitate is redissolved, a small amount of basic lead acetate added, and the new precipitate washed with water rendered very slightly alkaline. The filtrate and washings are now *saturated* with ammonium sulphate, and the resulting precipitate mechanically freed from the excess of solid ammonium sulphate by stirring it with pure chloroform. The salts fall to the bottom, while the light compound of the anti-bodies rises to the surface and is removed. In this way it was possible to concentrate the antitoxine to the extent of 300 to 400 times the strength of that originally present in the milk. A still further purification of the antitoxine can be effected by treating the filtrate, after removal of lead, not with ammonium sulphate, but first with sodium chloride, and then with sodium phosphate. Practically none of the antitoxine is precipitated with the sodium chloride precipitate, whereas it is nearly all carried down by the sodium phosphate precipitate.

The properties of the antitoxine have naturally received much attention. It does not differ materially from diphtheria antitoxine. It is partially destroyed at 68° C., though not completely so even at 80° C. Acids (hydrochloric acid, in the proportion of 1 part to 15 of antitoxine, and lactic acid) have also a destructive influence.

Very weak alkalies do not injure it, but, when concentrated, rapidly destroy it. It does not readily putrefy (Behring).

It is not dialysable, and is partially retained by Chamberland porcelain filters. Tizzoni and Cattani[2] attribute to it the nature of a ferment, for which, in my opinion, there is no justification (see under *Diphtheria*). They draw this conclusion from the fact that it can be precipitated by alcohol, and can be extracted, though slowly, from the precipitates by means of glycerin. Doubtless, like diphtheria antitoxine, it is also a substance closely allied to the globulins.

[1] Brieger and Cohn, "Beitr. z. Concentr. geg. Wundstarrkrampf schützenden Substanz.," *Zeit. f. Hyg.*, xv., 439, 1893.

[2] Tizzoni and Cattani, "Sur les proprietés de l'antitoxine du tétanos," *Arch. ital. d. Biol.*, xvi., 394 (abstract), 1891; "Ueber d. Eigenschaften des Tetanus-Antitoxins," *Centralbl. f. Bakt.*, ix., 685, 1891; "Fernere Unters. üb. das Tetanus Antitoxin," *ibid.*, x., 33, 1891; Tizzoni, "Ueb. d. experim. Immunität gegen Tetanus," *Festschr. für Virchow*, Berlin, iii., 29, 1892.

It had already been found by TIZZONI and CATTANI that it was precipitated by magnesium sulphate. They had also observed that the globulins that were precipitated by means of weak acids (acetic acid, carbonic acid) or those separated by means of dialysis did not carry down the antitoxine, but only those globulins that were precipitated by means of solid magnesium sulphate at 30° C.

PICK (*loc. cit.*) was able to confirm these statements by means of his more delicate methods. According to his results tetanus antitoxine is distributed in exactly the same manner as diphtheria antitoxine; thus, in horse serum, he found it to be combined exclusively with the *pseudoglobulin*.

The *value* of tetanus antitoxine has been calculated by BEHRING[1] in the following manner:—A "*single*" serum is taken to be that which will protect 1 grm. of an animal against the action of a certain lethal dose. Thus 1 c.c. of serum of the strength of 1 in a million will protect 50,000 mice of 20 grms. each; hence a mouse requires $\frac{1}{50000}$ c.c. or 0·00002 c.c., a sheep of 50 kilos. 0·05 c.c., and a horse of 400 kilos. 0·4 c.c. Recently, however, the serum has been tested as accurately as diphtheria serum in the Royal Institute for the Investigation and Testing of Serum. Test poisons are standardised upon a test serum that has been kept unaltered, and are then used for the valuation of the sera under examination.

A fact of the greatest importance for the estimation of the action of antitoxine in the organism was that established by HANS MEYER and RANSOM (*loc. cit.*)—viz., that the antitoxine, unlike the toxine, is *not* capable of penetrating the axis cylinder. Hence the antitoxine is absolutely powerless against the poison when *once the latter is in the nerves.* Nor can it penetrate even by way of the blood and lymph tracts into the centres. Thus it can only neutralise the *excess* of poison in the tissues. And this explains why even highly-immuned animals can be poisoned when the poison is introduced directly into their nerves. MEYER and RANSOM hoped to be able to *effect a cure by the direct injection of antitoxine into the nerves.*

[1] The values given by BEHRING (*Die Blutserumtherapie*, ii., 20) do not exactly tally. If 1 c.c. protects 50,000 mice, *one* mouse requires not 0·00005 c.c., but 0·00002 c.c., and a horse of 400 kilos. not 0·25, but 0·4 c.c.

BOTULISM TOXINE.

A third *true* toxine is the active principle in many cases of *flesh poisoning.*

Considerable light was thrown upon the fairly obscure etiology of botulism by the discovery of Van Ermengem,[1] who isolated from a poisonous ham a saprophytic bacillus, *B. botulinus*, which even then he concluded to be the producer of a specific, extremely active toxine. According to Kempner, it can also be detected in the fæces of swine, and Schneidemühl[2] regards it as the cause of the so-called *birth-paralysis* of cattle. Although substances had already been isolated at an earlier period, and asserted to be the active agents in the poisoning—*e.g.*, that isolated by v. Anrep[3] from sturgeon's flesh—yet botulism toxine was the first that was proved to be the *specific* poison of flesh poisoning, and a *true* toxine.

Van Ermengem isolated it by filtration of cultivations of his *Bacillus botulinus.* It is extremely poisonous, the lethal dose for man being as little as 0·035 mgm. This in itself points to its being a true toxine. A further proof is the specific nature of its action, the symptoms of illness produced corresponding exactly with those of botulism.

It produces the same symptoms in the eyes, aphonia, constipation, and retention of urine. Fever does not occur. Finally, symptoms resembling those of bulbar paralysis are manifested, and end in death.

Its action does not begin until after a certain period of incubation.

According to Forssman,[4] the mode of introduction has an influence upon the results. Thus, intercerebral injection did not produce the same characteristic form or intensity of illness as followed subcutaneous injection; there was a greater difference with intraperitoneal, and the greatest with *intrapulmonary* injection. Violent dyspnœa is then the predominating symptom of the poisoning. Moreover, after interpleural injection the toxine is five to nine times as poisonous, although the period of incubation after a single lethal dose is longer. On the other

[1] Van Ermengem, "Ueber einen neuen anaëroben Bacillus u. s. Bezieh. zum Botulismus," *Zeit. f. Hyg.*, xxvi., 1, 1897.

[2] Schneidemühl, "Ueb. Botulismus beim Menschen und die sog. Geburtsparalyse bei Rindern," *Centralbl. f. Bakt.*, xxiv., 619, 1898.

[3] v. Anrep, "Intoxication par les ptomaines," *Arch. Slaves de Biol.*, i., 341, 1886, quoted by v. Ermengem, *loc. cit.*

[4] Forssman, "Beitr. z. Kenntn. d. Bakt. d. Botulismus," Author's abstract in *Centralbl. f. Bakt.*, xxix., 541, 1901.

hand, the minimum period of incubation (with large doses) is six hours after subcutaneous injection, and four hours after intra-pulmonary injection.

Like all true toxines, it is very sensitive to external influences. It is rapidly weakened by air and light, and also by raising the temperature even to 58° C. for three hours. Alcohol, ether, and oxidising substances also destroy it rapidly, while reducing agents are relatively but little injurious. On the other hand, it is a remarkable fact that it can act by way of the intestinal canal, as was found by VAN ERMENGEM, and confirmed by FORSSMAN. The fluids of the stomach and small intestine do not injure it, but it is speedily destroyed by the *contents of the large intestine.*

BRIEGER and KEMPNER [1] have prepared the toxine in a concentrated form by Brieger's method :—

> The germ-free filtrate is partially neutralised with ammonia, and treated with twice its volume of a 3 per cent. solution of zinc chloride. The precipitate is carefully washed and cautiously treated with a 1 per cent. solution of ammonium bicarbonate until the mixture just shows an alkaline reaction, after which it is decomposed with ammonium phosphate, the liquid filtered from the precipitated zinc phosphate, and the toxine precipitated with ammonium sulphate.

In this way they obtained very small yields of solid toxine in quantitative experiments.

Botulism toxine is a specific *nerve poison.* KEMPNER and POLLACK [2] and MARINESCO [3] have simultaneously studied its action upon the anatomy, and especially the alterations produced in the cells of the anterior cornua, and the destruction, chromatolysis, and decomposition of Nissl's granule, with which I cannot deal here.

It is, however, very important that, owing to this strong affinity for the nerve substance, botulism toxine is fixed and rendered harmless by it in an analogous manner to tetanus poison.

KEMPNER and SCHEPILEWSKI [4] found that the brain and spinal cord were able to combine with considerable quantities of poison, and that that property could be utilised both in the prophylactic injection of brain substance, as well as for the neutralisation of

[1] Brieger and Kempner, "Beitrag z. Lehre v. d. Fleishvergiftung," *Deutsch. med. Woch.*, 1897, 521.

[2] Kempner and Pollack, "Die Wirkung des Botulismus toxine auf die Nervenzellen," *Deutsch. med. Woch.*, 1897, 505.

[3] Marinesco, "Lésions des Centres nerveux produites par la toxine du B. botulinus," *Soc. Biol.*, xlviii., 31, 1896 ; *Sém. Méd.*, 1896, 488.

[4] Kempner and Schepilewski, "Ueb. antitoxische Substanzen geg. d. Botulismusgift.," *Zeit. f. Hyg.*, xxvii., 213, 1898.

toxine already in the system for a period of twelve hours after its introduction. These properties distinguish the action of the substance of the central nervous system from that of certain simpler chemical substances, which, while equally combining with the botulism poison *in vitro*, completely lack its immunising and curative capacity. Examples of such substances are *lecithin* and *cholesterin*, but not *cerebrin*. This property of the brain substance is destroyed by boiling.

According to CHARRIN and BARDIER,[1] it also acts as a heart poison. It retards the action of the heart, and acts more rapidly than diphtheria poison in this respect.

KEMPNER[2] subsequently prepared an antitoxic serum against botulo-toxine by the immunisation of goats. The antitoxine acts in accordance with the law of multiples. It can, however, only have any curative effect when used within twelve hours; the dyspnœic form in particular is asserted by FORSSMAN to be incapable of cure. The practicability of the treatment is therefore as doubtful here as in the case of tetanus.

PYOCYANEUS TOXINE.

Bacillus pyocyaneus, which is very pathogenic for many animals, also produces a *true* toxine. The poisonous action of this toxine has frequently been investigated, as have also the phenomena of immunity that result on its introduction into the bodies of animals. There are also numerous researches on the "toxicity" of *B. pyocyaneus* in scientific literature, in which naturally no distinction was made between the poisonous effects of the cells and of the filtrate.[3] Essentially, however, immunity against *B. pyocyaneus* is *bactericidal;* no antidote to the poison is produced in immune bodies, but the bacilli themselves perish under the influence of a specific agent directed against them. Here, then, we meet with conditions similar to those that occur in *typhus* and *cholera*, with which we shall deal in a later page.

The conditions differ, however, from those of *cholera* in one essential particular. Whilst in that disease the supposed toxine can only be separated very sparingly from the vibriones, and, in

[1] Charrin and Bardier, "Action cardiaque, propriété speciale de la botuline," *Soc. Biol.*, l., 60, 1898.

[2] Kempner, "Weit. Beitr. zur Lehre von der Fleischvergiftung," *Zeit. f. Hyg.*, xxvi., 481, 1897.

[3] The most important literature on the subject is given by Breymann, "Ueb. Stoffwechselprod. des B. pyocyaneus," *Centralbl. f. Bakt.*, xxxi., 841, 1902.

the main, remains attached to the cells themselves, it can be separated almost completely from the cells in the case of *pyocyaneus*, so that we must assume that secretory processes occur here similar to those that produce diphtheria toxine.

B. pyocyaneus has a very characteristic behaviour, as was shown by WASSERMANN.[1] Frequently it develops in the body of an animal, and is thus *infectious*, and then the bactericidal protective forces come pre-eminently into action.

On the other hand, however, it develops in its cultivations a *true* toxine, which can be separated from the cell substance, and produces in the organism a true antitoxine.

B. pyocyaneus thus occupies a very interesting intermediate position between the purely toxic diphtheria bacillus, on the one hand, and the bacteria of the cholera type, on the other hand, in the case of which immunity is also pre-eminently bactericidal, *not antitoxic*, while the supposed endotoxines have not as yet been isolated in the free state.

WASSERMANN proved that this poison could be separated almost completely from the bacilli, so that the cells, just as in the case of diphtheria, contained practically no more toxine; but, above all, he showed that by injection of the living bacilli in small, but increasing, doses an immunising process results, which was directed *exclusively* against the bacilli themselves, and had absolutely no influence upon the poison.

But, on the other hand, he succeeded in producing a true *antitoxic* solution by means of the soluble poison. In this case the serum had absolutely no *bactericidal* power *in vitro;* yet, at the same time, an animal rendered proof against the poison was also immune against the living bacilli, just as in the case of diphtheria; for the bacilli, deprived of their keenest weapon, their *toxic function*, are merely harmless intruders in the organism rendered proof against the poison, and speedily perish without inflicting any injury.

WASSERMANN thus proved that *B. pyocyaneus* produced a true toxine, which in this respect was undoubtedly akin to diphtheria and tetanus toxine. It is somewhat more stable than these, especially as regards its behaviour towards heat. It is not *completely destroyed* even by boiling.

In consequence of this a fact of theoretical importance was established in the case of pyocyaneus poison.

It was found that in a neutral mixture of pyocyaneus toxine and antitoxine the latter could be completely eliminated by heat,

[1] Wassermann, "Unters. üb. einige theoret. Punkte d. Immunitätslehre," *Zeit. f. Hyg.*, xxii., 263, 1896.

so that the previously neutral serum became poisonous again. This proves beyond doubt that what CALMETTE had previously shown to be the case with snake poisons also applies to bacterial toxines—viz., that the antitoxine does not effect any destruction of the poison, but that there is a simple form of combination which renders the toxine incapable of attaching itself by means of its haptophore group to the cell and poisoning it.

WASSERMANN prepared his toxine by cultivating *B. pyocyaneus* on beef bouillon containing 2 per cent. of peptone, and subsequently sterilising it by means of toluene. Further investigation of the poison and its antitoxine are still required. In particular an answer is still needed to the question whether the toxic principle is identical with pyocyanolysine, with which we shall deal presently, or whether the *B. pyocyaneus*, as is probably the case, resembles the tetanus bacillus in producing two active substances, a toxine and a lysine.

It can be isolated by filtration through Chamberland filters. Its toxicity cannot be compared, at all events as yet, with that of tetanus toxine, for example. WASSERMANN found the lethal dose for guinea-pigs to be 0·5 c.c.

The quantitative ratios between pyocyaneus toxine and antitoxine show a very important peculiarity.

In this case the law of multiples only holds good up to about 10 times the lethal dose. From that point upwards even great doses of antitoxine no longer afford protection.

Undoubtedly WASSERMANN is right in concluding from the relatively slight toxicity of the poison, as well as from this restricted formation of antitoxine, that pyocyaneus toxine contains *secondary poisons of non-haptoid nature* derived from the toxine, such as have hitherto only been obtained *outside* the body in the case of cholera and typhus. In this respect, too, it would seem that *B. pyocyaneus* occupies an intermediate position between the diphtheria bacillus, which produces relatively stable true toxines, and the bacteria of cholera and typhus.

THE TOXINE OF SYMPTOMATIC ANTHRAX.

A weak immunising poison which did not cause death, and was only partially destroyed at 115° C., was obtained by ROUX [1] by filtration of cultures and from flesh juices of infected animals.

DUENSCHMANN [2] cultivated the bacilli of symptomatic anthrax

[1] Roux, "Immunité contre le charbon symptomatique," *Ann. Past.*, ii., 49, 1888.

[2] Duenschmann, "Étude expérim. sur le charbon symptomatique," *Ann. Past.*, viii., 403, 1894.

anaërobically on macerated flesh or ox serum, and found in the filtrate after seven days a poison with a specific action, which killed guinea-pigs in doses of 5 to 6 c.c. It had no protective action against *living bacilli*.

ARLOING,[1] in his latest researches on immunisation against symptomatic anthrax, makes no mention of any toxine being formed by the bacteria.

Our knowledge of this otherwise practically unknown poison has been greatly enlarged by the recent monograph of GRASSBERGER and SCHATTENFROH.[2] Their results show that a true toxine is produced by the bacillus of symptomatic anthrax which had not been discovered by the researches of previous workers. They find that the formation of the toxine by the bacillus only takes place under certain definite conditions, notably when the micro-organism shows itself as a typical producer of butyric acid. For this purpose the presence of fermentable sugar, or still better of calcium lactate, in the culture medium is a primary necessity. It very frequently happens that there is no formation of toxine at all; the "denaturalised" micro-organisms are no longer able to ferment the lactic acid and produce no trace of toxine. In such cases the power of sporulation may be either restricted or retained in full vigour. On the other hand, there may be an energetic formation of toxine in a quiet after-fermentation in which chiefly the lactic acid is decomposed, while none was formed in the first vigorous fermentation, and this may also occur in cultivations which are *free from sugar* but contain lactic acid. A further point of the utmost importance is the *purity* of the cultures, since other bacteria apparently injure the very unstable toxine. A temperature of about 37° C. is also necessary for the production of the toxine. The formation of toxine by the bacillus of symptomatic anthrax is a true free secretion. Filtration through infusorial earth was found to be the best means of separating the toxine from micro-organisms, since more compact filters absorbed too much of the poison.

The effects of the toxine on guinea-pigs are similar to those caused by infection with symptomatic anthrax—viz., œdema, areas of hæmorrhage, lowering of the temperature, œdema of the lungs—in fact all the general symptoms of toxine poisoning. The period of incubation is only a few hours, while the disease lasts for two to four days or, after very large doses, six to seven hours.

[1] Arloing, "Serothérapie du charbon symptomatique," *Comptes Rend.*, cxxx., 548, 1900; cxxxi., 319, 1900.

[2] Grassberger and Schattenfroh, *Ueber das Rauschbrand*, 1904.

The unit of measurement chosen was a solution of the poison, 0·01 c.c. of which killed a guinea-pig of 200 to 300 grms. on subcutaneous injection. In the case of rabbits death did not take place until after the lapse of an hour after the intravenous injection of even 1,000 lethal doses; the lethal dose for them on subcutaneous injection was 0·1 to 0·2 c.c. of the normal poison. Similar amounts per kilo. of body weight were also fatal to monkeys, dogs, hedgehogs, mice, hens, pigeons, sheep, and oxen. The lethal dose for a young ox was 40 c.c. of the normal poison, while sheep required about 2 c.c. Frogs were refractory, but retained the poison within their body.

The poison passes very slowly through a porcelain filter and is hardly dialysable. It resists freezing and thawing, and light has but little injurious effect upon it, but it is affected when heated even to 30° C. On the other hand, it can be dried in a vacuum in the cold. It is almost completely destroyed in an hour when exposed to a temperature of 50° C., so that very large doses (7 to 10 c.c.) then produce only local swelling. It becomes rapidly weaker even when kept in an air-tight vessel and still more rapidly when exposed to the air.

It is destroyed by permanganate added in the proportion of 0·15 per cent., by phenol (1 per cent.), and formaldehyde (0·1 per cent.), while "salting out" with ammonium sulphate and precipitation with alcohol and ether have a considerable injurious effect. Chloroform is absolutely without influence upon it.

The poison of symptomatic anthrax is also shown to be a *true toxine* by the fact that it is possible to produce an antitoxine to it. Guinea-pigs cannot be used for this purpose since they are too susceptible, but it can readily be done with rabbits and oxen. It is very easy to immunise the latter, and they yield sera of high antitoxic value (up to 400-fold), whereas even highly immune rabbits give sera which are only weak in antitoxine. The ratios between the toxine and antitoxine show numerical proportions exactly analogous to those observed, *e.g.*, in the case of diphtheria toxine (marked variations in the value of D, swellings caused by the injection of mixtures in the "differential zone," &c.), and thus point to the presence of *toxones*. On the other hand, there does not appear to be any *formation of toxoids*, since the decrease in the toxicity keeps parallel with the decrease in the combining power. Moreover, combination appears to take place very slowly.

The antitoxine appears to be very stable (it can be kept for two years). It is not dialysable; it can be dried and can resist a temperature of 60° to 65° C. for an hour.

Over-saturated mixtures of toxine and antitoxine are frequently still poisonous to guinea-pigs, though other animals can be immunised by their means, so that here too there appear to exist conditions of equilibrium about the neutral point, which need further investigation.

BACTERIAL HÆMOLYSINES.

Closely allied to the true toxines are those bacterial substances which exert a specific activity on the *red corpuscles of the blood*, altering their plasma in such fashion that the hæmoglobin exudes, the blood being thus "laked." They differ essentially, however, from blood poisons of the ordinary kind, such as, *e.g.*, phenylhydrazine, &c., in acting physiologically as true toxines—*i.e.*, producing *anti-bodies*, antilysines, in the organism. They thus approximate on the one hand to the true toxines, and on the other hand to other *hæmolytic haptines*, such as *ricine*, &c., as well as to the specific *hæmolysines* which are formed on the introduction of erythrocytes foreign to a body and also in normal sera. Whether these lysines are simple haptines or whether they do not rather consist of amboceptor and complement has not yet been definitely decided; yet in the case of staphylolysine, at all events, all the arguments up to the present support the view that they are *simple* haptines (Bordet, Ehrlich, and Morgenroth).

Of these bacterial hæmolysines only two are as yet definitely known—*tetanolysine* and *staphylolysine*. Other bacteria also exhibit hæmolytic activity, but it is not yet quite certain whether this is to be attributed to specific lysines, although it is true that in the case of coli-lysine, for example, anti-bodies are known. But the chief argument against their being of the nature of toxines is the fact that they can resist a temperature of 120° C.

A further interesting point is the fact that there is an extraordinary difference in the degree of resistance offered by the blood-corpuscles of different species to the various lysines, and that the erythrocytes of certain species are naturally more or less completely immune against each of them.

TETANOLYSINE.

Tetanolysine was discovered by Ehrlich[1] in cultivations of the tetanus bacillus.

[1] Ehrlich, *Ges. d. Charitéärzte*, [3], ii., 1898; *Berl. klin. Woch.*, 1898, No. 12.

The reasons that led EHRLICH to conclude that it was a different poison to the true tetanus poison, the convulsion-producing *tetanospasmine*, are as follows :—

The ratio between the amounts of *tetanolysine* and *tetanospasmine* in the cultivations and preparations derived from them is not constant. Some solutions of the poison are rich in the former and relatively poor in the latter, and *vice versâ*.

Tetanolysine is more sensitive to external influences than tetanospasmine.

Tetanolysine combines with the erythrocytes, while tetanus toxine is left by them in solution.

In proportion as the tetanus solution varies in the amounts of the two poisons, so also the anti-serum prepared by means of this solution contains varying relative quantities of the corresponding *anti-bodies*, so that it is sometimes more antitoxic and sometimes more antilytic in its action.

Tetanolysine was thoroughly investigated by MADSEN[1] in EHRLICH's Institute, and his results were confirmed by KRAUS and CLAIRMONT.[2]

MADSEN obtained, by means of precipitation with ammonium sulphate from a bouillon culture of tetanus, a preparation, of which 0·000001 grm. was the amount required for a lethal dose for a mouse.

This poison dissolves the blood-corpuscles of many animals; rabbits' blood, being particularly sensitive, is used for the experiments, in the form of a 5 per cent. emulsion in a physiological solution of sodium chloride.

The amount of solution is determined colorimetrically by comparison with a standard solution of blood. It depends, *ceteris paribus*, on the amount of poison added. There is a difference, however, in the sensitiveness of *individual* blood-corpuscles; moreover, tetanolysine has *much less* action in the cold than at the incubation temperature, whereas other hæmolytic poisons do not possess this property.

Tetanolysine is extraordinarily sensitive to external influences. Even at the ordinary temperature it becomes considerably weaker in less than an hour, especially in dilute solutions, but concentrated solutions also soon lose part of their hæmolytic power. Higher temperatures, even 50° C., have a very injurious effect. The lysine can be kept on ice without undergoing decomposition for twenty-four hours, and in the dry condition is absolutely stable.

[1] Madsen, "Ueber Tetanolysin," *Zeit. f. Hyg.*, xxxii., 214, 1899.

[2] Kraus and Clairmont, "Ueber Hämolysin und Antihämolysin," *Wien. klin. Woch.*, 1900, 49.

It is particularly interesting to note that MADSEN was able to show that this weakening was due to the *formation of toxoids.*

In his experiments he followed exactly the same methods as were used by EHRLICH in elucidating the constitution of *diphtheria poison—i.e.*, he determined the conditions of combination with the specific *anti-body* of tetanolysine. This antilysine is present in the preparations of antitoxine against tetanus. In the first place, MADSEN established a unit of toxic activity and a unit of the antilytic power of the anti-body, in accordance with Ehrlich's methods, though here obviously the experiment on an animal had to be replaced by a determination of the blood-solvent power in a test tube. Then, on investigating the conditions of the *partial neutralisation* of the poison with antitoxine, he found that they were quite analogous to those in the case of diphtheria poison, for the neutralisation did not take place regularly throughout the whole quantity of poison, but zones of different combining power with regard to the antilysine could be detected.

An addition of only one-thirteenth of the total amount of antitoxine required to neutralise the toxic unit was sufficient to reduce the hæmolytic power by a *half;* an addition of a fifth neutralised as much as nine-tenths of the poison; whilst one-half neutralised ninety-nine hundredths.

From this it follows that that part of the poison which has the *greatest* affinity for the antilysine is also endowed with the main proportion of the *activity*, that then come a second and third zone with less affinity and also smaller solvent capacities, and finally the "spectrum" is completed by a zone with slight affinity and little toxic activity.

We have here, then (to employ the terminology adopted in the case of diphtheria poison), a zone of highly active *prototoxine*, with then a broad zone of less active *deuterotoxine* (hemitoxine?), followed by the zone of *tritotoxine*, and, lastly, the *toxones*, which only act upon certain particularly susceptible erythrocytes, and also enter much more slowly and feebly into combination.

In general, *only prototoxine* and *deuterotoxine* act in the cold (MADSEN[1]). If a poison is so far neutralised with antilysine that these two groups remain inactive, the still remaining *tritotoxine* has absolutely no solvent action at 8° C., even when present in *the largest quantities.*

This, as MADSEN has shown, is due to the fact that at that temperature the *toxophore* group of the tritotoxine is inactive, for it combines with the erythrocytes even at that temperature,

[1] Madsen, quoted by Dreyer, *Zeit. f. Hyg.*, xxxvii., 274, 1901.

so that solution takes place after separating the mixture by centrifugal force and applying heat. MORGENROTH (p. 121) was able to demonstrate the occurrence of similar phenomena in the case of tetanus in the frog.

A further analogy with diphtheria poison is seen in the mode of the *formation of toxoids.* Thus, *tetanolysine* decreases in strength very rapidly, and mainly, as in the case of diphtheria poison, at the cost of the *prototoxine zone.* The *deuterotoxine zone* is relatively more stable.

Like tetanus poison, tetanolysine differs from diphtheria poison in requiring a certain amount of time, even several hours, to combine with the antitoxine.

Its action upon the erythrocytes also does not begin at once, but only after a certain period of incubation, which decreases with the increase in the amount of poison.

ARRHENIUS and MADSEN (*loc. cit.*) have made a closer examination of this incubation period, and attribute it to the restrictive effect of the membrane (see General Part).

According to ARRHENIUS and MADSEN it is not *absolutely indispensable* to assume the existence of poison spectra, as described above, in the case of tetanolysine, and, in their opinion, the quantitative ratios can be better explained by the hypothesis of *dissociated conditions of equilibrium* such as we described at length in the General Part. I have, however, cited MADSEN'S investigations for their bearing on EHRLICH'S "spectra" and the conclusions originally drawn from them, because it is not definitely decided that there is a plurality of poisons in this case.

The results obtained by TIZZONI and CENTANNI [1] show that the *toxoids* can also have an immunising effect, for they found that an anti-body to the lysine could be prepared by means of *tetanospasmine* which apparently contained no *lysine.*

Further experiments made by MADSEN [2] upon tetanolysine are very interesting, and have also an important bearing upon the question of the *therapeutic action of antitoxines—i.e.*, upon their power of again liberating the lysine from its combination with the cell. It was found that, by the addition of antilysine, lysine in combination with the blood-corpuscles could be liberated again, and that the blood platelet could be "healed" even after it had been attacked. The necessary dose, however, increased very rapidly, just as in the case of diphtheria poison and tetano-

[1] Tizzoni and Centanni, *Real Accad. Bologna*, 1900, quoted by Neisser and Wechsberg, *loc. cit.*

[2] Madsen, "Ueber Heilversuche im Reagenzglas," *Zeit. f. Hyg.*, xxxii., 239, 1899.

spasmine. Even after five minutes twice the dose was required; after fifteen minutes, three times; and after thirty minutes, five times the amount of a single protective dose. Beyond that time accurate measurements were not possible, since, prior to the addition of the antilysine, the amount of solution was so great that the shade of colour could no longer be accurately matched.

PYOCYANOLYSINE.

A similar bacterial hæmolysine has been obtained from cultivations of *B. pyocyaneus* by Bulloch and Hunter.[1]

They isolated it from eight different cultivations, the "results being practically the same."

The hæmolytic power was tested upon the blood-corpuscles of the most different species of animals, the amounts usually employed being 0·5 c.c. of the unfiltered culture, or 1·5 to 2 c.c. of that filtered through a Chamberland filter. Rabbit's blood offered some resistance.

There is very little pyocyanolysine present in young cultures, the filtrate being practically devoid of it. Cultivations three to four weeks' old yield a filtrate which also contains lysine, though invariably in a very small proportion as compared with that in the main cultivation.

Bulloch and Hunter conclude from this that the lysine is combined with cells of the bacilli, and is not liberated until the cultivations grow older. In consequence of this combination with the cells it is somewhat protected from the destructive effect of heat, so that the unfiltered cultivation can resist a temperature of 100° C. for a short period (fifteen minutes), whereas the poison in the filtrate is rapidly destroyed by boiling. The antilysine has not yet been detected.

These results were, in the main, confirmed immediately afterwards by Weingeroff,[2] who, however, also obtained the lysine by filtration of the cultures. Subsequently Marg. Breymann[3] found the lysine only in the filtrates, even in the case of young cultivations.

Weingeroff was able to give a direct proof that the lysine combined with the blood-corpuscles, while the *toxine* present in

[1] Bulloch and Hunter, "Ueber Pyocyanolysin," *Centralbl. f. Bakt.*, xxviii., 865, 1900.

[2] Weingeroff, "Zur Kenntnis des Hämolysins des B. pyocyaneus," *Centralbl. f. Bakt.*, xxix., No. 20, 1901.

[3] M. Breymann, "Ueb. Stoffwechselprod. d. B. pyocyaneus," *Centralbl. f. Bakt.*, xxxi., 481, 1902.

the same solution remained at liberty. This showed that the lysine and toxine were discrete.

Lubenau,[1] too, found that an old *pyocyaneus* cultivation (twenty-one months) had a very pronounced hæmolytic action; it was strongly alkaline; neutralisation perceptibly weakened the hæmolytic function, but did not destroy it.

Loew and Kozai[2] found that the admission of air and the addition of sugar promoted the formation of pyocyanolysine.

According to Breymann the *lysine resists the action of heat.*

COLILYSINE.

A principle that attacked the corpuscles of the blood, and *was not destroyed by heat*, was discovered by Kayser[3] in cultivations of *B. coli.*

It is produced when the reaction of the liquid is very faintly acid. Its action is most pronounced upon the blood of the dog, and then upon that of the horse, ox, and rabbit, while it has little or no effect upon the blood of man, the guinea-pig, sheep, goose, and pigeon. The order of the degree of activity upon the different kinds of blood differs from that of staphylolysine.

The poison is present in filtered cultivations of three days' growth, not in the cells of the bacteria. The hæmolysis is not preceded by agglutination.

The lysine can resist a temperature of 120° C. for thirty minutes without injury. It combines with the erythrocytes in the cold like a true toxine, and, on warming, solution takes place. Its *stability* on keeping varies very considerably.

On subcutaneous injection there is formed in the organism an *antilysine* which is stable at 56° C. Moreover, "*healing*"—*i.e.*, restriction of hæmolysis when once commenced—can be effected by means of antilysine (cf. *Tetanolysine*). Normal serum, *especially that of the horse*, also contains the *antilysine.*

STAPHYLOLYSINE.

The history of the investigation of the *staphylococci* poisons and their rôle is, in the main, a description of the same labyrynthine paths as have usually been followed in the examination

[1] Lubenau, "Hämolyt. Fähigkeiten einzelner pathog. Schizomyceten," *Centralbl. f. Bakt.*, xxx., 356.

[2] Loew and Kozai, "Ueb. d. Bild. des Pyocyanolysins," *Malys Jb.*, xxxi., 912, 1901.

[3] Kayser, "Ueb. Bakterienhämolysine, bes. d. Colilysin," *Zeit. f. Hyg.*, xlii., 118, 1903.

of bacterial toxines. Fortunately in this case, unlike that of the *streptococci*, for example, definite results have been yielded by the latest researches.

As regards the earlier investigations only three are really of importance. Rodet and Courmont[1] discovered in cultivations of staphylococci an immunising substance precipitable by alcohol. Reichel,[2] who made a closer study of the problem, was able, by filtration of cultivations of *Staphylococcus pyogenes aureus*, to isolate a *specific poison*, which was, however, not very toxic, and against the action of which immunity could be obtained.

Mosny and Marcano[3] found that staphylococci were slightly poisonous, and, on injection, produced an antitoxine.

We know now that *Staphylococcus pyogenes aureus* produces two specific poisons, one of which, *leucocidine*, has a poisonous action on the leucocytes, whilst the other, a *lysine*, affects the red corpuscles of the blood.

The first to indicate briefly the existence of a staphylotoxine acting on the red corpuscles, as a substance distinct from *leucocidine*, was Van de Velde,[4] and then Kraus and Clairmont.[5] This lysine was first systematically studied, however, in Ehrlich's Institute by Neisser and Wechsberg.[6]

Staphylococcus pyogenes aureus produces a blood-solvent toxine, which can be detected in bouillon cultures after three to four days' growth, the *optimum* period for its formation being ten to fourteen days. The best condition for its production is in the still faintly acid broth, to which has been added from a third to a half of the quantity of normal alkali required for its complete neutralisation. It can be separated by *filtration*.

The capacity for producing lysine varies very greatly with the different species of staphylococci, and apparently, as is also the case with the true toxines, stands in no direct ratio with regard to its virulence for man. The true pyogenic species, *albus* and *aureus*, invariably produce it, but there are numerous other non-pathogenic species that produce *no lysine*. The quantity of

[1] Rodet and Courmont, "De l'existence . . . dans des cultures du staphylocoque d'une substance vaccinante," *Comptes Rend.*, cxiii., 432, 1891.

[2] Reichel, "Ueb. Immunität gegen das Virus von Eiterkokken," *Arch. f. klin. Chirurg.*, xlii., 237, 1891.

[3] Mosny and Marcano, "De l'action de la toxine du staphyl. pyog.," *Sem. Méd.*, 1894, 544

[4] Van de Velde, "Étude s. l. mécanisme de virulence du staphylocoque pyogène," *La Cellule*, x.; *id.*, "Contribution à l'immunité des lapins contrè le Staphylocoque," *Ann. Past.*, x., 580, 1896.

[5] Kraus and Clairmont, "Ueber Hämolysine u. Antihämolysine." *Wien. klin. Woch.*, 1890, 49.

[6] Neisser and Wechsberg, "Ueber das Staphylotoxin," *Zeit. f. Hyg.*, xxxvi., 299, 1901.

lysine formed varies with the different species, as does also the period of time at which the maximum production occurs. According to LUBENAU[1] this fluctuates considerably within a few hours. The addition of glucose to the cultivations reduces the yield of lysine (KAYSER[2]).

Staphylolysine answers the requirements of the true toxines as regards the influence of external factors. While it can generally be kept for a long period unaltered in an ice chest (with the addition of phenol) it is destroyed in twenty minutes at 56° C., is injured at 48° C., and even when kept in an incubating chamber loses its activity within a few weeks.

It can resist the action of considerable quantities of alkalies, acids, and ordinary salt; but strong alkalinity, especially at the incubation temperature, is injurious.

The blood-corpuscles of different animals vary considerably with regard to their susceptibility towards the same lysine; the erythrocytes of the rabbit appear to be the most susceptible, while those of man, of the goat, and, above all, of the goose, offer much greater resistance. A still further complication is introduced into the conditions by the fact that the normal serum of most species of animals exercises a more or less pronounced protective action against the lysine, so that in order to obtain comparable results, it is necessary to use washed blood-corpuscles. Rabbit's blood, apart from its special susceptibility, is also the best medium for the test, owing to the fact that normal rabbit's serum has only a very insignificant protective capacity. Even with the same blood it is possible, as in the case of *tetanolysine*, to demonstrate differences in the susceptibility of the erythrocytes, so that even weak solutions of lysine dissolve certain blood-corpuscles, whereas considerably stronger solutions must be employed to obtain *complete* solution.

ANTISTAPHYLOLYSINE.

The protective force exerted by certain normal sera in varying degree against the action of staphylolysine must be attributed to the presence of a specific *anti-body* whose activity is exclusively directed against staphylolysine. Normal horse serum, in particular, is sometimes so rich in it that as little as 0·01 c.c. will afford protection against a dose of staphylolysine that would otherwise dissolve the corpuscles *en masse*.

[1] Lubenau, "Hämolytische Fähigkeit einiger pathogener Schizomyceten," *Centralbl. f. Bakt.*, xxx., 356, 1901.

[2] Kayser, "Einw. d. Traubenzuckers auf Staphylococcus," *Zeit. f. Hyg.*, xl., 21, 1902.

Normal horse serum also frequently affords protection against *tetanolysine*. From this KRAUS and CLAIRMONT have drawn conclusions as to the identity of the two lysines and their anti-bodies. NEISSER and WECHSBERG, however, were able to show that, on the one hand, immune sera that afforded protection against staphylolysine had no action at all upon *tetanolysine*, and that, on the other hand, *tetanus sera*, which acted energetically against tetanolysine, afforded under certain conditions much less protection than normal serum against staphylolysine.

Staphylolysine thus shows itself to be a true toxine in so far as it possesses a specific anti-body, which is present even in many normal sera including human serum, in varying amounts, and is *invariably* formed whenever an animal is rendered immune against the lysine.

This is attained by subcutaneous or intravenous, but *not peritoneal*, injection of small doses twice or thrice repeated into goats or rabbits.

A very interesting point is the protection afforded to the lysine by combining with the anti-body. Although the anti-body can resist temperatures up to 68° C., which speedily destroy the lysine, it is not possible to eliminate the toxine from a normal mixture by means of heat, and to leave the antitoxine free.

All these lysines, whatever their origin, produce the same anti-lysine effective against them all, so that it would seem that we have here to deal with a simple haptine. The evidence is insufficient for us to decide whether we have here products of the activity of staphylococci or *normal* side-chains in the stricter sense of the word. Yet anti-bodies of the most diverse kind occur in normal serum with such relative frequency as to render it probable that *normal* receptors may actually have an affinity for the poisons in question, which in this case may have the form of free amboceptors resembling the receptors of the blood-corpuscles.

The Constitution of Staphylolysine.—Staphylolysine has many analogies with *tetanolysine* in its constitution. It is not constructed on the type of Bordet and Ehrlich's hæmolysines—*i.e.*, it does not, like them, consist of two parts, the "*amboceptor*" and the "*complement*," for after being heated to 56° C., at which temperature it *becomes inactive*, it can not be rendered active again either by normal serum or any other means. Nor does this lysine, after being rendered inactive, still produce an anti-body, as do the intermediate bodies that withstand the heat in the case of the hæmolysines. Its structure is thus that of the *simple toxine;* the *haptophore* and *toxophore* groups are attached to *one* nucleus. As in the case of the simple toxines, the haptophore group is able to *enter into combination* even in the cold,

while *solution* does not take place owing to the *toxophore* group remaining inactive. If the erythrocytes of rabbits' blood be treated for some hours with staphylolysine at 0° C., no solution occurs; but on carefully washing the erythrocytes, and warming them to 37° C. solution, it immediately takes place. Perfectly analogous conditions of combination and activity have been observed by MORGENROTH (*loc. cit.*) in tetanus in the frog, and by MADSEN in the case of *tetanolysine*. Staphylolysine is thus closely allied to the true toxines.

This analogy goes still further. Adopting Ehrlich's method of determining the constitution of a toxine, NEISSER and WECHSBERG have treated a definite amount of toxine with increasing fractions of the antitoxic unit, and have thus obtained "spectra," which present many analogies with those of diphtheria toxine and tetanolysine. There is no need to go more fully into these details here.

Hæmolysines are also produced by other bacteria, as has been described—*e.g.*, by LUBENAU (*loc. cit.*), and KRAUS and CLAIRMONT (*loc. cit.*). Agents with a solvent action upon the blood are formed by *cholera micro-organisms* and other similar vibriones. LEVY[1] isolated from *typhus cultivations* a lysine which acted most upon dogs' blood.

By means of immunisation with typhoid cultures he succeeded in preparing an antilytic serum. Typhoid lysine also resists the action of heat. A *streptolysine* that was fairly stable when heated has been described by BESREDKA.[2]

It only occurs in young cultivations, and varies in its properties with the nature of the culture medium. It is only destroyed after two hours' heating at 70° C., and is not dialysable.

It does not form an anti-body under any condition, and thus is apparently not a *haptine* at all. For this reason I give no further particulars about it here.

A hæmolysine isolated from cultures of the *pneumococcus* has been described by CASAGRANDI.[3] It is characteristic of this that only the *non-pathogenic* varieties of this diplococcus should form a lysine. Its constitution is similar to that of the other toxines. and it forms an anti-body. Certain species are also stated to produce a specific *leucocidine* in addition to the lysine.

[1] E. and P. Levy, "Ueber die Hämolysine des Ty.-B.," *Centralbl. f. Bakt.*, xxx., 405, 1901.

[2] Besredka, "De l'hémolysine streptococcique," *Ann. Past.*, xv., 880, 1901.

[3] Casagrandi, "L'Emolisina e la Leucolisina Diplococcica," *Bull. Soc. Lancis. Rom.*, xxvii., 2; *Biochem. Centralbl.*, i., 402, 1903.

Lastly, specific *hæmagglutinines* obtained from the filtrates from bacteria—*e.g.*, from staphylococcus and different vibriones—have recently been described by KRAUS and LUDWIG.[1] They are destroyed at 58° C. and form specific anti-bodies. They must be kept quite distinct from the specific hæmolysines.

A lysine of the plague bacillus has also been described by URIARTE.[2]

THE LEUCOCIDINE OF THE STAPHYLOCOCCI.

Simultaneously with the production of lysine there is formed in the case of the typical species of staphylococci a second soluble toxine, *leucocidine*, which was independently discovered by VAN DE VELDE (*loc. cit.*) and by BAIL,[3] and was more closely studied by NEISSER and WECHSBERG.[4] Its specific activity is directed mainly against the leucocytes, which it kills and dissolves, and also against certain other cells, such as hæmatoblasts, ganglionic cells, &c. Owing to the fact that it appears to destroy the leucocytes in the living organism also, it produces infarct and other alterations in the kidneys. It does not appear, however, to have a specific action upon the epithelium of the kidneys. NEISSER and WECHSBERG tested its activity by means of their "*bioscopic method*," in which they took the reducing power of the leucocytes as the measure of their vitality, a dilute solution of methylene blue being used as the indicator. If the leucocidine was active no decolorisation of the methylene blue took place. In such determinations it is obvious that the quantity of leucocytes present must be taken into account; this was done by determining beforehand the single reducing dose, L_r, for each exudation from the living cells, and taking this result as the basis of the calculation.

Leucocidine is produced in bouillon cultivations in about four days, and reaches its maximum in about eight days. As regards the influence of the alkalinity of the culture fluid almost the same conditions appear to hold good for leucocidine as for staphylolysine.

Leucocidine invariably occurs in association with the lysine.

[1] Kraus and Ludwig, "Ueb. Bakteriohämagglutinine," *Wien. klin. Woch.*, 1902, 120.

[2] Uriarte, "Hémolysine du bac. pesteux.," *Soc. Biol.*, lvii., 254, 1904.

[3] Bail, "Ueber leukocide Substanzen in den Stoffwechselprodukten d. Staph. pyog. aureus," *Arch. f. Hyg.*, xxxii., 133, 1898.

[4] Neisser and Wechsberg, "Ueber eine neue einfache Methode z. Beob. von Schädigungen leb. Zellen und Organismen," *Münch. med. Woch.*, 1902, 1261.

The same species of staphylococci that produce the lysine also produce leucocidine, although far from invariably in corresponding quantity. As in the case of the lysine, passage through an animal increases the production of poison. It is, nevertheless, *a specific poison unmistakably distinct from the lysine*, and possesses its own haptophore and toxophore groups. The lysine does not combine with the leucocytes. Leucocidine passes into the *sterilised filtrates* and is thus a *soluble poison*.

It is somewhat less stable than the lysine. At 50° C. it is destroyed in twenty minutes, and at 58° C. in ten minutes. When kept, preserved with phenol, in an ice chest it very rapidly becomes weaker, its activity being from twenty-five to sixty times less after sixteen days. Eventually it becomes quite inactive.

Its action is fairly slow, so that it is essential to extend the period of observation to two hours. It is shown to be a true toxine by the fact that it is capable of forming an antitoxine. Denys and Van de Velde[1] were the first to prepare an *anti-leucocidine* by means of the injections of the filtrates from cultivations. Then Neisser and Wechsberg discovered anti-leucocidine in normal horse serum and human serum, but not in that of the rabbit; in like manner they invariably obtained an antileucocidine of uniform character by immunising rabbits and goats.

We have thus in the leucocidine of staphylococci a true *toxine*, which has specific action upon leucocytes—characteristic activity which has hitherto only had an analogy in the pneumococcus leucocidine discovered by Casagrandi (*loc. cit.*).

[1] Denys and Van de Velde, "Sur la production d'une antileucocidine, &c.," *La Cellule*, xi., quoted by Neisser and Wechsberg.

II.—THE ENDOTOXINES.

CHOLERA VIRUS.

THE question of the nature of cholera virus, and of its position with regard to the true toxines, is still far from being definitely settled. Apart from the unfortunate fact that we meet with direct contradictions in the experimental results, there is also one very important circumstance that increases the difficulty of making a survey in this and many very similar cases, such as the poisons of typhus, pneumococcus, &c. The earlier investigators of immunity were not at that time able to draw the fundamental distinction between antitoxic and bactericidal immunity, and thus at first no systematic research was made to determine the existence of a cholera toxine and antitoxine. But even at the present day the difficulty of investigation is greatly increased by the fact that immunity against cholera is undoubtedly, in the main, *bactericidal*, and that antitoxic immunity, even if it exists at all, occupies an absolutely secondary position. Even inoculation with the *dead* cells apparently produces a purely antibacterial immunity.

The history of cholera virus begins with R. KOCH,[1] who had long regarded cholera as a disease due to toxine poisoning, although it was only after tedious experiments that he succeeded in causing rapid poisoning, and then only by means of *living* bacteria. On the other hand, NICATI and RIETSCH[2] obtained poisonous filtrates devoid of specific activity, as was also done by VAN ERMENGEM.[3] Then followed the usual investigations of the soluble crystalloid substances of a ptomaine character which had been isolated from the cultures, but were soon recognised as not responsible for the toxic action of the vibriones. The toxalbumins, too, which BRIEGER and FRÄNKEL isolated by their method from cholera cultivations, were found

[1] R. Koch, "Vort. über die Cholera," *Berl. klin. Woch.*, 1884, 498; *id.*, "Zweite Conferenz z. Erört. d. Cholerafrage," *ibid.*, 1895 [37*a*], 8.

[2] Nicati and Rietsch, "Effets toxiques des produits, &c.," *Comptes Rend.*, xcix., 929, 1884.

[3] Van Ermengem, "Sur l'inoculation des produits de culture du bacille virgulæ," *Bull. Acad. Méd. Belg.*, [3], xviii., 1221, 1884.

by them to be substances having only a slight toxic action, and not producing any *specific* effects. Their cholera toxalbumin, in particular, was insoluble in water and not poisonous to rabbits.

The first successful attempt to isolate a poisonous and approximately specifically active product from Koch's vibrio was made by PETRI.[1] He discovered in peptone cultivations a soluble poison, which killed guinea-pigs with hypothermia and other symptoms of cholera poisoning, although fairly large doses (2 c.c.) were required. His "*toxopeptone*," it is true, differed widely from the true toxines in that it could resist boiling. He also confirmed the far-reaching observation first made by CANTANI,[2] that the *cells after being killed* invariably still contained an abundance of virus, so that the filtered cultivations were never as poisonous as the original sterilised cultures. KLEMPERER[3] also found that the dead cells were still poisonous and could act *per os* when the acidity of the stomach was partially neutralised and the intestine in a state of rest.

Next come the experiments of HUEPPE[4] and SCHOLL[5] to obtain a "toxine" by means of anaërobic cultivations in eggs and precipitation with alcohol, but *their* results were contradicted by GRUBER and WIENER,[6] WESBROOK,[7] and DÖNITZ,[8] who were unable to prepare active specific poisons by these means.

GAMALEÏA[9] concluded that there were two cholera poisons. He cultivated the vibriones for fifteen days in calf's-foot bouillon and sterilised the culture at 120° C. The poison thus obtained caused death preceded by a marked fall of temperature and paralysis, as well as by hyperæmia of the abdominal organs. There was absolutely no acclimatisation to this poison, which

[1] Petri, "Unters. üb. die d. d. Wachstum der Cholerabakt. entstehenden chemischen Umsetzungen," *Arb. Kais. Ges.-Amt.*, vi., 374, 1890.

[2] Cantani, "Giftigkeit der Cholerabazillen," *Deutsch. med. Woch.*, 1886, 789.

[3] Klemperer, "Ueb. künstlichen Impfschutz gegen Choleraintoxikation," *Berl. klin. Woch.*, 1892, 789.

[4] Hueppe, "Ueb. d. Aetiologie u. Toxikologie der Cholera asiatica," *Deutsch. med. Woch.*, 1891, 417.

[5] Scholl, "Unters. üb. giftige Eiweisskörper bei Cholera asiatica," *Arch. f. Hyg.*, 1892, 172.

[6] Gruber and Wiener, "Ueb. d. intraperiton. Cholerainfektion," *Wien. klin. Woch.*, 1892, 543.

[7] Wesbrook, "Contrib. à l'étude d. toxines du Choléra," *Ann. Past.*, viii., 318, 1894.

[8] Dönitz, "Ueb. d. Verhalten d. Chol.-Vibr. im Hühnerei," *Zeit. f. Hyg.*, 1895, 20, 31.

[9] Gamaleïa, "Recherches expér. sur les poisons du Cholera," *Arch. de Med. Exper.*, 1892, 173.

originated from the cells of the vibriones and was of a nucleic character. In addition to this, however, the cultivations contained a poison, a *nucleo-albumin*, which was unstable when heated; it occurred in the cultures that had been sterilised at 58° C., while the filtrates were only very slightly poisonous. It produced very violent diarrhœa and other choleraic symptoms.

WASSERMANN[1] found that the dead cells when used in the proportion of eight to ten times the quantity of the living vibriones produced the typical symptoms, ending in death, in a guinea-pig. By evaporation of the cultures and precipitation with alcohol he obtained a *poison* which, in a dose of 0·02 grm., killed guinea-pigs but produced no antitoxic immunity. PFEIFFER and WASSERMANN[2] and ISSAEFF[3] assert that the serum of immune animals does not confer antitoxine immunity. KLEMPERER[4] found filtered cultivations to be slightly poisonous.

The question was then further investigated by WESBROOK. He cultivated the vibriones on alkali albuminate which gave no biuret reaction. After three weeks there was an unmistakable biuret reaction. The poison, when filtered through porcelain, was fatal to guinea-pigs in doses of 0·5 to 1·5 c.c. and possessed *immunising* capacity.

He attempted to isolate it by neutralising the alkali albuminate with hydrochloric acid so that it was precipitated, concentrating the filtrate *in vacuo* at 40° C., and dialysing it after the addition of alcohol. Both the precipitated albuminate and the albumose left in the filtrate were poisonous.

He also obtained from a rich growth of the bacteria in proteid-free culture-media (like those employed by USCHINSKY), to which a certain proportion of sodium hydroxide had been added, a poison which, after imperfect purification, yielded a brown substance that gave no biuret reaction and only a faint xantho-proteid reaction. It had a *poisonous action* and *immunising* capacity. From these results he concluded that cholera virus was only in combination with proteids, but was *not* a proteid itself.

This poison was invariably only sparingly produced in the cultivations, and was but very slightly poisonous in comparison

[1] Wassermann, "Unt. üb. Immun. geg. Chol. asiatica," *Zeit. f. Hyg.*, xiv., 35, 1893.

[2] Pfeiffer and Wassermann, "Unters. üb. das Wesen d. Choleraimmunität," *Zeit. f. Hyg.*, xiv., 46, 1893.

[3] Issaeff, "Unt. üb. d. künstl. Imm. geg. Cholera," *Zeit. f. Hyg.*, xv., 287, 1894.

[4] Klemperer, "Schutzimpf. d. Menschen geg. asiat. Cholera," *Berl. klin. Woch.*, 1892, 970.

with *diphtheria toxine* and *tetanus toxine*, and hence PFEIFFER[1] asserted that cholera virus possessed a totally different nature and mode of production to these *true* toxines. He put forward the view that *cholera virus* was *not an excretion product of the vibriones*, but a substance firmly retained within the cell in the normal condition, an "*endotoxine*," which only left the bacteria *after the death* of the latter. This would account for the relatively small toxic action of the filtered cultivations compared with the highly poisonous effects of the living cultures, and, as was shown by PFEIFFER, of the dead bacilli.

PFEIFFER advanced, in support of his view, the following facts which he had observed:—The germ-free filtrate of bouillon cultures is only about half as poisonous as the original cultivation before boiling. If boiled cultivations are filtered the germ-free filtrate is *more poisonous* than that from the unboiled culture. But if, on the other hand, the filtrate from the untouched cultivations is boiled, it loses its *toxic property*.

If the vibriones are killed by means of chloroform or thymol the poisonous property is retained, whereas the addition of alcohol and precipitation with ammonium sulphate have an injurious effect upon it. When the vibriones are allowed to dry slowly, so that they die, they retain their toxic power. If they are then heated with water and the liquid filtered through a Chamberland filter, the filtrate is non-poisonous; nor is glycerin any more effective in extracting the poison from the dead cells. PFEIFFER[2] concludes that there is a *primary* very unstable poison which is converted by heat into the *secondary* poison, prepared by SCHOLL and others, which while acting in the same manner is ten to twenty times weaker.

PFEIFFER[3] still firmly maintained his theory even after RANSOM[4] had claimed to have prepared, from cultivations, an active cholera toxine possessing true immunising powers, and after SOBERNHEIM[5] had, at an earlier period, obtained by filtration of *old* cultivations in a rapid state of decomposition a poison that acted *per os*, and also produced *immunity*. RANSOM himself

[1] Pfeiffer, "Unters. üb. d. Choleragift," *Zeit. f. Hyg.*, xi., 393, 1892.

[2] See also Pfeiffer, "Studien zur Cholera-Aetiologie," *Zeit. f. Hyg.*, xv., 268, 1894.

[3] Pfeiffer, "Ueber die spezifischen Antikörper der Cholera," *Zeit. f. Hyg.*, xx., 217, 1895; *id.*, "Ein neues Grundgesetz der Immunität," *Deutsch. med. Woch.*, 1896, Nos. 7 and 8.

[4] Ransom, "Choleragift u. Choleraantitoxin," *Deutsch. med. Woch.*, 1895, 457.

[5] Sobernheim, "Experim. Unters. üb. Choleragift und Cholerschutz," *Zeit. f. Hyg.*, xiv., 485, 1893.

gave no further details as to the preparation of his poison, but Behring[1] subsequently stated that Ransom's poison was obtained from cultivations of five to ten days' growth, by means of a short heating at 100° C., filtration through a Pukall's filter, and precipitation with alcohol.

This solid poison is stated to produce violent choleraic symptoms and death when tried upon animals. It causes in the organism of the animal a slight *formation of antitoxine* (protection against four to six times the lethal dose), and in this respect should be regarded as a true toxine. Against this, however, must be placed its stability when heated, which, according to Ransom, is one of its characteristics, and also its relatively small toxic action (0·07 grm. of the solid poison is the lethal dose for guinea-pigs). Pfeiffer, therefore, regards Ransom's poison as a *secondary product*, and is of opinion that its reputed antitoxic power is not greater than that of the normal serum.

Metschnikoff, Roux, and Taurelli-Salimbeni[2] brought forward the following interesting experiments in opposition to Pfeiffer's theory:—They placed 3 to 4 c.c. of a peptone solution, part of which contained living vibriones and in part of which the micro-organisms had been killed by means of chloroform, in sterilised collodion capsules, which were then hermetically closed and placed in the abdominal cavity of guinea-pigs. The animal treated in this way with the dead vibriones became slightly ill, whereas that poisoned with the living micro-organisms perished in three to five days with typical choleraic symptoms. Post-mortem section showed the usual alterations found in cholera, but in none of the organs were there any vibriones, although these were *alive* in the capsule.

Some of the animals survived and then showed an increased resistance.

The inevitable deduction to be drawn from this experiment is as follows:—

In the culture medium contained in this closed capsule which thus represented a model intestine, there was formed a poison which could diffuse through the collodion membrane whilst the vibriones developed vigorously inside the capsule. On the other hand, but little poison was liberated from the dead cells—only sufficient to produce slight illness.

[1] Behring, "Untersuch. Ransoms üb. die Agglutination der Cholera-vibriones," *Deutsch. med. Woch.*, 1898, 294.

[2] Metschnikoff, Roux, and Taurelli-Salimbeni, "Toxine et antitoxine cholérique," *Ann. Past.*, x., 257, 1896.

At first the vibriones showed vigorous increase within the capsule, but soon they assumed other forms and then *no longer increased;* they did not die, however, but could still be cultivated months afterwards, on nutrient media. Passed through the body of an animal after intraperitoneal injection they again yielded cultures of a high degree of virulence.

They then endeavoured to obtain this poison also from cultivations of the vibriones thus made virulent. Old cultures have relatively little toxic action, and they, therefore, used quite young cultivations of two to twenty-four hours to three or four days' growth.

These when filtered had an average toxicity of 0·3 c.c. per 100 grms. of animal. The addition of serum to nutrient liquids increased the degree of toxicity and also the development of a *torula form* in the cultivation.

The poison thus obtained was also *stable when boiled*, but was soon rendered inactive by air and light. When hermetically sealed up it kept for a long time. Alcohol and ammonium sulphate did not precipitate it. It behaved in a manner analogous to Ransom's poison. By injection of increasing doses it was possible to produce an *antitoxic* immunity, which although greater than that of normal serum, was yet *exceedingly insignificant* compared with that of diphtheria and tetanus antitoxines. In the most favourable case 1 c.c. neutralised six times the lethal dose! In this respect its behaviour is the very opposite of Pfeiffer's bactericidal antiserum, obtained by means of immunisation with dead vibrio cells, which is devoid of any neutralising effect upon the toxine. This is an argument against the view that the dead cells contain any appreciable amount of immunising toxine, since otherwise this, when liberated, would necessarily show its power of forming antitoxine.

Courmont and Doyon[1] also obtained a soluble poison that could be filtered; it had an exceedingly small toxic effect, the lethal dose for a rabbit being 4 c.c. (!). It produced hypothermia and hæmorrhagia, also paralysis and peripheral neuritis. It was extremely sensitive to the influence of light and air. Cultivations sterilised at 50° C. proved somewhat more poisonous.

Hahn,[2] on the other hand, employing Buchner's method of trituration and expression at a pressure of 4 to 5 atmospheres, isolated from quite young cultivations, a *cholera plasmine*, in the form of a yellowish-brown liquid, which when injected in doses of 0·5 to 0·6 c.c. protected guinea-pigs after eight days against ten times the lethal dose. Particularly interesting

[1] Courmont and Doyon, "Effets de la toxine cholér.," *Arch. de Phys.*, xxviii., 785, 1896.

[2] Hahn, "Immunisierungs- u. Heilversuche mit den plasmat. Zellsäften, &c.," *Münch. med. Woch.*, 1897, 1344.

points in connection with these plasmines are that they are only *very slightly poisonous*, and that the immunity that they confer is apparently a true *bactericidal* one; it is not as yet possible to prove that the active substances expressed are those that cause the formation of the bactericidal intermediate products. This question is of great theoretical importance and will be specially dealt with when we come to consider *Tuberculine* (*q.v.*). Buchner's plasmines cannot help us in the matter of the *poisons* and antitoxines of cholera.

It is not altogether easy to form a definite conception of the nature and mode of action of cholera virus from these apparently contradictory experimental results.

For this purpose the question must be formulated in a more precise manner in accordance with our theoretical conceptions concerning bacterial toxines.

The question is then no longer: "Do the vibriones produce a *soluble poison* that gives rise to choleraic symptoms?" but it is necessary to ask whether they produce a toxine—*i.e.*, a poison which enters into combination in a specific manner, and eventually produces specific *antitoxic* immunity.

When we put the question in this form the following conclusions may apparently be drawn with some degree of probability from the experimental results.

It appears to be the fact that the vibriones produce a *true toxine*, which is very sensitive to all external influences, and even through the ageing of the cultivations undergoes a change in a manner that has yet to be further investigated. This toxine differs materially, however, from those of the diphtheria type, in that it is not, like such toxines, *secreted* in the free state and almost without residue by the vibriones, but appears to be energetically retained by them *intra vitam*. We here meet with peculiarities similar to those that occur in the case of true ferments. While, for example, yeast *excretes* only one enzyme, yeast diastase, it also contains others—*e.g.*, *invertase* and *maltase*—which are not given up by the living yeast cell, and can only be extracted after the cell has been killed, or after its membrane has been ruptured by means of glass powder.

In addition to these the yeast cell also contains E. Buchner's *zymase*, which can only be set at liberty by the very drastic means of trituration and expression under high pressure.

Perfectly analogous conditions are found in the case of the inverting enzyme of *Monilia candida*, which is also active after the addition of toluene, but has not yet been isolated from the cell of the mould-fungus by any of these methods.[1]

[1] For further details of these "Endoenzymes" see my *Ferments and their Actions*, Griffin & Co., London.

And the cholera vibrio retains such ferment within itself in firm combination, but capable of isolation by drastic means, as was shown by GERET and HAHN when they isolated a proteolytic enzyme. In like manner, then, the *toxine* also appears to be firmly combined with the living cell, to be an *endotoxine* analogous to the *endoenzymes*. After the death of the cell, however, it passes, partially at all events, into the culture medium, just like yeast invertase, so that, as PFEIFFER has shown, cultures killed by thymol, &c., or by drying, give indications of the poison they contain. But that the toxine, after the death of the vibriones, is really *free*, dissolved in the medium, and diffusible, is shown, again, by the experiments of METSCHNIKOFF, ROUX, and TAURELLI-SALIMBENI, in which it was found that living vibriones enclosed in a collodion capsule exerted their toxic activity outside the membrane. For, without doubt, in an experiment on these lines, of luxuriant growth on a restricted culture medium, a process of death and decomposition would take place simultaneously with the development of new vibriones. And since in this case the toxine can, immediately after its production, exert its activity upon the organism, while itself protected from every injurious influence, we may conclude with some degree of probability that here the primary true *cholera toxine* is the active agent. Probably this presumably true toxine is also active in cases of real cholera in the living organism. And this toxine ought, therefore, also to produce an *antitoxic* reaction in the organism. That this reaction is so slight ought not to excite surprise, for, in the first place, the amount of toxine thus set free can only be very trifling, so that a high degree of immunisation is not to be expected; and, further, this degree of antitoxic activity is lacking in a complete poison that has undergone secondary alterations, and, as we shall see below, probably contains numerous toxoids, the result being that the antitoxic power of the serum must appear too small. Thus the cholera vibriones, after death and decomposition, give up part of their endotoxine to the culture media; at the same time it is highly probable that they still retain the larger proportion of the toxine, just as zymase is retained by yeast. Possibly the method of *aseptic autolysis* tried by CONRADI[1] upon *typhoid bacilli* may enable us to prepare the primary cholera poison.

This primary poison, a *true toxine*, is extraordinarily sensitive to external influences, in which respect it resembles zymase, and its full activity can therefore only be demonstrated under con-

[1] Conradi, "Ueber lösliche, durch Autolyse erhaltene Giftstoffe," *Deutsch. med. Woch.*, 1903, No. 2.

ditions as favourable as those of the above-described experiment of METSCHNIKOFF and his collaborators.

A further conclusion to be drawn is that cultivations that have been killed contain only a very small amount of the toxine, and this accounts for the want of success in the attempts to isolate it as a chemical entity, and also the but trifling toxic action of the collodion capsules containing the *dead* cells.

As the vitality of the cells grows weaker the proportion of true toxine present also decreases very rapidly, so that in old cultivations or those that have been destroyed there is little poison present except that *secondary heat-resisting* product that has been described by various observers. This is certainly no longer a true toxine, since it resists boiling and is relatively *but slightly poisonous*. This poison, too, is only secreted to a small extent, but is, at all events, sufficiently stable to be isolated as a definite substance distinct from the body cells of the vibriones. It has, moreover, a slight power of forming an antitoxine; it thus shows all the characteristics that belong to Ehrlich's *toxoids*—viz., *greatly reduced toxic capacity, greater stability, and the power of forming an antitoxine, as well as of entering into combination.*

Hence, from these considerations, we can conclude with *some degree of probability* that the cholera vibrio *produces* a *true* toxine, an *endotoxine*, comparable with yeast invertase, which is only separated scantily, if at all, from the living cell, and that this toxine is extremely unstable, and is very readily transformed into a *secondary* mixture of poisons rich in toxoids.

At the same time, it is highly probable that, in addition to this poison so scantily secreted, the cholera vibrio, presumably, like all bacteria, also contains in its protoplasm a simple *non-specific* bacterial protein, which produces symptoms of inflammation, as, for example, that of the *diphtheria bacillus*. Only in this case it is not possible to separate the enzymic poisons so quantitatively as in the case of diphtheria, so that it is difficult here to obtain objective proof whether any such poisonous protein is present, *in addition to* the specific poisons.

CHOLERA ANTITOXINE.

We have shown above that it is probable that there is a cholera toxine which produces antitoxine to a small extent. No decisive unquestionable proof, however, that the serum of the animals in the experiments contains an active *antitoxic* substance has been given; and so long as cholera antitoxine has not been actually

detected in the fluids of the body, the whole question whether Koch's vibrio produces a true toxine must be regarded as an open one.

The question whether or no the serum of the animals used in the experiments confers antitoxic immunity does not necessarily coincide with that which is *alone* important, from the theoretical point of view—viz., Does it contain *antitoxine?* This antitoxine may possibly be very unstable, or, owing to its small quantity and its great dilution when introduced into the animal, it may become inactive. This would explain the negative results obtained by Pfeiffer, Kolle, Wassermann, Isaeff, and others in experiments on animals, and of Lazarus[1] on convalescent cholera patients, assuming that there really exists a cholera antitoxine, proof of which has certainly not yet been brought.

TYPHOID VIRUS.

Almost the same description might be given of *typhoid* as of cholera virus, only that in this case the experimental material is far more scanty, and the question of the existence of a toxine and antitoxine has hardly passed the first stages of discussion.

All that is certain is that *typhoid bacilli* can, under certain conditions, produce true toxine poisoning, and that the bacteria must therefore produce an active poison. On the other hand, it is equally certain that *immunity* against typhoid bacilli is essentially, as in the case of cholera, not *antitoxic*, but *bactericidal*, and antitoxic immunity, if it occurs at all, is only of slight importance.

Here, too, the history of the investigations of the poison begins with the researches of Brieger,[2] who first isolated *typhotoxine*, and subsequently his *toxalbumin*. These and similar preparations are *not* the specific typhoid poison.

On the other hand, as was found by Beumer and Peiper,[3] and subsequently by Chantemesse and Widal,[4] cultures sterilised at 100° to 120° C. in autoclaves are poisonous, the dose being from five to six times that of the living cultures; the older they are the more toxic they become.

[1] Lazarus, "Ueb. antitoxische Wirksamkeit des Blutserums," *Berl. klin. Woch.*, 1892, Nos. 43-44.

[2] Brieger, *Weiteres über Ptomaine*, Berlin, 1885.

[3] Beumer and Peiper, "Bakt. Stud. üb. Typhusbaz.," *Zeit. f. Hyg.*, ii., 110, 1887.

[4] Chantemesse and Widal, "L'immunité contre le virus de la fièvre typhoide," *Ann. Past.*, ii., 54, 1888. *Id.*, "Ét. expérim., &c., de l'infection typhique," *ibid.*, vi., 755, 1892.

Soluble poisons were prepared by BRIEGER, KITASATO, and WASSERMANN (*loc. cit.*) by heating the liquid cultures to 80° to 90° C. and precipitating with alcohol, and also by concentrating them at 37° C. before the addition of the alcohol. These had slight toxic and immunising properties, while the filtrates were absolutely inactive. SIROTININ[1] obtained a poison possessing *specific*(?) activity by *filtration*, but PFEIFFER and KOLLE'S[2] results were a direct contradiction of this. BITTER'S[3] preparation was obtained by extraction with concentrated glycerin and evaporation *in vacuo* at 36° C.

SANARELLI[4] cultivated very poisonous typhoid cultures on glycerin bouillon for a month at 36° C., and then sterilised them; these cultures were frequently rendered more poisonous by intraperitoneal injection into mice. From them he isolated, by means of maceration for several days at 60° C., a very *weak* soluble poison which produced specific symptoms in the mucous membrane of *guinea-pigs* and *monkeys*, especially that of the intestine. The lethal dose for *rabbits* amounted to 10 c.c. per kilo. There was nothing specific, however, in the symptoms in the case of this animal.

RODET[5] found that filtered typhoid cultivations were slightly toxic, causing elevation of temperature and local signs of necrosis; the residual cells had hardly any poisonous action. Special importance attaches to the results obtained by CHANTEMESSE,[6] who obtained a very active *typhoid toxine* speedily decomposing in the air, by cultivation of the bacilli on an *extract of spleen* which had been digested with pepsin and again neutralised. Here, too, we find the same behaviour as in the case of cholera, the poison being not completely destroyed at 100° C.—*i.e.*, doubtless being converted into a less poisonous, but more stable, modification. It is possible that in this case also we have to deal with toxoids.

It acts upon susceptible animals, producing drowsiness, para-

[1] Sirotinin, "Die Uebertrag. von Typhusbazillen auf Versuchstiere," *Zeit. f. Hyg.*, i., 465, 1886.

[2] Pfeiffer and Kolle, "Ueb. d. spez. Reaktion d. Typhusbazillen," *Zeit. f. Hyg.*, xxi., 203, 1896.

[3] Bitter, "Ueb. Festig. v. Versuchstieren geg. d. Intoxikation durch Typhusbazillen," *Zeit. f. Hyg.*, xii., 298, 1892.

[4] Sanarelli, "Études sur la fièvre typhoide expérim.," *Ann. Past.*, viii., 193, 1894.

[5] Rodet, "Sur les proprietés toxiques des cultures des bacilles d'Eberth," *Soc. Biol.*, l., 774, 1898.

[6] Chantemesse, "Toxine typhoide soluble," *Prog. Méd.*, 1898, 245; *id.* "Lösliches Typhustoxin," *Wien. med. Blätter*, 1898, 18 *et seq.*

lysis, and also, especially on intravenous injection, acceleration of the pulse and lowering of the blood-pressure. Introduced *per os* it is harmless.

VINCENT[1] found that filtered cultures of EBERTH's bacillus were very poisonous when injected into the cranium, and that even animals rendered immune to typhoid were not protected against its action.

Hens and pigeons are almost refractory, while other animals show varying degrees of susceptibility. Rabbits are three times as susceptible as guinea-pigs.

Soluble poisons were also found by MARTIN[2] in filtered typhoid cultivations; he concluded that the poison retained in the cells was identical with this. He cultivated the bacilli in a bouillon partially prepared from extract of spleen and containing alkali albuminate.

The collodion capsule method has been tried by RODET and GUÉCHOFF,[3] but with little success.

CONRADI[4] obtained *typhoid poison* by *aseptic autolysis* of the bacilli, mixing them with a 0·8 per cent. solution of sodium chloride and leaving them for at most forty-eight hours in the incubating chamber. In this way he prepared a solution of a cell-free poison, which in a dose of 2 c.c. killed guinea-pigs in twenty-four hours.

This method and also that of MACFADYEN and ROWLAND[5] are particularly promising for the elucidation of this question in the future. MACFADYEN triturated the bacteria at the temperature of *liquid air* and thus obtained a *poison* that *caused acute symptoms*, and also, according to his preliminary experiments, produced antitoxic immunity.

This typhoid endotoxine has been described more fully by MACFADYEN[6] in a detailed investigation recently published. It was found to be very poisonous. In immunisation experiments

[1] Vincent, "Inoculation intercranielle du bacille Eberth, &c.," *Soc. Biol.*, lv., 1214, 1903.

[2] Martin, "Die chem. Prod. path. Bakt.," *Wien. med. Blätter*, 1898, No. 25 *et seq.*

[3] Rodet, A. and G. Guéchoff, "Versuche die Methode der Kollodiumsäckchen auf die Kenntnis der toxischen Produkte des Eberthschen Bacillus und des B. coli anzuwenden," *Soc. Biol.*, lii., 962, 965, 1900.

[4] Conradi, "Ueber lösliche, durch aseptische Autolyse erhaltene Giftstoffe," *Deutsch. med. Woch.*, 1903, No. 2.

[5] Macfadyen and Rowland, "An intracellular Toxine of the Typhoid Bacillus," *Proc. Roy. Soc.*, lxxi., 77, 1902, also *Centralbl. f. Bakt.*, xxxiv., 618, 1903.

[6] Macfadyen, "Über das Vorkommen intracellularer Toxine," *Z. für allgem. Phys.*, iii., 303, 1904.

it had a protective action against both the toxine and infection with living bacilli.

Whether these soluble poisons are true toxines or toxoids—*i.e.*, whether or no they produce antitoxines in the body—is still more doubtful than in the case of cholera. PFEIFFER and KOLLE absolutely deny that there is any excretion of a free soluble poison or formation of antitoxine, whilst BITTER concludes that there is a slight production of antitoxine. On the other hand, CHANTEMESSE observed an unmistakable and energetic formation of antitoxine, after the injection of his typhoid toxine, especially in the case of the horse.

We have thus, as in the case of cholera, a poison firmly retained in the cells of the bacilli, and only to be separated from them with difficulty. Our theoretical conclusions must, therefore, with all reserve, be very similar to those we have drawn in the case of cholera virus—viz., that there possibly exists a typhoid toxine, but that it is hardly excreted in the free state, and that the poisons that *have been prepared* are greatly altered *secondary* products.

BACILLUS COLI COMMUNIS.

The only statements that the *coli* bacillus produces a toxine are those of BARBA-MORRHY[1] (of whose results only a short abstract giving no particulars of the nature of the poison was accessible to me), and that of RODET, who discovered them at the same time in typhoid cultures (*q.v.*). According to MARTIN's results (*loc. cit.*) the *coli* bacillus and also Gaertner's *B. enteritidis* appear to behave in quite an analogous manner to the typhoid bacillus as regards the production of their poison.

VAUGHAN[2] by heating the cells with dilute (1 per cent.) sulphuric acid, obtained a poisonous substance which was certainly not a *toxine*. With regard to *colilysine*, *vide supra*.

DYSENTERY.

CONRADI (*loc. cit.*) has succeeded, by means of the method of *aseptic autolysis*, in preparing a soluble dysentery poison.

After eighteen hours' autolysis he obtained poisons which killed large rabbits after the intravenous injection of 0·1 c.c. The symptoms were violent diarrhœa, paralysis, decrease of temperature, &c. In cases of chronic poisoning with smaller doses there were also intestinal ulcers and all the pathological and anatomical appearances of dysentery.

[1] Barba-Morrhy, *Baumgartens Jb.*, 1897, 403.

[2] Vaughan, "The intracellular Toxines of some Pathogenic Bacteria," *J. Amer. Med. Assoc.*, 1903, 828; *Biochem. Centralbl.*, i., No. 1,056.

PLAGUE TOXINE.

The plague bacillus also produces soluble poisons which probably belong to the true toxines, although the question is still unsettled.

The filtrates of quite young cultivations have no poisonous action, as was shown by the concordant results of the *German Commission*,[1] WERNICKE,[2] ALBRECHT and GHON.[3]

On the other hand, toxic properties are developed in old cultivations (even after five days), and increase with their age. The cultures cause death, preceded by emaciation, degenerative changes in the liver, and weakness of the heart's action. The plague toxine has been most thoroughly studied by MARKL.

MARKL[4] found, first of all, that the cells of bacilli that had been killed by chloroform were very poisonous, but subsequently made use of the filtrates from bouillon cultures instead, which proved fully as poisonous.

The toxic power was particularly great in the case of old cultures that had been grown at a lower temperature (about 20° C.), and it increased up to about the second month, after which it became stationary, and then decreased until eventually the toxine disappeared. Plentiful admission of air to the cultures was most essential. The temperature of incubation had an injurious effect upon the toxines.

The lethal dose of the most active poisons amounted to 0·005 to 0·01 c.c. for mice and to about 0·1 c.c. for rats.

In the case of these animals the poisoning proceeded rapidly, with symptoms of collapse, but without any anatomical alteration except fatty degeneration of the liver; with rabbits and guinea-pigs, however, it only acted in this way when very large doses had been given, and otherwise its action was more protracted, extending over several weeks. Occasionally a splenic tumour and pigmentary atrophy of the liver were produced. In the case of a cat he observed, in addition to marasmus, loss of hair and wide-spread necroses of the skin. In guinea-pigs it produced rigor and extreme lowering of the temperature (25° C.).

[1] "Bericht der deutschen Pest-Comm.," *Arb. Kaiserl. Ges. Amt.*, xvi., 1899.

[2] Wernicke, "Ueb. Immun. Vers. b. d. Beulenpest," *Centralbl. f. Bakt.*, xxiv., 1894.

[3] Albrecht and Ghon, "Bakt. Unters. üb. d. Pestbac.," *Wiener Akad.*, lxvi., 1898.

[4] Markl, "Beitrag z. Kenntnis der Pesttoxine," *Centralbl. f. Bakt.*, xxiv., Nos. 18-20 (Bibliography), 1898; *id.*, "Weit. Unters. üb. Pesttoxine," *Zeit. f. Hyg.*, xxxvii., 401, 1901.

The poison is very sensitive to external influences. Even at the ordinary temperature the toxic power of the solutions rapidly decreases, and this loss takes place more rapidly at high summer temperatures (25° C.) and immediately at 70° C. It is true that filtrates thus heated are still poisonous to guinea-pigs and rabbits when introduced in large doses, but in quite a different way, possibly on account of the formation of toxoids.

Markl has also endeavoured to purify plague toxine, but his experiments (precipitation with alcohol) are little more than preliminary. He has found that it is intimately associated, like all true toxines, with albuminous substances.

It is probable that he was dealing, in the main, with secondary poisons which are formed in such old cultures. They cause a formation of antitoxine, even if only to a slight extent.

Kossel and Overbeck,[1] too, succeeded in producing immunity by means of filtered cultivations that had been heated to 60° C.

PNEUMOTOXINE.

The state of affairs with regard to pneumococcus, the cause of croupous pneumonia, appears to be very similar to that found in the case of cholera and typhoid.

The first important experiments throwing light upon the poison of this diplococcus were those carried out by the brothers Klemperer.[2]

They attempted to produce immunity by means of cultures which, although weakened or inhibited, still contained the cells of the cocci; but they also employed germ-free solutions for the purpose.

They tried not only the simple filtrates from cultivations, but also a purulent pleural exudation of which the pneumococcus was the exciting cause, and which had been proved experimentally to be free from bacteria, in addition to the heated sputum of pneumonia patients and glycerin extracts of agar cultivations filtered from the cells of the bacteria.

In this way they obtained fairly weak toxic substances producing no specific effects; by heating these products to 60° C.

[1] Kossel and Overbeck, "Bakt. Unters. über Pest," *Arb. Kaiserl. Ges.-Amt.*, xviii., 1901.

[2] G. and F. Klemperer, "Vers. über Immunisierung u. Heilung bei der Pneumokokkeninfektion," *Berl. klin. Woch.*, 1891, Nos. 34, 35; G. Klemperer, "Die Bezieh. versch. Bakteriengifte z. Immunität u. Heilung," *Zeit. f. klin. Med.*, xx., 165, 1892.

they succeeded in eliminating their toxic power almost completely without destroying the immunising capacity. The serum of the animals used in the experiments contained a specific antitoxine which neutralised the dissolved poison *in vitro* and after previous injection. At the same time, both the toxic capacity and formation of antitoxine were very limited in extent.

Almost identical results were obtained about the same time by Foà and Carbone[1] and Scabia,[2] who also succeeded in producing antitoxic immunity by means of germ-free filtrates and glycerin extracts of blood from animals infected with pneumonia.

Belfanti,[3] too, produced immunity, though only to a very slight extent, by means of filtered germ-free sputum.

Foà also succeeded in separating the immunising principle by precipitation with alcohol or ammonium sulphate.

Pane[4] found filtered cultivations to be very slightly poisonous. Washbourne[5] states that he, too, was able to produce immunity with filtered cultures.

Isaeff[6] found that pneumococci produced only slightly toxic products in the ordinary culture media, but that their toxic power could be considerably increased by frequent, at least twelve, passages through rabbits. From the blood of the heart of such rabbits he obtained, by the addition of 1 per cent. of glycerin and some sodium carbonate, followed by filtration through a Chamberland filter, a *poison* which killed rabbits when introduced in the proportion of 1 per cent. of their body weight, and was greatly weakened when heated at 70° C., and destroyed at 100° C. The fluid from the peritoneum of such animals was very poisonous, but not fatal, after being sterilised by heat. Although he, too, produced a certain degree of immunity by means of germ-free filtrates, he yet asserted that he was absolutely unable to observe any *antitoxic* immunity, and that it was only antibacterial.

[1] Foà and Carbone, "Sulla immunità verso il diplococco pneumonico," *Gazz. med. di Torino*, 1891, 1 (*Centralbl. f. Bakt.*, x., 768).

[2] Foà and Scabia, "Sulla immunità della pulmonite," *ibid.*, 1892, 13-15 (*Centralbl. f. Bakt.*, xi., 615).

[3] Belfanti, "Sulla immunisazione per mezzo di filtrati di sputo pneumonico," *Rif. Med.*, 1892, 126 (*Centralbl. f. Bakt.*, xii., 401).

[4] Pane, "Ueber d. Heilkraft d. antipneumon. Serums," *Centralbl. f. Bakt.*, xxi., 664, 1897.

[5] Washbourne, "Experiments with the Pneumococcus," *Journ. of Path.*, iii., 142; *Baumgartens Jb.*, 1895, 62.

[6] Isaeff, "L'immunité contre le pneumocoque," *Ann. Past.*, vii., 259, 1893.

MENNES[1] obtained toxines which, in large doses, caused death, preceded by fever, diarrhœa, and loss of weight; he claims to have produced antitoxic immunity by means of innoculation with cultivations weakened at 50° C.; the serum is stated to neutralise the toxine *in vitro*.

CARNOT[2] succeeded in producing typical croupous pneumonia by *intrapulmonary* injection of 2 to 6 drops of *pneumotoxine*.

CARNOT and FOURNIER[3] were able to keep pneumococci alive and virulent for a long time, when they used as culture media, blood, serum, or, still better, fresh cerebral substance, while sterilised culture-media prepared from these were less satisfactory. They separated without delay, by means of dialysis, the toxines that were formed, and in this way obtained poisonous dialysates, which they concentrated by evaporation *in vacuo*, or by precipitation with nascent calcium phosphate. The symptoms produced by them were similar to those caused by poisoning with the living cocci. The same investigators[4] found that their poison had an intense action upon the muscular tissue of the *heart* and *blood-vessels*; even after the introduction of small doses very acute inflammation and perforations were produced.

The general conclusion to be drawn from all these observations is that the existence of a secreted true pneumotoxine has not been established beyond doubt.

GONOCOCCAL POISON.

The gonococcus also produces a poison which appears to belong to the group of cholera-typhoid poisons inasmuch as it only passes to a slight extent into the culture fluids, and rather is mainly retained in the bacteria, and is probably only given up in a slight degree to the liquid media after the decomposition of the cells. The first investigations into this poison were made by A. WASSERMANN.[5]

He succeeded in cultivating the gonococci on a nutrient medium which was prepared from pig's serum, to which had been

[1] Mennes, "Das Antipneumokokkenserum," *Zeit. f. Hyg.*, xxv., 413, 1897.

[2] Carnot, "Reprod. expérim. de la pneumonie fibrineuse," *Soc. Biol.*, li., 927, 1899.

[3] Carnot and Fournier, "Sur le pneumocoque et ses toxines," *Arch. Med. Exper.*, 1900, 357.

[4] Carnot and Fournier, "Lésions Cardiaques et musculaire par la toxine pneumon.," *Soc. Biol.*, lii., 143, 1900.

[5] A. Wassermann, "Gonokokkenkultur und Gonokokkengift," *Berl. klin. Woch.*, 1897, 685; "Weitere Mitteilungen über do.," *Zeit. f. Hyg.*, xxvii., 298, 1898.

added 2 per cent. of nutrose, and contained peptone and serum albumin as necessary ingredients.

While he found that the living gonococci distributed in a physiological solution of salt had absolutely no toxic effect upon animals, the sterilised culture, on the other hand, had a fairly energetic poisonous influence, and killed the animals by peritonitis on intraperitoneal injection, though it also produced, *e.g.*, keratitis with hypopyon in the eyes, &c.

The filtrate had but a slight poisonous action. Hence it would seem that the poison, just as in the case of cholera, is retained within the cells. In its resistance to heat, even at the temperature of boiling, it also resembles the so-called cholera poisons.

Very similar results were obtained, independently of Wassermann, by Nicolaysen,[1] who, by means of sterilised, non-filtered cultivations, produced purulent gonorrhœa in animals.

He found the lethal dose for a mouse to be 0·3 c.c. He, too, is of opinion that the poison is firmly retained within the cells, for he was unable to separate it either by extraction with distilled water or with dilute soda solution.

Almost simultaneously Schäffer[2] succeeded in obtaining from a cultivation of four days' growth an aqueous decoction of ascites and flesh, a poison which, *after filtration* through a porcelain filter, produced on the urethral mucous membrane acute suppuration which rapidly disappeared. At about the same time the question of *gonotoxine* was studied by Christmas.[3] He obtained, by means of filtration through porcelain, from cultures of ten to fifteen days' growth, in media of ascites and bouillon, a poison which he was able to concentrate by precipitation with alcohol. He also prepared a poison by evaporating the cultures with glycerin at 50° C. This was stable at 50° C. to 70° C., and could be kept for six months in the dark. It had a very virulent effect upon animals, producing, in addition to local inflammation, cachexia, ending in death. It only acted upon the mucous membrane of the urethra in the case of *man*.

Christmas[4] subsequently made a new investigation of the subject. He found that the living gonococci contained poison, but that, on the other hand, the dead cells yielded none when macerated at 20° C. He cultivated the gonococci on a nutrient

[1] Nicolaysen, "Zur Pathogenität und Giftigkeit der Gonokokken," *Centralbl. f. Bakt.*, xxii., 305, 1897.

[2] Schäffer, "Beitr. z. Frage d. Gonokokkentoxine," *Fortschr. d. Med.*, 1897, 813.

[3] Christmas, "Le Gonocoque et ses toxines," *Ann. Past.*, xi., 609, 1897.

[4] Christmas, "Contrib. a l'étude du Gonococcus," *Ann. Past.*, xiv., 331, 1900.

medium consisting of 75 per cent. of ascites and 25 per cent. of bouillon, and filtered the cultures through talc on the filters or through kieselguhr, since the porcelain filter retained the poison. The maximum of toxic power was reached on the twentieth day. He then precipitated the poison with ammonium sulphate or alcohol. If subsequent dialysis was employed the symptoms of poisoning, it is true, were different, but the lethal dose remained unaltered.

The poison was completely destroyed at 75° to 80° C., but could resist a temperature of 60° C.

Its action, on subcutaneous injection, was very weak, the lethal dose for guinea-pigs being 5 to 10 c.c. On the other hand, it acted very energetically after intercerebral injection, producing convulsions and dyspnœa, and killing a guinea-pig, even in a dose of 0·002 c.c., within six hours.

It is stated to possess strong *immunising* properties, affording protection up to one hundred times the dose of poison after intercerebral injection, but to a much smaller extent after subcutaneous injection. This *antitoxic immunity* has not been observed in the smallest degree by other investigators.

Moltschanoff[1] prepared a toxine from a culture in hydrocele fluid and yeast peptone bouillon. He made use of unfiltered cultures, which he sterilised at 70° C. He made a special study of the action of the poison upon the *central nervous system*, but I cannot give the details of his results here.

Grosz and Kraus[2] and Scholtz[3] absolutely deny the specific nature of gonotoxine, and simply regard it as a non-specific, pus-producing bacterial protein; they point out that Schäffer's *urethritis*, at all events, can also be produced by the living or dead cells of other bacteria (*pyocyaneus*, *coli*, &c.).

We have, therefore, again to draw the same conclusions about gonococci poison as in previous instances. The existence of a specific antitoxine-producing gonotoxine has not yet been proved.

STREPTOTOXINE.

In considering the metabolic products of the streptococci we meet with the same obscurity as in the case of gonotoxine. The existence of a true antitoxine-forming toxine is still open to question.

[1] Moltschanoff, "Ueber das Gonokokkentoxin und seine Wirkung auf das Centralnervensystem," *Münch. med. Woch.*, 1899, 1013.

[2] Grosz and Kraus, "Bakteriol. Studien über den Gonococcus," *Arch. f. Dermatol.*, xlix., 3, 1899.

[3] Scholtz, "Beitr. z. Biologie d. Gonococcus," *ibid.*

Certain investigators categorically deny that streptococci produce any specific poison at all.

Aronson[1] not only found the sterilised filtrates from cultures of streptococci to be devoid of toxic power, but was also unable to detect any poison in the sediment of cells of the cocci that had been killed by chloroform; local infiltrations were the only results of his inoculations. The existence of a streptococci poison is also denied by de Giaxa and Pane.[2]

At the same time, so many investigators have found *poisons*, at all events, in streptococci cultivations, that further proof is required before this radical conclusion can be accepted.

One of the first poisons to be demonstrated in *filtered* cultures was found by Manfredi and Traversa[3] in cultivation of erysipelas streptococci, grown preferably at 28° to 30° C. They described it as a readily oxidisable poison, which produced convulsions and paralysis, and soon became inactive on exposure to the air. In like manner poisonous metabolic products of streptococci were found in filtered cultures by Roger,[4] Marmorek,[5] Homén,[6] Friedrich,[7] Laitinen,[8] Claude,[9] Parascandalo,[10] and Schenk.[11]

As regards the nature of the poison little or nothing is known. Roger precipitated his poison from the cultivation by the addition of ten times the volume of alcohol, redissolved the precipitate in a solution of common salt, and endeavoured to

[1] Aronson, "Ueber Antistreptokokkenserum," *Berl. klin. Woch.*, 1896, 717.

[2] de Giaxa and Pane, "Contributo alla cognizione sulla immunis. contre la infez. da streptococco," *Riform. Med.*, xii. [4], 5; *Baumgartens Jb.*, 1896, 23.

[3] Manfredi and Traversa, "Sull'azione dei prodotti di cultura dello streptococco," *Giorn. Inter. delle Scienze Mediche*, 1888.

[4] Roger, "Action des produits solubles streptocoque d'erysipéle," *Soc. Biol.*, xliii., 538, 1891.

[5] Marmorek, "Le streptocoque et le sérum antistreptococcique," *Ann. Past.*, ix., 593, 1895.

[6] Homén, De l'action du streptocoque et de ses toxines sur les nerfs," *Sem. Méd.*, 1896, 211 (*Soc. Biol.*, xxiii., [5], 1896).

[7] Friedrich, "Beobacht. üb. d. Wirkung von subkutan einverleibten Streptokokken-Toxinen, &c.," *Berl. klin. Woch.*, 1895, 1065.

[8] Laitinen, "Das Streptokokkentoxin u. seine Wirkung auf das Nervensystem," *C. f. allg. Pathol.*, 1896, 358.

[9] Claude "Myelite aïgue par toxines streptocoq," *Soc. Biol.*, xlviii., 122, 1896.

[10] Parascandalo, "E. neue Versuchsreihe üb. de Serotherapie bei Infektionen mit pyogenen Mikroorg," *Wiener klin. Woch.*, 1897, 937.

[11] Schenk, "Ueb. Streptok.-Serum und Streptok.-Toxine," *Wien. klin. Woch.*, 1897, 937.

obtain by means of this preparation, insoluble in alcohol, the typical general symptoms produced by the living cultivations. His "poison" had, however, only a slight toxic activity (13 to 20 c.c. per kilo. of body weight); it was weakened, but not destroyed, at 104° C.

Most of these investigators, however, simply contented themselves with investigating the toxic action of *filtered cultures*, or of cultures after sterilisation at 65° to 70° C., or by the addition of 0·5 per cent. of phenol.

Schenk *alone* (*loc. cit.*) endeavoured to obtain a purer preparation by means of precipitation with zinc chloride and subsequent treatment by Brieger's method.

Marmorek found that the toxine was weakened even at 58° C. But the most important question, whether the poison thus obtained does or does not produce antitoxic immunity against the streptococcus, has not yet been definitely settled.

Roger (*loc. cit.*) found that his poison not only failed to produce any immunity, but that it even increased the susceptibility to infection.

Sieber-Schoumowa[1] was unable to effect immunisation by means of filtered cultures.

On the other hand, Laitinen (*loc. cit.*) cultivated streptococci on a 5 per cent. bouillon of peptone containing 2 per cent. of glycerin and 0·3 per cent. of ordinary salt, and having an alkalinity of 0·2 to 2 per cent., and precipitated from these cultures by means of ammonium sulphate or amyl alcohol toxine preparations, which, when injected into the peritoneum in the proportion of 0·1 to 0·4 c.c., or even of 0·01 c.c. on direct injection into the nerves, killed large rabbits, while they also had the power of conferring a certain degree of immunity against infection.

Parascandalo (*loc. cit.*) laid special stress on the point that he was best able to produce immunity against streptococci by means of soluble toxines.

He cultivated them on sugar bouillon, and, after sterilising them by means of 0·5 per cent. of phenol, filtered the cultivations after twenty-four hours through paper. He had found that simple filtration through porcelain did not remove all the living micro-organisms, which was also the case on heating them to 60° or 70° C., although a higher temperature injured the toxine. By making the first inoculations with the filtrates of young and only slightly toxic cultures, he was able to immunise animals gradually against large doses of very poisonous cultures, and in this way obtained a true therapeutic serum.

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[1] Sieber-Schoumowa, "Les sérums thérapeutiques anticocciques," *Arch. des Sciences Biol.*, iv., 415, 1896.

Yet even his results are open to question, inasmuch as it has been found by SCHENK (*loc. cit.*) that 0·5 per cent. of phenol is also insufficient to kill all the micro-organisms. He found that, although the cultures were apparently sterile, there were yet some living micro-organisms in the blood of the heart of the poisoned animals, so that it is quite possible that PARASCANDALO produced immunity by means of isolated, weakened, but yet living streptococci. SIMON,[1] too, was only able to detect the production of toxine after the decomposition of the cells. Thus proof has yet to be brought of the existence of streptotoxine, and the question is as open as that of gonotoxine.

THE POISON OF THE TUBERCLE BACILLUS.

The poisonous substances produced by the bacillus of tuberculosis require separate treatment for the following reasons:—In the case of other poison-producing bacteria the true toxines, or rather those specific poisons that it is possible to describe with more or less certainty as of the nature of toxines, can be separately described and differentiated from the so-called *bacterial proteins*, in the wider sense, that remain behind in the cells of the bacilli themselves after the extraction of these poisons which the micro-organisms have produced. This sharp differentiation is not possible on historical grounds in the case of tuberculosis, for since the time when the poisons of the tubercle bacillus were first seriously studied, it has been the almost invariable practice to investigate both kinds of poisons without separating them.

The first preparations of tubercle poison, and notably R. Koch's tuberculine, were thus a mixture of *all* the specific and non-specific poisonous products of this bacillus, and no investigation was made to determine the question of the existence of a true toxine. At that time, too, it was not possible to formulate the question in such precise terms, since the definition of a true toxine, and of the true antitoxic immunity that it involved, had not then been given with sufficient clearness.

These toxic preparations, therefore, consisted of the more or less altered substances of the cell itself. In addition to these, however, they also undoubtedly contained other toxic products, some of which possibly we must regard as primary secretion products, and eventually as true toxines, and others as secondary products of a simpler nature, with which we shall also deal later.

[1] Simon, "Über die Gifte der Streptococcus," *Centralbl. f. Bakt.*, xxxv., 318, 1904.

Here we must first determine whether there is a true tuberculosis toxine.

Until quite recently the only preparations actually tested with regard to this point were those of MARAGLIANO.[1] By means of filtration through bacterial filters he obtained from fresh cultures grown at the ordinary temperature a poisonous bouillon, which in large doses caused death, preceded by hypothermia and sweating. The poison was destroyed at 100° C. Similar insignificant results were obtained by BERNHEIM[2] with cultures filtered through a Kitasato filter.

MARAGLIANO and his pupils also prepared therapeutic sera and antitoxines against tuberculosis, but he himself concluded that the antitoxine did not have a direct neutralising effect upon the "toxine," but only acted as a stimulus to other therapeutic forces; hence it loses all interest for us.

These experiments appeared of great importance, and their results were very thoroughly tested as regards their therapeutical application, though without any material success. But this did not do much to advance the knowledge of the toxine question itself.

LEDOUX and LEBARD[3] also found in cultivations that had been filtered through a Chamberland filter substances which had, it is true, a pyrogenic and toxic effect on intraperitoneal injection, but their action was so slight that they were inclined to attribute it exclusively to the culture medium itself.

More recently the results of an investigation into tuberculosis toxine have been published by FRENKEL and BRONSTEIN.[4] They cultivated tubercle bacilli on a glycerin bouillon of 5 per cent. strength, and thus obtained solutions of poison which, after being freed from the bacilli by filtration, killed a guinea-pig in a few days when injected in a dose of 1·5 to 2 c.c.

The poison was rapidly weakened by light and air. They prepared it in a concentrated form by means of precipitation with alcohol.

They claim to have produced immunity by means of inocula-

[1] Maragliano, "Heilung d. Lungentuberkulose durch Heilserumtherapie," *Berl. klin. Woch.*, 1895, 689; *id.*, "Ueber das tuberkulöse Heilserum u. seine Antitoxine," *ibid.*, 1896, 773; *id.*, "Ueb. d. Tuberkelantitoxin," *Malys Jb.*, 1900, 1044.

[2] Bernheim, "Immun. tuberc. et sérumthérapie," *Soc. Biol.*, xlviii., 291, 1896.

[3] Ledoux and Lebard, "De l'action sur la tempér. du bouillon des cultures tuberc.," *Arch. d. Méd. Expér.*, x., 601, 1898.

[4] Frenkel and Bronstein, "Ueber Tuberkulosetoxin und Antitoxine," *Berl. klin. Woch.*, 1901, 861.

tion with this poison, not only against all the tubercle poisons, but also against the proteïn.

The chief point that makes one sceptical of the results of these investigations is that the filtrates of the cultures had so slight a toxic action. The authors had practically nothing more than tuberculine in their hands, and this is fairly poisonous when employed in the same manner. None of the investigators determined whether he had obtained a specific toxine which produced in the organism an antitoxine acting only against the toxine itself. An antitoxic immunity against the proteins is *a priori* hardly conceivable, but, in any case, could not have been produced by the supposed true toxine, and it is just this omnipotence of the "anti-bodies" of these therapeutic sera that lays these statements open to the gravest suspicion. It is quite certain that the existence of a true haptophore toxine of the tubercle bacillus has *not* been proved by these experiments.

On the contrary, it appears more and more probable that the tubercle bacillus does not produce any true toxine in the sense of our definition. Hence, notwithstanding its intrinsically very great importance, we can only deal briefly here with the subject of the tubercle poisons.

TUBERCULINE.

Under this name are included preparations of different kinds, having in common the characteristic that they are obtained from cultures of tubercle bacilli, and contain their bodies in a nearly unchanged condition.

These substances are probably, for the most part, *not* true toxines. They are, as is shown by their mode of preparation, proteids resisting heat, closely allied to the albumoses, and possibly possessing a certain amount of specific activity. Their chemical nature and relationship to the albumoses has been thoroughly studied by KÜHNE.[1] They have but little toxic action upon a healthy organism, but they produce considerable reactions in tuberculous subjects.

The original tuberculine of ROBERT KOCH [2] was prepared from a culture of tubercle bacilli grown at 38° C. on the surface of a 4 per cent. glycerin bouillon to which as much air as possible was admitted. After six to eight weeks the cultivations were evaporated to a tenth of their volume, and filtered through a

[1] Kühne, "Erfahrungen über Albumosen u. Peptone," *Z. f. Biol.*, xxix., 24, 1892; xxx., 220, 1894.

[2] R. Koch, "Mitt. über ein Heilmittel gegen Tuberk.," *Deutsch. med. Woch.*, 1891, 101, 1189.

porcelain filter. The extract thus obtained, containing 40 per cent. of glycerin, was very stable.

After many fruitless attempts KOCH abandoned the idea of further purification. His method has been frequently modified. NOCARD[1] first heated the cultures to 110° C. before treating them in the same way; he contented himself with filtration through paper, since his preparations were obviously already sterile. MARAGLIANO,[2] and, subsequently, FRENKEL and BRONSTEIN (*loc. cit.*), used a method of boiling, eventually in autoclaves, in the preparation of products very similar to tuberculine.

BEHRING[3] extracted from tuberculine a substance akin to mucin by means of soda solution, and fatty substances by means of ether, and in this way claimed to have obtained a preparation about twenty times as poisonous as before.

NIEMANN,[4] too, has endeavoured, though without any particular success, to obtain strong tuberculine preparations by precipitation with alcohol.

As regards the toxic value of these preparations the results obtained by these investigators have been supplemented by GRAMATSCHIKOFF,[5] who proved that tuberculine was a blood poison, and by v. LINGELSHEIM,[6] who proposed to determine a normal unit of toxic activity by means of intercerebral injections.

CARRIÈRE[7] found that fairly considerable alterations in the liver and kidneys followed the injection of tuberculine.

PEIPER[8] found that in non-tuberculous subjects it had a considerable pyrogenic effect, but was only slightly toxic. Even doses of 0·01 grm. produced only insignificant symptoms, such as pains in the head and limbs, &c.

All these experiments were put upon a new basis by the fact that the attempts to extract the poisonous substances in an unaltered form from the tubercle bacilli were largely abandoned in favour of experiments with the bacilli themselves. KOCH[9]

[1] Nocard, "Des injections revélatrices de la tuberculine," *Rec. de Med. Vet.*, lxxii., 369; *Baumg. Jb.*, 1895, 705.

[2] Maragliano, "Extrait aqueux des bacilles de la tuberc.," *Soc. Biol.*, l., 94, 1898.

[3] Behring, "Bekämpfung der Tuberkulose," *Münch. med. Woch.*, 1898, 580.

[4] Niemann, "Ueber Tuberkuloseheilserum," *Münch. med. Woch.*, 1897, 59.

[5] Gramatschikoff, "Ueber einige physiolog. Wirk. des kochschen Tuberkulins," *Arb. pathol. Inst. Tübingen*, i., 287; *Baumg. Jahrb.*, 1897, 548.

[6] v. Lingelsheim, "Ueber die Wertbestimmung der Tuberk. Giftpräparate," *Deutsch. med. Woch.*, 1898, 583.

[7] Carrière, "Étude expér. des altérations histolog. du foie, &c.," *Arch. Med. Expér.*, ix., 65, 1891.

[8] Peiper, "Ueber die Wirkung des Kochschen Mittels auf Gesunde," *Deutch. med. Woch.*, 1891, 160.

[9] R. Koch, "Ueb. neue Tuberkulinpräparate," *Deutsch. med. Woch.*, 1897, 209.

started from the observation that dead tubercle bacilli remained undecomposed in the body for a very long period, and were therefore not absorbed. But, according to him, so long as they retain their form, immunity against the bacilli cannot be produced; and it is only after their decomposition, but too late for any curative action, that a slight *bactericidal* immunity is attained. Koch, therefore, endeavoured to get nearer to his goal by breaking up the bacilli and using an emulsion of their cell constituents for the inoculation. For this purpose he first dried the bacilli rapidly, and then triturated them mechanically. The masses were treated with distilled water, and repeatedly subjected to centrifugal force. The first portion separated was soluble in water, but was not only non-poisonous, but also devoid of any immunising power (T.O.). It was only the second and third "centrifugates" that contained the substance with specific action. These preparations were termed "T.R." by Koch. They were found to possess immunising and therapeutic powers without any particular toxic action, and notably without abscess formation or inflammation. It was found best to use young cultivations dried *in vacuo*, and to protect the preparations from light as far as possible.

H. Buchner,[1] working alone and also in collaboration with Hahn,[2] isolated from the bacilli by means of E. Buchner's method of applying enormous pressure, expressed fluids "*plasmines*," with which he believed that he obtained specific effects, although he himself regarded the question as still unsettled.

Behring[3] described a poison obtained by means of glycerin water at 150° C. from the finely-divided bacteria after extraction of the fat, and stated that 1 grm. of this was sufficient to kill 1,250 mice. It was neutralised by the serum of a cow cured of tuberculosis (antitoxine formation?).

Landmann[4] claimed to have discovered an active tubercle poison in his "*tuberkulol*" and asserted that it destroyed a guinea-pig of 250 grms. when injected in a dose of 0·1 grm.

He macerated tubercle bacilli for a long time at 40° C. with a physiological solution of salt, distilled water and glycerin; then decanted the

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[1] H. Buchner, "Zur Koch's Mitt. über neue Tuberkulinpräparate," *Berl. klin. Woch.*, 1897, 322; *id.*, "Die Bedeutung d. aktiven lösl. Zellprodukte, &c.," *Münch. med. Woch.*, 1897, 12.

[2] Hahn, "Immun. u. Heilungvers. mit den plasmat. Zellsäften," *Münch. med. Woch.*, 1897, 1344.

[3] Behring, "Autoreferat über den Vortrag in Madrid," *Deutsch. med. Woch.*, 1898, 293.

[4] Landmann, "Ueb. eine neue Meth. der Tuberkulosetoxin-Behandlung," *Hyg. Rundschau*, x., 168; *Centralbl. f. Bakt.*, xxvii., 280, 1900.

liquid, and treated the residual bacilli in the same way successively at 50°, 60°, &c., up to 100° C. The different extracts were then united and mixed with culture fluid, and the whole evaporated at 37° C. *in vacuo* and filtered through porcelain. In this way he obtained a mixture of all the products of the tubercle bacilli, including both the toxines destroyed by heat as well as the hot-water extracts, with the proteins. He claimed to have produced therapeutic results by means of this mixture. It is obvious that such mixtures as this cannot advance our theoretical knowledge.

Subsequently KOCH,[1] in a later research, published a new method of making tuberculine preparations.

He triturated 0·1 grm. of the absolutely dry tubercle bacilli in an agate mortar with a solution of 0·5 grm. of phenol and 0·85 grm. of sodium chloride in 100 c.c. of water, a few drops being used at first, and then more and more of the liquid until the total amounted to 100 c.c. The mixture was then whirled for six minutes in a centrifugal machine, decanted from the deposit, and diluted with ten times its volume of the solution, so that eventually the liquid contained in a litre the extract from 0·1 grm. of tubercle bacilli.

This liquid was *rendered turbid* by the addition of agglutinating serum. By itself it also served the purpose of diagnosis.

The question of the nature of the poisons of the tubercle bacillus was thoroughly studied by RUPPEL.[2] He found, first of all, that the filtrates were absolutely *non-specific*, and, with the exception of albumoses, chiefly deuteroalbumose, was unable to discover anything toxic in them. Nor was he more successful in his attempts to isolate any specific poison from the bacilli by extraction. This really gives a definite answer in the negative to the question whether there exists a soluble specific toxine of the nature of diphtheria toxine.

On the other hand, he isolated from the crushed bacilli two *poisonous substances*—viz., a nucleic acid containing 9·42 per cent. of phosphorus, *tuberculinic acid*, and also a *protamine*, which he recognised as such by its being precipitated with picric acid, and termed *tuberculosamine*. The proportion of the finely-divided bacilli dissolved after treatment in a centrifugal machine amounted to 50 per cent. The solution gave no proteid reactions and probably contained only a compound of the nucleic acid with the protamine. According to NEUFELD,[3] however, the action of tuberculosamine is absolutely non-specific and v. Lingelsheim's method of valuation (*vide supra*) cannot be used.

[1] Koch, "Ueb. d. Agglut. d. Tuberkelbacillen," *Deutsch. med. Woch.*, 1901, 829.

[2] Ruppel, "Zur Chemie der Tuberkelbazillen," *Z. f. phys. Chem.*, xxvi., 218, 1898.

[3] Neufeld, "Zur Werthbestimmung der Tuberkulosepräparate," *Deutsch. med. Woch.*, 1899, 13.

RUPPEL and KITISHIMA[1] made a further investigation of tuberculinic acid. It was from three and a half to four times as poisonous as dry old tuberculine. It was much more poisonous on intercerebral injection, especially in the case of tuberculous guinea-pigs (1 grm. killed 40,000 kilos.). Other nucleic acids were less poisonous. Tuberculinic acid prepared by the older somewhat drastic method of KOSSEL was found to be five times less poisonous than the new preparation. RUPPEL and KITISHIMA then prepared from tuberculinic acid by Kossel's method, a substance similar to thymic acid, *tuberculothymic acid*, "1 c.c. of which contained as much poison specific for tuberculous individuals as 20 c.c. of Koch's tuberculine" (BEHRING), and also a still more simple poisonous substance of as yet unknown nature, *tuberculosine*, 1 grm. of which was equivalent to 25 to 30 c.c. of Koch's tuberculine.

According to BEHRING this represents the *poison-nucleus*, around which the other substances are grouped in some way or other, and without which there can exist no poison with the specific action of tuberculine.

Important as these researches are with respect to the toxic action of the tubercle bacilli, they have yet little claim upon our attention here, since there can be no question of these substances obtained by drastic chemical methods being true *toxines*.

A further objection to drawing comparisons between these poisons and diphtheria poison is that the former are relatively only slightly poisonous at all events to the healthy organism.

There is never any *formation of antitoxine* in the case of these poisons.

Tuberculine is invariably but slightly poisonous to a healthy organism, and its toxic action can be still further reduced by processes of purification without injuring its specific activity.

On the other hand, it has frequently been shown by RÖMER,[2] BUCHNER,[3] and others that the action of tuberculine, as regards its *toxic* effects, is non-specific. The same pyrogenic and local inflammatory symptoms could also be produced, in the same degree, by the proteins of other micro-organisms (*pyocyaneus*,

[1] Behring, *Die Diphtherie*, Berlin, 1901, 91; see also Behring, "Ueber die spez. giftigen Eigensch. d. Tuberkulinsäure," *Berl. klin. Woch.*, 1899, 537.

[2] Römer, "Ueb. d. formativen Reiz d. Proteine Buchners," *Berl. klin. Woch.*, 1891, 886; "Tuberkulinreaktion d. Bazillenextrakte," *Wien. klin. Woch.*, 1891, 835.

[3] Buchner, "Tuberkulinreakt. d. Proteine nicht spezif. Bakt.," *Münch. med. Woch.*, 1891, 841.

prodigiosus, *pneumobacillus*) that had been prepared in an analogous manner.

It is thus hardly conceivable that the old tuberculine preparations should possess any specific *toxic* activity.

Moreover, their specific protective activity is scarcely to be attributed to an antitoxic process. There appears, rather, to be a liberation of specific *bactericidal* protective forces, by means of these specific proteids, whether it be that by this treatment similar substances are set free and can be utilised, just as specific bacteriolytic processes are also brought about in the case of *cholera*, &c., by such substances—viz., the receptors of the bacteria, which concentrate their activity upon the *intermediate products* as defined by EHRLICH; or whether we have here to deal with specific proteids concentrating their activity upon the *precipitines* or *agglutinines*, which act upon the bacilli and are able to bring about specific reactions of a protective or curative nature in the diseased organism. The fact that specific agglutinines were present in the serum of tuberculous subjects was made known by ARLOING and COURMONT, and more recently confirmed by KOCH in another way (*vide supra*). It would thus seem conceivable that the substances extracted from the bacilli were in this case able to bring about bacteriolytic processes similar to those produced by the *whole* bacteria in the case of cholera, typhus, rinderpest, &c. There has, as yet, been no sufficient reason for concluding that in these immunising processes a separate position is occupied by inoculation with unbroken bacteria, since these delicate organisms are readily absorbed, and when decomposed within the body bring about the curative immunising processes. And the only attempts to produce immunity with "plasmines," in the case of cholera (HARN, *loc. cit.*), also support the view that in those cases bactericidal, *not* antitoxic, phenomena play the chief part. On the other hand, the tough tubercle bacilli protected by a thick membrane of fat-like substance (*cf.* RUPPEL, *loc. cit.*) are, as KOCH has shown, attacked either not at all or only with great difficulty in the organism, and are thus incapable of bringing about any bactericidal immunising processes. It has, however, been shown by KOCH that slight phenomena of bactericidal immunity appear during their ultimate decomposition. It would appear, then, that by drastic means, especially mechanical trituration of the bacilli and injection of the extracts obtained from them, substances are liberated which lead more rapidly to the production of antibacterial immunity. Still more effective is the crushing of the bacilli and expression of their cell content,

which causes a still more plentiful liberation of those specific receptors, which can then develop their specific reactions in the organism. These intermediate bodies, however, withstand heat and can thus resist high temperatures at which a true toxine, which produces antitoxic immunity, would certainly be destroyed.

On this assumption, the specific tuberculine reaction in tuberculous men, as a reaction of a *bactericidal* nature, would have no connection with the toxic pyrogenic effects in healthy men; these poisonous principles would be only *interfering* impurities, and indeed we have seen that, as a matter of fact, KOCH and others have made successful attempts to prepare an almost *non-toxic and yet specifically active tuberculine.* Moreover, the latest researches of KOCH (*loc. cit.*), from which it appears that the serum of tuberculous subjects gives a precipitate with his new tuberculine preparation, appear to support this assumption of specifically active proteids and their precipitines. This hypothesis, that the action of tuberculine in its most recent form is due to the liberation of specific amboceptors, materially elucidates and simplifies the problems. Tuberculine occupies a different position, according to BUCHNER, from the series of specific bacterial poisons, and yet has claims to be regarded as a specific therapeutic and immunising agent, just as much as those dead cells that produce antibacterial immunity in cholera, &c.

Nevertheless, the question of *tubercle poisons* needs further study. This hypothesis of the action of tuberculine does not by any means do away with the necessity for such research; for, as in the case of other bacteria, there may in addition to this be a true toxine which can also bring about specific antitoxic immunity.

That such a toxine has *not* yet been discovered may possibly be due to the methods; but, on the other hand, it is not improbable, as was suggested above, that *tubercle bacilli do not produce any true toxine at all.* Possibly they form poisons of another kind, which, although not of the nature of toxines, have yet a *specific* character.

The results of BEHRING and his pupils in the preparation of very toxic tubercle poisons also point in the same direction, and their discovery of *tuberculosamine* and its combination with nucleic acid must also be regarded as valuable evidence in support of this theory, although these were undoubtedly not the specific poisons of the tubercle bacillus. For RUPPEL himself concludes that in these compounds we have to deal with derivatives of the *cell nucleus.* These are undoubtedly not the specific bacterial poisons, which we must certainly

regard as protoplasmatic or paraplasmatic *products*. It is not improbable that perfectly analogous substances might be isolated from cell nuclei or cells of other bacteria.

On the other hand, we cannot recognise as the specific poison the products obtained by AUCLAIR,[1] who claims to have isolated, by extraction of the cultures with ether, a poison which produces caseous degeneration of the lungs on intratracheal injection; and, by extraction with chloroform, a second poison which, he asserts, produces fibrous pneumonia.

We have therefore, unfortunately, to own that the question of a specific tubercle poison has not yet been decided, even if we exclude the hypothetical action of tuberculine, as described above, entirely from the discussion.

MALLEINE.

Malleine contains the cell constituents of the *bacilli of glanders* in much the same way that the old tuberculine contained those of the tubercle bacilli. The subject of malleine has been investigated almost exclusively by veterinary surgeons from the practical point of view, so that scientifically little or nothing is known about its *nature*. In the absence of further knowledge we shall do well to apply to malleine the considerations that we have taken into account in the case of the old tuberculine.

HELMAN,[2] in 1890, was the first to prepare an extract of glanders bacilli, and he was followed by KALNING, who boiled pure cultivations repeatedly with water and filtered them through Chamberland filters. PREUSSE, and subsequently PREISZ, employed glycerin extract of *potato cultures*.

JOHNE and PEARSON were the first to use bouillon as the culture medium; they cultivated the bacilli for fourteen days at 37° C. and sterilised the filtrates, or first concentrated the cultures at 80° C. and then filtered them (PEARSON).

ROUX sterilised very virulent cultivations at 110° C. and filtered them after evaporation.

FOTH (*loc. cit.*) proceeded in a similar manner. He passed the glanders bacilli several times through animals to render them as virulent as possible, and then cultivated them for twenty days as a surface cultivation on Löffler's meat broth of 45 per cent. strength. The cultures were evaporated to a tenth

[1] Auclair, "La Sclérose pulmonaire, &c." *Arch. de Méd. Exper.*, 1900, 189; see also *Baumgartens Jb.*, 1898, 475, 476.

[2] I have taken all these references, which were not readily accessible to me in the original, from Foth's paper, "Das Mallein, &c.," *Fortschr. d. Med.*, 1895, 637.

of their volume at 80° C., filtered, and treated with thirty times their quantity of alcohol. The white precipitate thus obtained was left to dry over calcium chloride *in vacuo*, and furnished a non-hygroscopic white mass.

GUINARD[1] made a closer study of the action of malleine. He found that the action of the heart was first stimulated and then lowered, and that a similar stimulation and subsequent depression of the nervous organs also occurred. Sweating was also observed.

SCHATTENFROH,[2] on the other hand, regards malleine as absolutely non-specific in its action.

The question whether or no malleine has a *specific* toxic action is as little decided as in the case of tuberculine. It is possible that here, too, we have non-specific symptoms similar to those invariably produced by proteins foreign to the body.

ANTHRAX POISON.

We are quite ignorant whether or no there exists a true anthrax toxine. As in the generality of cases, the answer to this question has been rendered extraordinarily difficult by the fact that many investigators made their experiments with the sole object of *producing immunity* against the anthrax bacillus, by any possible means, and that consequently they made use of living bacilli or the dead cells without troubling themselves about the possible existence of a specific antitoxine-producing poison. At the earliest period it was hardly to be expected that the problem could be stated in such precise terms, but even many later investigators have given almost exclusive attention to the *practical* question of immunisation. They have endeavoured, as, for instance, has been done by SOBERNHEIM in his researches into anthrax immunisation, to obtain in every possible way the desired immunity by inoculation, without investigating whether this protection was due to a true *antitoxic* immunity. We meet with this difficulty in all other investigations into less known poisons; yet nowhere has the toxine problem remained in such obscurity or the results been so contradictory as here. I must, therefore, content myself with mentioning the most important researches that have dealt more or less expressly with the hypothetical toxine, and

[1] Guinard, "Effets physiolog. du Malleïne," *Journ. Med. Vet.*, xlvi., 454; *Baumgartens Jb.*, 1895, 311.

[2] Schattenfroh, "Ueber die Wirkung von Bakterien-Proteïnen," *Zeit. f. Hyg.*, xviii., 456, 1894.

for this purpose the unusually careful bibliography compiled by CONRADI [1] has naturally been of the greatest service to me.

The first attempts to isolate poisons from anthrax bacilli by filtration were made by PASTEUR,[2] but with negative results. The filtrates were absolutely inert, while those obtained by W. KOCH [3] produced only elevation of temperature and dyspnœa.

The first published account about a substance obtained from anthrax cultures, and stated to have an immunising action, was that of WOOLDRIDGE.[4] He cultivated anthrax bacilli for two to three days at 37° C. upon a bouillon of thymus or scrotum extract. The liquid thus obtained had an immunising non-toxic effect after filtration. He also made very similar experiments [5] with a solution of his tissue fibrinogen prepared from scrota, &c., and sterilised by means of heat, care being taken that it had only a slightly alkaline reaction; for vigorous growths of the micro-organisms in strongly alkaline media yielded no poison or immunising substance to the solution. But the filtrates of cultures grown on weakly alkaline solutions of tissue fibrinogen undoubtedly contained an immunising non-toxic substance, which *only* afforded protection on intravenous injection. Yet he asserts that he obtained almost analogous results by simple injection of his solution into animals without previous cultivation of anthrax bacilli, and claims that the tissue fibrinogen by itself affords protection against anthrax. This completely destroys the value of his work as regards the discovery of a possible toxine.

Nor does any greater importance attach to the theoretical proof of CHAUVEAU [6] that anthrax poison must exist in the soluble form, since the placenta, being impermeable to bacteria, allows none of the bacilli to pass, and yet the embryo of a sheep infected with anthrax is immune; rather its importance is less, since in rare instances anthrax bacilli can pass through the placenta.

HANKIN attempted in a series of investigations [7] to prove that his anthrax albumoses were the chief toxic principle. He cultivated the bacilli on a bouillon containing 0·1 per cent. of meat extract, and a large proportion of fibrin (10 to 50 per cent.).

[1] Conradi, "Z. Frage d. Toxinbild. bei d. Milzbrandbakt.," *Zeit. f. Hyg.*, xxxvii., 287, 1899.

[2] Pasteur and Joubert, "Étude sur la maladie charbonneuse," *Comptes Rend.*, lxxxiv., 900, 1877.

[3] W. Koch, "Milzbrand und Rauschbrand," Stuttgart, 1886, quoted by Conradi (*loc. cit.*).

[4] Wooldridge, "Note on the Protection in Anthrax," *Proc. Roy. Soc.*, xlii., 312, 1887.

[5] Wooldridge, "Vers. ü. Schutzimpf. auf chemischem Wege," *Du Bois Arch.*, 1888, 527.

[6] Chauveau, "Sur le mecanisme de l'immun.," *Ann. Past.*, iii., 66, 1888.

[7] Hankin, "On Immunity produced by an Albumose isolated from Anthrax Cultures," *Brit. Med. J.*, 1889, ii., 65; *id.*, "On the Conflict between the Organism and the Microbe," *ibid.*, 1890, ii., 810; Hankin and Wesbrook, "Albumoses et toxalbumines du bacille charbonneux," *Ann. Past.*, vi., 633, 1892.

The sterilised liquid was inoculated and allowed to stand for eight days, after which it was filtered, and the filtrate treated with ammonium sulphate. The precipitate was dialysed at 42° C., and the liquid evaporated *in vacuo*, or treated with alcohol. This yielded an *albumose*, which weakened bacteria did not give. Very small doses (one part of a 1 : 1 million solution per kilo. of body weight in the case of mice) immediately produced immunity against anthrax, but this was not the case with large doses, which had only a toxic, but not immunising action.

Similar *toxic* albumoses isolated almost at the same time from anthrax cultures and the organs of animals by MARTIN [1] and BRIEGER and FRÄNKEL,[2] caused symptoms somewhat resembling those produced by the living bacilli. Yet the definite results of all these investigations of toxalbumins [3] were very insignificant, as was eventually the case with all the toxalbumins. Martin's albumose, for example, had to be given in doses of 0·3 grm. per mouse (!) to produce death.

According to the views now accepted, there can certainly be no toxine activity here. The anthrax bacilli have naturally decomposed the proteids of the culture medium, and either traces of poison have been retained by the resulting albumoses, or the proteids foreign to the body that have been produced have in themselves a slight toxic action, as is also the case with other proteids.

In any case, PETERMANN,[4] who repeated HANKIN'S work, was unable to detect *any* trace of toxic or immunising activity in his albumose, though HANKIN and WESBROOK (*loc. cit.*), observing under more accurately specified conditions, and, in particular, avoiding temperatures above 20° C., still maintained their position, and also again obtained frequently, though not invariably, transient immunisation.

An important point in PETERMANN'S investigation is the proof that anthrax cultures when simply filtered have a slight and transient, but unmistakable, immunising effect; this would seem to point to the presence of a real toxine, which, however, can only be present in very slight quantity.

[1] Martin, "The Chemical Products of the Growth of the Bacillus Anthracis," *Proc. Roy. Soc.*, xxii. [5], 1890.

[2] Brieger and Fränkel, "Untersuch. über Bakteriengifte," *Berl. klin. Woch.*, 1890 [11-12].

[3] Similar investigations with equally scanty results were also made about the same time by, *e.g.*, Balp and Carbone (1891), Landi (1891), G. Klemperer (1891), Maltzew (1891); for particulars about these the reader must be referred to Conradi (*loc. cit.*).

[4] Petermann, "Recherches sur l'immun. contre le charbon au moyen des albumoses extraites des cultures," *Ann. Past.*, vi., 32, 1892.

Quite in agreement with this are the results obtained by ARLOING.[1] He merely allowed bouillon cultures of anthrax to stand, and then decanted the liquid at frequent intervals from the deposited bacteria, thus obtaining a germ-free fluid, in which obviously there was no possibility of the very considerable loss of poison retained by the filters usually employed. On the other hand, the toxine might very easily undergo decomposition during the long standing. The fluid thus obtained had an immunising effect when given in large doses.

There are also the results obtained by SCLAVO[2] and MARCHOUX,[3] who produced immunity by means of the living bacilli; but these have apparently nothing to do with the question of a toxine. At all events, MARCHOUX asserts that his serum acts by means of phagocytosis.

The next serious attempts to discover the *toxine* were those made by MARMIER,[4] who, from cultivations on peptone-glycerin (1 litre of water, 40 grms. of peptone, 15 grms. NaCl, 40 grms. of glycerin, 0·5 grm. of sodium bisulphate, and 0·2 grm. of potassium bisulphate), preferably grown at 20° C., obtained a substance, which, when separated by precipitation with ammonium sulphate and dialysis, or by extraction with glycerin and precipitation with alcohol, formed a pulverulent brown mass giving neither proteid nor alkaloidal reactions, and showing no enzymic activity.

It was toxic to the extent of 0·08 grm. per kilo. of animal (though *not* constantly so), and caused elevation of temperature, diarrhœa, emaciation, convulsions, slowed respiration, and suffocation. A dose of 0·2 grm. per kilo. was invariably fatal. According to MARMIER the symptoms here were sufficiently similar to those caused by infection with living bacteria as to justify the conclusion that they were due to a *specific* anthrax toxine.

There are two reasons, however, against the view that there was a true toxine here, apart from the fact that the lethal dose was surely a little too high for a true toxine.

In the first place, MARMIER stated that the poison could be boiled for an hour without alteration, and could even resist being heated for five minutes at 120° C., and hence it could not have

[1] Arloing, "Sur la présence de la substance phylacogène dans les liquides du bac. anthr.," *Bull. Med.*, 1892, 1038, quoted from *Centralbl. für Bakt.*, xiii., 561, 1892.

[2] Sclavo, "Ueber die Bereitung des Serums gegen den Milzbrand," *Centralbl. f. Bakt.*, xviii., 744, 1895.

[3] Marchoux, "Sérum anticharbonneux," *Ann. Past.*, ix., 9, 1895.

[4] Marmier, "Sur la toxine charbonneuse," *Ann. Past.*, ix., 533, 1895.

been a true toxine. The poison was weakened by gold chloride, platinum chloride, and calcium chloride.

And, in the second place, it apparently produced only a slight immunity, and that, too, not invariably. Hence, in any case, it could not have been a weakened toxine, since then the reduction in toxic power must have been accompanied by the more energetic immunising effect.

CONRADI (*loc. cit.*) has recently once more investigated the question whether anthrax bacilli form a soluble diffusible poison, and has, if we may anticipate, arrived at an absolutely negative result. He started from the point of view that it was the multiplicity of the nutrient substrata used by previous investigators that was frequently responsible for the diametrically opposite results. Hence he decided to search for the anthrax poison in the *body of the animal.* For this purpose he used peritoneal exudations and extracts of organs (liver and spleen) of infected animals, and found both, after filtration through a Kitasato or Chamberland filter, to be invariably devoid of any toxic effect.

Moreover, he introduced virulent bouillon cultures into the abdominal cavity of guinea-pigs, which was then enclosed in a bacteria-proof bag made with the inner membranes of the reed (*Phragmites communis*), and in this case, too, there was no toxic action.

Two objections can be offered to CONRADI'S results.

The reed experiments only prove that there does not exist any soluble and *diffusible* anthrax poison, but do not exclude the possibility of there being a non-diffusible toxine, just as other haptines—*e.g.*, certain ferments—are also indiffusible. And, indeed, the results obtained by ARLOING (*vide supra*), who found a poison in unfiltered cultures, whereas the majority of investigators found *nothing* in filtered cultivations, render it not improbable that anthrax toxine may be readily retained by filters and membranes.

The second objection appears to me more important. Even granting the existence of a *true* haptophore toxine of anthrax, it is possible that under certain conditions it may not be found again in the organs and fluids of the infected bodies of *susceptible* animals, owing to its entering into stable combination with the receptors; the poisons of tetanus and diphtheria can, even under normal conditions, only be found again in the blood and organs of infected animals after the introduction of large doses.

It is true that living micro-organisms were present in these exudations, but, at the same time, these could not have been able to have used their toxine-producing powers for any length of

time; and, moreover, the toxines produced in these exudations would surely have undergone renewed metabolism, and have been fixed by the receptors, so that only very slight traces could have been left behind. In addition to this the exudations in this case would doubtless contain *antitoxines*, which would render a correct deduction still more difficult. At the same time, it is possible that such exudations may be used as suitable natural culture-media, in which, eventually, toxines may be detected.

Be this as it may, it is impossible to draw any conclusion from these experiments by CONRADI as to the non-existence of a *true* toxine. Speaking generally, such experiments are not suitable for determining the question of the production of poison by a bacterium; for other poisons of simpler nature may also be so altered or destroyed after producing injurious effects, or even by their physiological action, that no trace that can be detected remains in the fluids of the tissues. Even many alkaloids, such as *cytisine*, disappear in the organism without leaving a trace. It is not safe, in view of the manifold attacks to which a poison is exposed in the body, to draw *negative* conclusions as to the non-existence of this poison; only positive results would be conclusive here. Thus CONRADI's experiments only contribute negative evidence towards a decision on this point; once more a means of discovering the hypothetical poison has proved unworkable; the question of the existence of the poison still remains open. And, in this connection, it must not be lost sight of that the existence of an anthrax poison is really an etiological postulate; for there are many cases of anthrax with fatal results where general bacterial infection is out of the question, and where it has been very difficult or impossible to detect living bacilli in the organism. Surely such cases can only be explained as cases of *poisoning*. Hence, notwithstanding Conradi's results, further search must be made for the poison.

On the other hand, no objection can be offered to the experiments made by CONRADI to determine whether the anthrax bacillus produced a specific *endotoxine*, which is only liberated after the death and decomposition of the cell in an analogous manner to yeast, invertase, and zymase. He killed the bacilli by means of *toluene*, or by *freezing*, or disintegrated them by Buchner's method. In every instance the result was absolutely negative. Nor could he isolate *toxalbumins* from the organs, either by means of the method of Brieger and Fränkel or by that of Marmier.

Hence, the present state of affairs is as follows:—

Convincing proof of the existence of a *true* anthrax toxine,

either in a state of free secretion or as an *endotoxine* retained by the cells, has not yet been adduced, and the existence of an endotoxine is practically disproved. With the exception of substances causing slight pyrogenic effects due to the bacterial proteins of the cell itself and similar to those produced by all bacteria, whether pathogenic or harmless, no anthrax poisons are known. At the same time we must assume that poisons that cause the symptoms of the disease are formed; yet these appear to be of such a subtle character and to be produced so sparingly during the growth of the bacilli in the attacked organism as to escape detection. CONRADI made no experiments with regard to the immunising power of his sterile filtrates; and it is conceivable that they contained *toxoids* which had immunising capacity but were no longer toxic, and that this is a possible explanation of HANKIN'S results. These obvious possibilities still remain unsettled.

OTHER SOLUBLE BACTERIAL POISONS POSSIBLY OF THE NATURE OF TOXINES.

When we consider that the investigation of the specific poisons of some of the most important pathogenic micro-organisms is for the most part still in its infancy, it is not surprising that, in the case of less important bacteria, we have very scanty results, to which I will only briefly refer here. It is quite possible that on closer investigation one or more *true* toxines may still be discovered as a product of these bacteria, but as yet there are no certain indications of this.

As regards the majority of those *infectious* bacteria that grow within the organism, we can come to the same conclusion as in the case of cholera, streptococci, &c.—viz., that if the cells secrete any true toxines they do so only in minute quantities, that these are extremely unstable, and that the poisons must be regarded as consisting in the main of firmly retained endotoxines. Immunity, as in the case of all infectious micro-organisms, is brought about almost exclusively by bacteriolytic processes, any eventual formation of antitoxine being insignificant and occupying an altogether inferior position, both from the practical and theoretical point of view.

HOG CHOLERA.

METSCHNIKOFF[1] and also SELANDER[2] found the sterilised

[1] Metschnikoff, "Zur Immunitätslehre," *Congr. f. inn. Med.*, 1892, 282; "Études sur l'immunité," *Ann. Past.*, v.; vi., 289, 1892.

[2] Selander, "La maladie infectieuse des porcs.," *Ann. Past.*, iv., 545, 1890.

blood of diseased animals to be very poisonous. It is extraordinarily easy to produce immunity against the bacteria, but this does not increase the power of resisting the sterile poison. The micrococci *grow* in immunised serum, but with reduction of their virulence, which is restored, however, by inoculating fresh bouillon. Hence, the serum would seem to contain *antitoxine*, which neutralises the production of toxine by the growing bacteria.

MALIGNANT ŒDEMA.

Roux and Chamberland [1] found that cultures of the septic vibrio freed from the micro-organisms were slightly poisonous, while sterilised peritoneal fluid was very poisonous and had an immunising power. But they also obtained "poisons" which still had a very weak toxic action and some immunising power after exposure to a temperature of 110° to 120° C. Besson,[2] too, has prepared weak poisons (5 to 10 c.c. being required to kill a guinea-pig) by filtering cultivations and in particular those grown on flesh.

SWINE PLAGUE.

Silberschmidt[3] discovered a weak poison with a specific action in filtered cultivations. It was not completely destroyed at 120° C., but was gradually weakened even at 60° C. The filtrates produced immunity.

Selberg[4] found poisonous substances with a slight specific action attached to the cells of the bacilli of swine plague.

METSCHNIKOFF'S VIBRIO.

A very interesting report on this vibrio was published by Sanarelli.[5] It grows vigorously on immunised serum after first apparently dying, but is then almost completely innocuous, though it again becomes toxic when grown in fresh bouillon. In like manner the vibriones from an immunised animal can be

[1] Roux and Chamberland, "Immunité contre la septicémie," *Ann. Past.*, ii., 561, 1887.

[2] Besson, "Contrib. à l'étude du vibrion septique," *Ann. Past.*, ix., 179, 1895.

[3] Silberschmidt, "Contrib. à l'étude de la swine plague," *Ann. Past.*, ix., 65, 1895.

[4] Selberg, "Beitr. z. Kenntn. d. Giftwirkung d. Schweineseuchenbakterien, &c.," *Diss.*, Berlin, 1896.

[5] Sanarelli, "Defense de l'organisme contre les microbes," *Ann. Past.*, vi., 225, 1892.

cultivated. If the serum of an infected animal be separated from the vibriones that it contains the latter are far more virulent than when the serum is also present.

From these results it appears that the vibrio is capable of increasing in the body of the animal, and that it produces toxines there which are injurious. But in an immune animal or in immunised serum, and also in the serum of the diseased animal, there are antitoxines which combine with the poison and render the vibrio harmless, although it continues to grow in the immune animal. If the antitoxine be removed the poison again becomes active, as is also the case when the vibrio is cultivated upon new culture media.

Unfortunately, SANARELLI did not attempt to isolate, by means of *filtration*, the toxine that was probably present. He only found cultures killed at 60° C. and 120° C. to be poisonous, and naturally was unable to produce any antitoxic immunity against the secondary poison which he obtained at 120° C. The latter is certainly not a toxine, although it would seem that a true toxine is produced by these bacteria and may possibly be demonstrated by careful work.

DYSENTERY TOXINE.

A toxine supposed to be that of dysentery was found by ROSENTHAL[1] in the filtrates of cultures three weeks old. The filtrates, in doses of 0·1 to 0·2 c.c., had a fatal effect upon rabbits. The poison is stated to be fairly resistant to heat and to form an antitoxine.

[1] Rosenthal, "Das Dysenterietoxin," *Deutsch. med. Woch.*, 1904, No. 167.

III.—THE VEGETABLE TOXINES (PHYTOTOXINES).

RICINE.

The seeds of the castor-oil plant, *Ricinus communis*, produce an extremely virulent poison, which is in every way closely allied to the bacterial toxines. It is present in the embryo endosperm but not in the husk (Werner). Poisons with completely analogous action are also present, according to Stillmark,[1] in ten other kinds of *Ricinus*—viz., *R. sanguineus*, *africanus*, *guyanensis nanus*, *altissimus*, *communis major*, *philippinensis*, *brasiliensis*, *borboniensis arboreus*, *spectabilis*, and *jamaicensis*. The seeds of *Jatropha curcas* (*Curcas purgans*), a West Indian Euphorbiaceous plant, popularly termed the "purging nut" or "Barbadoes seed," also yield a toxic product which is possibly identical with ricine (Stillmark).

Ricine was first investigated by Dixon.[2] He prepared a poisonous product either by extracting the seeds with hydrochloric acid and precipitation with a solution of soda, or by precipitating the substance from an aqueous extract by means of alcohol. It was obtained in a purer state by treating the aqueous extract with lead acetate and ammonia, suspending the precipitate in water and removing the lead by means of hydrogen sulphide, and then precipitating the toxine with alcohol. In addition to the toxine a non-poisonous glucoside was also detected in the seeds.

Ricine was more thoroughly studied by Stillmark,[1] working under the direction of Rudolf Kobert. He extracted the seed with a 10 per cent. solution of ordinary salt, treated the extract with sodium or magnesium sulphate, and removed the salts by means of dialysis. The yield amounted to 2·8 per cent., calculated on the decorticated air-dried seeds.

Cruz[3] washed the crushed seeds with chloroform and alcohol,

[1] Stillmark, "Ueber Ricin," *Arb. pharm. Inst. Dorpat*, iii., 59, 1889 (contains a complete bibliography of the earlier literature on the castor-oil plant).

[2] Dixon, *Australian Med. Gaz.*, 1887, 156, quoted by Cushny (*vide infra*).

[3] Cruz, "La ricine," *Ann. d'Hyg. Publ.*, xl., 344, 1898.

dried them, extracted them with water, and treated the extract with alcohol.

Great theoretical interest attaches to *ricine* owing to the fact that EHRLICH [1] has made it the starting point of his fundamental investigations of antitoxic immunity. He, too, separated it from the seeds by extraction with a 10 per cent. solution of sodium chloride, and purified it in exactly the same way as STILLMARK. Merck's preparation is precipitated from the sodium chloride solution by means of ammonium sulphate.

Chemical Nature of Ricine.—While the older investigators regarded ricine as a proteid, the progress of discovery appears to follow the same course here as was described in the case of the bacterial toxines, and, with the increase in the accuracy of the investigations, the belief continues to gain ground that this toxine, although a body of high molecular weight, is not a proteid in the narrower sense of the term.

STILLMARK regarded it as a *globulin*. CUSHNY [2] made many tedious experiments to prove that ricine was really a proteid, or to free it from proteid impurities, but did not obtain any definite results. It is quite impossible to effect a separation of the active principle from the proteids by the usual methods, either because the coefficients of precipitation and re-solution are the same, or, more probably, because *ricine*, like so many colloidal substances, is mechanically carried down by many precipitates as they fall, and especially by coagulated albuminous substances, &c.

Eventually the resistance offered by ricine to *trypsin* afforded a means of preparing a substance which, in all probability at least, was not of a proteid nature. This property, which was denied by STILLMARK, but demonstrated by CUSHNY and MÜLLER,[3] formed the starting point for JACOBY'S [4] striking experiments.

He first showed that, even after being digested for a week with *trypsin*, the toxic power of ricine remained unaltered, the same result being also obtained with *papaïn*. But he did not, of course, draw any definite conclusion from this alone, since it was possible that the proteids might also be able to resist the action

[1] Ehrlich, "Exper. Unters. über Immunität," *Deutsch. med. Woch.*, 1891, 976, 1218; "Zur Kenntnis d. Antitoxinwirkg.," *Fortschr. d. Med.*, 1897, 41.

[2] Cushny, "Ueb. das Ricinusgift," *Arch. exper. Path.*, xli., 439, 1898.

[3] Müller, "Beiträge z. Toxikol. des Ricins," *Arch. exper. Path.*, xlii., 302, 1899.

[4] Jacoby, "Ueb. d. chem. Natur des Ricins," *Arch. exper. Path.*, xlvi., 28 (reprint).

of trypsin. Accordingly he modified his experiment in a very ingenious fashion.

Ricine is precipitated by ammonium sulphate on saturating the solution to the extent of 60 per cent., whereas the active principle of trypsin is not separated until the solution has been completely saturated with ammonium sulphate. JACOBY, therefore, separated from an extract of pancreas by means of fractional precipitation with ammonium sulphate the proteids that were precipitated at a saturation of 60 per cent., and used this purified trypsin preparation for the digestion of ricine that had been separated by saturating its solution to the extent of less than 60 per cent.

Hence this mixture, before the digestion, would have the following composition :—

Precipitated at	60	per cent.,	. Ricine, and proteids combined with it.
,,	100	,,	. Trypsin.

After the action of the digestive enzyme, however, the composition would be different; the proteid impurities in the ricine would have been digested by the trypsin, and would only be precipitated with *more difficulty* by the trypsin, and possibly not at all; and the composition of the mixture would then be :—

Precipitated at	60	per cent.,	. Ricine.
,,	100	,,	. Trypsin and part of the digested products.

Under these conditions, then, the precipitation at a saturation of 60 per cent. must yield a *pure* ricine.

As a matter of fact, JACOBY obtained only a very slight precipitate by saturating the mixture to the extent of 60 per cent. after a five weeks' digestion, and he purified this substance by re-precipitation.

The proteid reactions were no longer obtained, while the toxic properties were quantitatively retained. Here, then, we have proof that pure ricine is not a proteid. And this result also disposes of one of the remaining "toxalbumins," and hence this term may well be regarded as now only possessing historical value. It has played an important part in the investigations of this subject, especially since it led to the proof that these poisons possessed quite different properties to the crystalloid poisons; but surely the time has arrived for us to discard it, since now it can only create confusion. In its place let us use the general

term "toxine," in the precise connotation that we have described, which covers all the properties of this characteristic class of bodies.

Properties of Ricine.—Ricine, like the bacterial poisons, presents many close analogies with the *enzymes*, so much so that it was classified with them by STILLMARK, although it does not bring about any true fermentative processes, such as diastatic action, &c. Among these analogies is its property, mentioned above, of being carried down by all kinds of precipitates; and this mechanical precipitation takes place with special readiness in the case of subsiding proteid precipitates. Its precipitation by *nucleic acid* must also be attributed to a mechanical process of carrying down, and not to a characteristic reaction of the toxine itself, such as TICHOMIROFF[1] claimed to have observed both in ricine and in other toxines. It also resembles the enzymes in its sensitiveness to physical and chemical influences.

The temperature of boiling water is extremely injurious to it, although, according to JACOBY, it does not entirely destroy its toxic property, and the purified poison offers greater resistance than that containing proteid impurities. JACOBY considers it not impossible that, on boiling, a different poison is produced, possibly a *toxoid*. The toxine is not destroyed by dry heat, 110° C. (STILLMARK), and is almost unaffected by pepsin-hydrochloric acid (MÜLLER).

We have already described its behaviour towards *trypsin*, and it is particularly interesting to note that JACOBY found that his pure *ricine was rapidly destroyed by trypsin*, but that this did not take place when fresh quantities of trypsin were added to digested mixtures containing the unpurified ricine. Hence, it would seem, the decomposition products of the proteids exert a protective influence.

Hydrogen peroxide has an energetic action upon pure ricine, but is only slightly injurious to the impure substance.

Alcohol does not dissolve ricine and does not injure it.

Ricine appears to be absolutely indiffusible, as was first shown by STILLMARK.

Action of Ricine.—Ricine has two of the most important properties of toxines—extraordinarily great activity and a period of incubation; but, on the other hand, does not show their strictly specific action. Hitherto no animal has been found that is completely immune against ricine, but the susceptibility is not

[1] Tichomiroff, "Ueber die Fällg. v. Toxalbuminen durch Nucleïnsaure," *Zeit. f. physiol. Chem.*, xxi., 90, 1895.

the same with all animals, though the differences are no greater than can be observed in the case of the crystalloid poisons.

Its toxic action is enormous. According to EHRLICH it is fatal when introduced subcutaneously in the proportion of 0·03 mgrm. per kilo., while 0·18 grm. is a lethal dose *per os* for a full-grown man. Subcutaneous injection of 1 grm. would kill $1\frac{1}{2}$ millions of guinea-pigs, though the minimum lethal dose shows some variation. Mice are less susceptible; rabbits somewhat more so. In measuring the toxic power EHRLICH invariably uses an injection of 1 c.c. for each 20 grms. of body substance; on that basis a dilution of 1 : 200,000 would be undoubtedly fatal to mice.

In like manner CUSHNY found the lethal dose for *rabbits* to be 0·04 mgrm. per kilo., while JACOBY found that 0·5 mgrm. of Merck's ricine per kilo. of body weight was required to kill rabbits.

According to OSBORNE and MANDEL[1] the lethal dose per kilo. for rabbits of their purified ricine was 0·002 mgrm.

It also shares with toxines the property of acting much more weakly by way of the digestive tract. Yet its action is always plainly perceptible even when introduced in this way, though doses a hundred times as great are required (STILLMARK, p 185). This property depends on the much more considerable resistance offered by ricine than by the bacterial toxines to the digestive enzymes.

The effects produced by ricine can be classified in four groups —viz., the *local action* at the point of application, the *general effects*, the action on the *conjunctiva*, and the action on the *blood-corpuscles*.

Ricine, like many bacterial toxines, frequently produces severe indurations, inflammation, abscesses and necroses at the point of inoculation. Whether these pathological changes are due to the ricine itself or to impurities has not yet been definitely determined. There is, however, some support for the view that these symptoms are possibly only produced, as is frequently observed in other cases, through the simultaneous introduction of proteids foreign to the body.

The general changes that take place in ricine poisoning had already been studied by the older investigators, and subsequently by FLEXNER[2] and FRANZ MÜLLER (*loc. cit.*) in particular.

The first symptom is an increase in the temperature and a

[1] Osborne and Mandel, "Ricine," *Amer. Journ. Physiol.*, x., 36, 1903.

[2] Flexner, "The Pathology of Toxalbumin Intoxication," *Johns Hopkins Hospital Record*, 1897 (reprint).

rapid decrease in the weight of the body, while, with these exceptions, no abnormal phenomena are noticed during the first twenty-four hours.

The decrease in the weight of the body is much greater than can be accounted for merely by a condition of hunger. Rabbits in a state of hunger lose only about $\frac{1}{14}$ of their total weight in twenty-four hours, $\frac{1}{8}$ after forty-eight hours, and $\frac{1}{7}$ after seventy-two hours, whereas rabbits poisoned by ricine lose $\frac{1}{7}$ to $\frac{1}{8}$ of their weight even after twenty-four hours. Nor can this decrease be explained solely by increased decomposition of proteids.

According to MÜLLER the conditions are similar to those observed in animals suffering from fever. The stools contain blood and albumen, and frequently blood passes into the urine.

After twenty-four to thirty hours the fatal symptoms of poisoning suddenly appear. They begin with clonic convulsions and weakening of the reflexes. Then follows paralysis. After fifteen minutes the convulsions are repeated, and dyspnœa and feeble inspiration precede death, which results within about half an hour after the first attack.

Sometimes the convulsions do not occur. An increase in the dose does not alter the symptoms, but shortens the period of incubation.

At the last there are serious central disturbances of the medulla oblongata: paralysis of the vasomotors and eventually of the respiration. The pressure of the blood does not fall until quite late in the attack; and death is then near. Ricine has no effect upon the heart.

Post-mortem dissection shows very characteristic results. Swelling and reddening of the subcutaneous lymph glands, and great stasis in the region of the blood-vessels in the abdomen; great enlargement of and red flecks in the mesentery lymph glands and of Peyer's patches, and numerous ecchymoses in the intestine, but no ulcerations. The spleen is much swollen and soft. Histologically there are characteristic changes in the blood, and notably severe leucocytosis, decomposition of cells in the spinal marrow, and necrotic foci in numerous organs, notably the liver. Thromboses cannot be observed. As a rule, the muscle of the heart shows fatty degeneration.

CRUZ (*loc. cit.*) observed, in particular, serious alterations in the kidneys, but STEPANOFF was unable to detect the toxine in the urine. Hæmorrhage of the suprarenal bodies is also a characteristic symptom.

Death from ricine is thus primarily due to central paralyses, but in addition to this the local irritant effects of the poison are

shown by the necroses of the cells. The intestinal ecchymoses, &c., can also be accounted for in the same way, since STEPANOFF[1] has shown that ricine is excreted into the intestine, and CUSHNY (*loc. cit.*) has found that in frogs ricine causes a discharge of blood containing the poison into the stomach.

Ricine has no action upon the isolated heart of the frog or upon the nerves, while it has a slight paralysing effect upon the muscle (STILLMARK).

The action of ricine upon the *connective tissue* of the eye is characteristic. It produces severe conjunctivitis similar to that caused by *abrine* (*vide infra*), which also frequently leads to permanent derangement of the cornea. Panophthalmitis is also not uncommon.

The Action of Ricine upon the Blood.—Ricine has a very characteristic action upon the red blood-corpuscles as was first noticed by KOBERT and STILLMARK (*loc. cit.*); and this can be observed both in fresh blood and in the case of erythrocytes that have been repeatedly washed. The erythrocytes agglutinate under the influence of ricine, and fall to the bottom as a coherent flocculent mass resembling clotted blood, so that the supernatant serum is clear.

According to KOBERT,[2] a combination takes place between the agglutinine and the arterine of the blood, and the clumping together is due to the fact that this compound is viscous. The action is very intense, and can be recognised even in a dilution of 1:600,000. This agglutination has absolutely nothing to do with the hæmoglobin, since it also occurs in the case of the dissolved blood-corpuscles—*i.e.*, with the *stroma* by itself. The serum appears to contain substances that have a restrictive influence upon the action of the ricine; at least it acts more energetically in diluted blood.

This ricine coagulation is quite distinct from the true coagulation of the blood. Any possibility of its being a simple coagulation is indeed excluded by the fact that it can be observed in defibrinated blood; but apart from this, the process, as such, can be distinguished from coagulation by the fact that it is not influenced by sodium chloride, potassium nitrite, and potassium chlorate. Fibrin coagulation is even retarded by ricine. Moreover, the so-called ricine fibrin—*i.e.*, the colourless washed ricine precipitate—is absolutely different from true fibrin.

[1] Stepanoff, "Études sur la ricine et l'antiricine," *Ann. Past.*, x., 663, 1896.

[2] Kobert, "Ueber vegetabilische Blutagglutinine," *Sitzungsber. d. naturf. Ges.*, Rostock, 1900, xxv. [5] (reprint).

With large doses of ricine the agglutination is followed by diffusion of the colouring matter of the blood, so that ricine has thus also a *hæmolytic action*. According to JACOBY this hæmolysis is only an intensified form of agglutination and not a separate process.

The agglutinating action is not restricted to the blood. LAU found that *ricine* also caused the agglutination of pus cells and the cells of organs, that it coagulated milk, and produced coagulations in solutions of white of egg and of plasmon, but did not affect solutions of myosin and human serum; while STILLMARK obtained precipitates by means of ricine in the sera of the dog, cat, ox, and hen.

The blood-corpuscles of different species of animals vary as regards their susceptibility, just as they do in the case of bacterial lysines.

The blood of *highly immune animals*—*e.g.*, goats—is yet completely susceptible to ricine poisoning, so that immunity through lack of receptors, comparable with natural immunity, does not occur.

LAU[1] concluded that the blood-corpuscles of fish were absolutely proof against ricine, but FRANKEL[2] showed that this was only relative and that the introduction of larger doses of ricine caused agglutination of the blood of even the barbel (*Barbus fluviatilis*). This resistance is due to the presence of a normal anti-body in fish serum. Ricine antitoxine from goats' blood also possesses this protective power. Yet, on the other hand, normal barbel serum has no antitoxic effect upon the action of ricine in cats' blood.

Great interest attaches to the question of how far this action upon the blood *in vitro* has a bearing upon the occurrence of *general ricine poisoning*. The older investigators, notably STILLMARK, were inclined to believe that the hæmorrhage and necroses could be explained by a similar process of coagulation within the lumen of the vessels. Yet, on the one hand, thromboses have never been found; and, on the other hand, as was shown above, these symptoms must be regarded as irritant effects of the poison in *loco*. Nor is any stoppage of the brain arteries suggested by the central symptoms.

But, above all, this theory that the action of ricine upon the blood might be made responsible as the cause of death is disproved by the fact that MÜLLER was able to show that this

[1] Lau, "Ueber vegetabil. Blutagglutinine," *Dissert.*, Rostock, 1901.

[2] Fränkel, "Ueb. d. Wirkg. d. Ricins auf Fischblut," *Hofm. Beitr.*, iv., 224, 1903.

agglutination never occurred at all in the living blood. He also discovered that pepsin-hydrochloric acid checked this action upon the blood without affecting the toxic power—a result which, it is true, was denied by LAU, but was conclusively confirmed by JACOBY (*vide infra*).

This question is particularly important because it should furnish an argument for deciding whether ricine is an individual substance, or whether its action upon the blood ought to be regarded as quite distinct from its toxic power, in the sense that there are two separate constituents of the castor seed, exerting their activity in a different way—a point of view put forward, *e.g.*, by CUSHNY and MÜLLER.

It is, however, extremely probable that the question in this form is not sufficiently precise. It is quite in accord with Ehrlich's theory that ricine should be a receptor of the second class of a somewhat complex constitution, possessing only *one* haptophore group but *two* ergophore groups—viz., a toxophore group which causes the poisoning, and another group producing the agglutination, and for which the term "agglutinophore" group has been suggested.

This theory can be readily supplemented by the further hypothesis that these two ergophore groups differ in their degree of stability, a difference of susceptibility to digestion with pepsin-hydrochloric acid being in fact capable of detection, so that even to explain this phenomenon it is unnecessary to assume the existence of two substances.

On the other hand, however, it is not as yet possible to demonstrate more fully the manifold nature of ricine poisons. As FRÄNKEL has shown, the antitoxine of normal barbel serum is absolutely devoid of protective power against the action of ricine upon cats' blood on the one hand, and, on the other hand, also against its *toxic* action upon rabbits and even upon the *barbel itself*, so that here there is evidence in support of the view that there is a distinct fish blood agglutinine and fish poison.

We cannot discuss this question more fully until we are able to consider the action of ricine in the light of the side-chain theory. As in the case of all true toxines there are four points that justify a poison being included among the haptines.

We have already touched upon the small lethal dose, the incubation period, and the variable toxicological behaviour. But the most important point of all is the *formation of anti-toxine* in the organism, the production of active and passive acquired *immunity*.

In this respect, also, the behaviour of ricine exactly corresponds with that of the bacterial toxines. It was, in fact, experiments

with *ricine* that led EHRLICH to his epoch-making researches upon antitoxic immunity.

Immunity against Ricine.—EHRLICH[1] succeeded in rendering white mice and rabbits immune against ricine by first introducing small doses *per os* (and, in the case of rabbits, also by way of the conjunctival sac), and then, after attaining a certain degree of immunity, he was able by cautiously increasing the doses in subcutaneous injection to reach a fairly high state of immunity, the process taking about four months. Mice previously treated in this way were capable, after eight weeks, of resisting the lethal dose for a man. While a dose of 1 c.c. in a dilution of 1 : 200,000 per 20 grms. of body weight was absolutely fatal to the animals used in control experiments, Ehrlich was able to give doses of 1 c.c. of solutions of 1 : 500, or even 1 : 250, to the immunised animals, so that from 400 to 800 times the original immunity was obtained. It was no longer possible to produce panophthalmitis in immune animals even by the introduction of large doses, although there was still a frequent occurrence of the local necroses.

Now, the serum of these immunised animals contains an *antiricine*, which, just like the bacterial antitoxines, is capable of combining with the ricine *in vitro* in such wise that both its toxic and agglutinating action are prevented in accordance with definite numerical laws. A special point of importance in this connection is the fact, also established by EHRLICH, that the same quantity of serum can influence *both* actions in the same way. A further proof that there is here a simple *combination* between the toxine and antitoxine has been brought by DANYSZ,[2] who found that on treating a neutral mixture of ricine and antiricine with proteolytic enzymes the antitoxine could be destroyed, so that the toxic power again appeared; animals that had been given an exactly neutral mixture *per os* died of typical ricine poisoning.

EHRLICH's fundamental experiments have since been confirmed and extended by M. JACOBY[3] in a very exact and theoretically far-reaching investigation.

JACOBY agrees with EHRLICH's conclusion that there is a true quantitative combination in the action of *antiricine* upon ricine,

[1] Ehrlich, "Exper. Unters. üb. Immunität," *Deutsch. med. Woch.*, 1891, 976, 1218; "Zur Kenntnis d. antitox. Wirkg.," *Fortschr. d. Med.*, 1897, 41.

[2] Danysz, "Mélanges des toxines avec les antitox.," *Ann. Past.*, xvi., 331, 1902.

[3] Jacoby, "Ueber Ricinimmunität," *Hofm. Beitr. z. chem. Physiol. u. Pathol.*, i., 51, 1901.

and that antiricine consists of broken-off *normal receptors*. He has observed a very interesting deviation from the bacterial toxines. If an active immune serum be brought into contact with solutions of ricine a distinct *precipitate* is formed, which is also the case when solutions of antiricine are used (*vide infra*). On the other hand, there is no precipitate produced when active ricine is treated with normal serum or with destroyed antiricine, or when ricine rendered inactive by boiling is brought into contact with antiricine.

DANYSZ (*loc. cit.*) simultaneously investigated this precipitate, and found that there was invariably an optimum of admixture at which the strongest precipitation occurred. We shall return presently to the conclusions that he drew from this result.

This combination of ricine with antiricine is, as JACOBY rightly concludes, a *primary* one. The resulting neutral compound is, however, in this case only sparingly soluble, and thus forms a precipitate which also carries down, as usual, other proteids of the serum; for this is the only way of explaining the *amount* of the precipitate. But there can be no question of any precipitation of proteid by some *precipitine* that has been formed, and of this precipitate then carrying down with it the mixture of ricine and antiricine; for even ricine that is free from albumin produces in the organism a serum that gives the albumin reactions. Moreover, the strictly quantitative combination that is formed is opposed to the view that there might be any removal of the toxic power of the ricine by adsorption during a simultaneous precipitation of proteids; for the proteid precipitates containing ricine that have been formed in another manner—*e.g.*, by nucleic acid (*vide supra*)—are poisonous, whereas this precipitate is not. But as soon as there is any excess of ricine present, the filtrate remains poisonous in exactly the degree corresponding to that excess. A removal of toxic power so quantitatively regulated cannot be accounted for by adsorption.

An apparently paradoxical phenomenon can be simply explained by the theory. The *washed erythrocytes of highly-immunised animals* are at least as susceptible, and apparently still more susceptible than the normal erythrocytes. It is theoretically conceivable that the power of forming receptors may become temporarily spent, so that the erythrocytes may contain few or none at all, with the result that their susceptibility may be *very insignificant*, or *nil*; this appears to happen frequently in the case of *eels' blood* (*q.v.*).

Conversely, the theory can also assume the possibility of the erythrocytes being endowed with the power of a very vigorous

formation of erythrocytes, so that they thus contain more than the normal quantity, and that their susceptibility is therefore unmistakably *increased*.

On the other hand, it is obvious, and has been confirmed by the facts, that the erythrocytes in the natural serum of immune animals receive very considerable protection from the presence of anti-bodies in their serum. In one experiment JACOBY found such blood to require ten times as much ricine to produce the maximum agglutination as in the case of normal blood.

This phenomenon of the haptines combining with the free side chains in preference to those in combination has been observed in the case of *all* toxines. The free receptors appear almost absolutely to have a greater degree of affinity than those attached to the cells.

This fact forms the basis of every "*cure*" in cases of toxine poisoning—*i.e.*, the disruption by means of the antitoxine of a combination *already formed* with the cell. We have already shown in their respective places that this curative action is only possible for a very short time after the combination in the case of diphtheria and tetanus; a very similar state of things is also found with the blood poisons, for MADSEN was able to detect in the case of tetanolysine (*q.v.*), and JACOBY, in the case of *ricine*, an arrest of agglutination through the subsequent addition of antiricine.

But, in spite of its slight affinity, the haptophore group of ricine combines in the absence of *free* receptors just as quantitatively with those attached to the erythrocytes; and just as tetanus poison becomes fixed to the central nervous system so is ricine quantitatively attached to the erythrocytes, so that the mixture has no action upon a fresh quantity of blood.

The *agglutinating* action is thus completely paralysed through the quantitative combination of its substratum with the erythrocytes. We ought, therefore, to conclude *a priori* that if *ricine* is an individual substance the toxic power of such mixtures should also be destroyed. This, however, is not the case. MÜLLER found that the *filtrates* from the precipitates produced by ricine were devoid of agglutinating power, but yet were *poisonous*, although to a considerably reduced extent, and without producing the typical appearances on *post-mortem* dissection.

These experiments were not regarded as quite conclusive by JACOBY. In his opinion it was possible that there might be still some ricine mechanically carried down in the precipitate formed in the blood, and that this ricine might again be liberated during

the slow filtration.[1] But even JACOBY's own experiments, made with unfiltered blood rendered non-coagulable, gave the same results. The mixture had the same degree of toxic power. He himself, however, raises the very important objection that although the poison in this way reaches the cells of the tissues in a combined form, these cells may very well possess the power of decomposing this combination and of attracting the poison to themselves to their own injury. For the *receptors of the tissues*, like the free receptors, may possess greater affinity for the toxine than do the *erythrocytes*, although, again, it must be less than that of the free receptors; otherwise no immunity against the toxic action could be produced.

This view is also supported by an argument which, to my surprise, was overlooked by JACOBY. If we assume that the tissue receptors combine more readily with the haptophore group of the ricine than does the latter with the receptors of the blood-corpuscles, we have an explanation why in cases of the poisoning of living animals with ricine the symptoms produced in the blood are so slight in comparison with the general symptoms, a fact for which it is otherwise difficult to account.

These experiments, too, do not decide the question whether or no ricine consists of two separate substances.

JACOBY, in a further research,[2] made use of Ehrlich's method of separating hæmolysines, by subjecting mixtures of ricine with blood-corpuscles to centrifugal force. The agglutinating power was invariably found to have *disappeared* from the liquid, whereas the *amount of toxine* varied from 25 to 90 per cent. of the original amount. The poison had never combined *quantitatively* with the receptors. Its action was also qualitatively unchanged.

JACOBY next rendered animals immune by means of this poison freed from the agglutinine. The immune serum thus obtained showed *not only antitoxic, but also* anti-agglutinating power, although the plasma poison freed from agglutinine required less antitoxine than ordinary ricine.

Exactly similar results were obtained with ricine that had been previously treated with pepsin-hydrochloric acid. This agglutinine-free poison also produced an antitoxine which had an influence upon both functions of the ricine. The action of pepsin-hydrochloric acid upon ricine had already been studied by JACOBY in his earlier investigation, and he had been able to confirm MÜLLER's assertion that the agglutinating power was considerably reduced, even to $\frac{1}{60}$ of its original amount.

[1] *Loc. cit.*, p. 68. That is, if I have correctly understood the drift of the argument, which is somewhat too briefly expressed.

[2] Jacoby, "Ueber Ricinimmunität," *Hofm. Beitr.*, ii., 535, 1902.

But the *same amount of antitoxine was required* after the experiment as before to *neutralise* the greatly reduced agglutinating power, as well as the unaltered toxic activity. One c.c. of antitoxine was required to neutralise the extraordinarily small agglutinating power of 5 c.c. of pepsin-ricine; but the same quantity was also sufficient to destroy the *toxic* activity of the same quantity corresponding to at least 15 mgrms. of ricine (30 lethal doses). But, more than that, it was possible still to add enormous quantities of poison (up to 8 c.c. of pepsin-ricine) without killing the animals, though they certainly became emaciated.

One is strongly reminded by these facts of the conditions observed in the case of bacterial toxines, especially those of tetanus and diphtheria. There, too, EHRLICH found that more than a single lethal dose had to be added to a completely neutral mixture (L_0) in order to obtain L_+ (see the value D in the General Part).

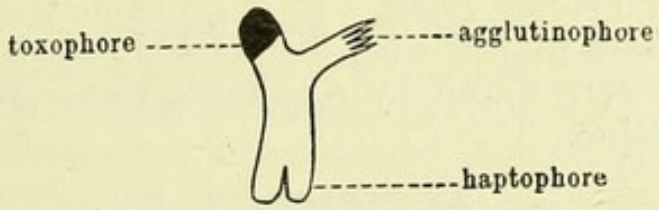
Another very interesting result was shown by JACOBY's experiments—viz., that whereas 1 c.c. of antitoxine was required to neutralise 0·26 c.c. of ricine solution *before* the treatment with pepsin, the same quantity was sufficient for 5 c.c. *after* the treatment. At the same time the toxic action remained unchanged, so that any possible destruction of the ricine molecules was out of the question.

It surely follows from these experiments that a large number of the *haptophore groups must have disappeared*, which, in the untreated ricine, had combined with the receptors of the serum, but, subsequently, no longer had any attraction for the anti-ricine. But these haptophore groups could not have corresponded with the toxophore groups, since the toxic power remained unchanged. We are thus inevitably driven to the conclusion that *non-poisonous haptophore groups* must have been present in the crude ricine. JACOBY is thus justified in his conclusion that there are *ricine toxoids which are destroyed by pepsin-hydrochloric acid.* And they must also be *syn-* or *protoxoids*, since they are simultaneously saturated on neutralisation.

The conditions are quite the reverse in the case of the *agglutinating function.* It is true that the number of haptophore groups also shows a decrease here, but to a smaller extent than that of the ergophore groups. If, now, we assume that the haptophore groups are the same, it is probable that there are here formed *new toxoids* of a *peculiar kind*, which *consist of only the haptophore and toxophore group, and have lost their agglutinophore group.*

And in support of the view that the haptophore groups are actually identical, and that ricine is thus an individual haptine, though of complex structure, we have, above all, the fact that the antitoxic and anti-agglutinating action remain just the same after the treatment with pepsin, and the results of immunising experiments with ricine devoid of agglutinine (*vide supra*). In the case of haptines that were undoubtedly distinct, such as, *e.g.*, tetanospasmine and tetanolysine, EHRLICH was unable to observe any trace of such a parallelism.

On the other hand, much can also be said *against* their identity, so that the question cannot yet be regarded as definitely settled. But if we grant their identity *commercial ricine* must consist of the complex groupings represented in the diagram—



and also of toxoids, either *without* any ergophore group,[1] or with only the agglutinating group thus—

These are eliminated by pepsin, possibly with the formation of toxoids with groupings in this form—

Fresh ricine, like diphtheria poison, appears to contain less toxoids, for according to JACOBY, Merck's preparation gradually decreases in its activity.

In addition to these bodies, which we are obliged to regard as pro- or syn-toxoids, there appear to be also *toxones* of ricine, though JACOBY has not touched upon this point; at least this conclusion can be drawn from the ratios of D $(L_+ - L_0)$ given

[1] This is really the more probable; it is difficult to conceive that there should be toxoids containing such a sensitive group as the agglutinophore group. This question, however, is of quite secondary importance.

above, which exactly recall those observed in the case of diphtheria poison.

It is as doubtful in the case of ricine as in that of the bacterial poisons whether there are not cases of dissociated equilibrium here. DANYSZ (*loc. cit.*), in fact, observed very striking ratios. The fact that there is an *optimum* point of mixture (*vide supra*) for the production of the largest precipitate is only a fairly weighty argument; and a far more important reason is that, according to DANYSZ, there is never an *absolutely neutral* mixture. He found that these mixtures invariably possessed both *toxic* and *antitoxic* action—*i.e.*, that although they themselves had a slight toxic action they were yet able to retard or altogether prevent death on the addition of a whole lethal dose. His speculations based on these facts are very similar to those of BORDET (see *General Part*). It is probable that the truth is that we have here to deal with dissociated conditions of equilibrium, such as were found by ARRHENIUS and MADSEN in the case of tetanolysine.

ANTIRICINE.

JACOBY has also made attempts to isolate antiricine. It may at once be mentioned that any separation of an *antitoxine* from an *antiagglutinine* has not been found possible by any method, and that, notwithstanding the theoretical considerations given above, we have thus in practice to deal with an individual substance.

Antiricine when salted out with ammonium sulphate is quantitatively precipitated in the fraction that falls when the degree of saturation reaches $\frac{1}{4}$ to $\frac{1}{3}$ of the total amount. In this way it can be separated from a large proportion of the other colloids.

Experiments with *trypsin* on the same lines as described above in the case of ricine showed that it absolutely resisted the action of that enzyme.

Antiricine was quite unaltered after two hours' heating at 60° C., and after digestion for thirty minutes with an equal amount of N/10 sulphuric acid or N/10 soda solution at 37° C. Pepsin-hydrochloric acid had no effect upon antiricine after an hour at 35° C. On the other hand, it was destroyed by acids at 60° C.

Antiricine thus appears to be a substance resembling the bacterial antitoxines in stability. It is a simple receptor of the first class, but provided with only one haptophore group.

STEPANOFF (*loc. cit.*) was able to detect antitoxine in the blood of rabbits, even twenty-four hours after the injection of serum

containing antiricine. After seven days both the antitoxine and the power of resisting the poison had disappeared. He found it neither in the urine nor in the intestinal tract, and there is thus no evidence of its being excreted; presumably it was oxidised in the system.

ABRINE.

Abrine, a toxine closely resembling ricine, is contained in the seeds of the jequirity (*Abrus precatorius*), a plant indigenous to the East Indies, and possibly also to Brazil. It is found in almost every part of the plant (Henseval [1]).

It was discovered by Bruylants and Vennemann [2] who were the first to announce that the active principle of jequirity seeds had nothing to do with bacteria, but was an enzyme, a toxic *proteid*. The same results were obtained by Warden and Waddell [3] working under the direction of Robert Koch in Calcutta. Sidney Martin [4] found that abrine consisted of a globulin and an albumose. The action of the albumose resembled that of the globulin, but was much weaker—*i.e.*, according to our present views more poison attaches itself to the globulin than to the albumose, while the true poisonous principle is distinct from either.

Its hæmolytic capacity was discovered by Kobert in 1889 and investigated by his pupil Hellin.[5]

Its action is so similar to that of ricine that Ehrlich (*loc. cit.*) considered it necessary to prove its individuality. Some differences were observed which showed beyond doubt that *abrine*, although very similar to ricine, was yet a distinct substance.

It is much less poisonous than ricine, especially *per os*. Ehrlich found that solutions of the same strength (1:100,000) killed mice, on subcutaneous injection, in six days, whereas in the case of ricine they caused death even after sixty hours. On the other hand, Calmette (*vide infra*) found 0·5 mgrm. per kilo. fatal to rabbits within forty-eight hours, while the

[1] Henseval, "L'abrine," *La Cellule*, xvii., 139; *Malys Jb.*, xxxi., 910.

[2] Bruylants and Vennemann, "Le Jequirity," *Bull. Acad. Méd. Belg.*, [3], xviii., 147, 1884.

[3] Warden and Waddell, *Non-bacillar Nature of Abrus Poison*, Calcutta, 1884.

[4] Martin, "The Proteids of the Seeds of Abrus," *Proc. Roy. Soc.*, xlii., 331, 1887; Martin and Wolfenden, "Physiol. Action of the Seeds of Abrus prec.," *ibid.*, xlvi., 94, 1889-90; Martin, "The Toxic Action of the Albumose from Seeds of Abrus prec.," *ibid.*, xlvi., 100, 1889-90.

[5] Hellin, "Der giftige Eiweisskörper Abrin.," *Dissert.*, Dorpat, 1891.

lethal dose for a mouse was 0·001 mgrm. The lethal dose for 1 grm. of mouse was found by Römer[1] to be 0·0005 mgrm.

Like ricine it produced inflammation, but necroses were seldom observed. On the other hand, a specific action of abrine is that it causes the hair to fall out rapidly around the point of injection, until complete baldness results.

The appearance on *post-mortem dissection* is almost the same as in the case of ricine; an additional effect being a characteristic hydropic degeneration of the muscle of the heart (Werhofsky[2]). Unlike ricine it also acts in small doses on fishes' blood (Lau, *loc. cit.*).

But the conclusive fact is that a state of immunity can be produced against abrine, which does *not* afford protection against ricine; in like manner animals rendered proof against ricine are not immune to abrine.

Action upon the Eye.—The action of abrine upon the *conjunctiva* is much more energetic than that of ricine, injection being followed by serious permanent lesions of the cornea or by panophthalmitis. In consequence of its energetic irritant action abrine is sometimes used in ophthalmic medicine, the violent inflammation produced frequently causing the disappearance of new inflammatory vessels and leucoma in the cornea. The maximum initial therapeutic dose is given by Römer[3] as about 0·01 mgrm. for rabbits.

According to Ehrlich the action of abrine can be regulated by immunising the conjunctiva itself with increasing doses. For practical purposes Römer has carried this immunisation to such a pitch that it is possible to avoid all the more serious symptoms without affecting the therapeutic action. Antitoxine is then produced in the *conjunctiva itself.*

Part of the poison is also absorbed from there and produces a general formation of antitoxine and immunity, although not to so pronounced an extent as after subcutaneous injection. The greatest degree of immunity attained was 500 antitoxic units, and local immunity was produced more rapidly than general immunity. Passive immunisation of the connective tissue with antiabrine serum only affords protection so long as the antitoxine itself is still present. On the other hand, the subcutaneous injection of large doses of antitoxine also protects the eye. An attack of abrine ophthalmia can be successfully treated by local

[1] Römer, "Ueber Abrinimmunität," *Arch. f. Ophthalm.*, lii., 90, 1901.
[2] Werhofsky, "Beitr. z. pathol. Anat. der Abrinvergiftung," *Zeiglers Beitr. z. pathol. Anat.*, xviii., 115, 1895.
[3] Römer, "Ueber Abrinimmunität," *Arch. f. Ophthalm.*, lii., 72, 1901.

application of therapeutic serum, even in very severe cases. The subcutaneous introduction of large doses also has a therapeutic action. The local immunity of the eyes lasts longer than the general immunity.[1]

Properties of Abrine.—Experiments to obtain an insight into the constitution of *abrine* have been made by HAUSMANN,[2] who has applied to this toxine the method used by JACOBY for ricine (*q.v.*).

Abrine is precipitated by saturating its solution with ammonium sulphate to the extent of 60 per cent., and can be freed from part of the proteid thrown down with it by repeated precipitation. Abrine thus purified has a very energetic toxic action, so much so that animals frequently die before the appearance of necroses and intestinal symptoms.

Abrine resembles ricine in resisting the action of trypsin. Hence it was found possible, as in the case of ricine, to obtain a preparation which, while possessing a high degree of toxic power, no longer gave the biuret reaction.

On the other hand, its agglutinating power is very much more resistant to the action of pepsin-hydrochloric acid than is that of ricine (*q.v.*), and when the action of the enzyme is very energetic the toxic and agglutinating functions eventually disappear almost simultaneously.

With this exception there is nothing special to be said with regard to the chemical reactions of abrine; so far as is known it behaves in exactly the same manner as ricine in this respect. According to CALMETTE[3] it is rendered inactive by iodine tincture, gold chloride, and hypochlorites.

It is apparently but little affected by the digestive fluids. HELLIN, alone, has found that it is destroyed by the enzymes of the intestine. On the other hand, according to NENCKI and SCHOUMOW-SIMANOWSKI[4] pepsin has no influence upon abrine.

In like manner it was found by RÉPIN[5] that the diluted digestive fluids and also the living mucous membrane of the stomach and intestine and the intestinal bacteria were inactive. He is inclined to believe that the smaller toxic effects on introduction of the poison *per os*, which, according to HENSEVAL (*loc.*

[1] Rehns, "Immunité acquise contre l'abrine," *Soc. Biol.*, lvi., 329, 1904.

[2] Hausmann, "Zur Kenntnis des Abrins," *Hofm. Beitr.*, ii., 134, 1901.

[3] Calmette and Déléarde, "Sur les toxines non-microbiennes," *Ann. Past.*, x., 675, 1896.

[4] Nencki and Schoumow-Simanowski, "Die Entgiftung d. Toxine d. d. Verdauungskanal," *Centralbl. f. Bakt.*, xxiii., 840, 1898.

[5] Répin, "Sur l'absorption de l'abrine par les muqueuses," *Ann. Past.*, ix., 517, 1895.

cit.), are from 200 to 250 times less than in the case of subcutaneous injection, are due to the fact that, on the one hand, abrine is extremely susceptible to the action of acids and is thus partially destroyed in the stomach; and that, on the other hand, it is extremely indiffusible. He found that after forty-eight hours' dialysis the proportion that had passed into the water did not amount to 1:250. Hence it remained in the intestine, and he was able to find it again in the fæces. HENSEVAL, however, asserts (*loc. cit.*) that it is also absorbed by way of the intestine and rectum, as well as of the bladder and peritoneum.

CALMETTE and DÉLÉARDE investigated the excretion of abrine. The blood from the heart of poisoned animals was found to be toxic in large doses (10 mgrms. introduced by intravenous injection); but the urine was absolutely free from toxine. On the other hand, however, they found that abrine appeared again unaltered in the intestinal tract, when injected intravenously into rabbits. Neither the blood from the heart nor the contents of the intestine of immunised animals were found to contain any poison that had been introduced.

An interesting fact which CALMETTE[1] was able to demonstrate with the aid of specific antitoxines is that the poisoned wood shavings with which the Indians, with malevolent intent, poison cattle are smeared with particles of jequirity; the poison on the rags impregnated with snake-venom and inserted into the rectum of oxen so as to poison them, could also be identified in the same way.

Some experiments made with antiabrine serum by CALMETTE and DÉLÉARDE may also be mentioned here.

Antiabrine loses its activity at 58° C. Calcium chloride and gold chloride have no effect upon it.

According to HAUSMANN (*loc. cit.*) the agglutinating power is considerably increased by the addition of very slight doses of antiabrine, which is doubtless to be attributed to the elimination of inactive proagglutinoids (protoxoids), which have a restrictive influence.

On mixing abrine and antiabrine a copious precipitate is formed, as in the case of ricine, even when abrine that gives no biuret reaction is used.

[1] Calmette, "Sur le sérum antivénimeux," *Comptes Rend.*, cxxii., 203, 1896.

CROTINE.

A third toxine closely allied to ricine is found in the seeds of *Croton tiglium*, an East Indian plant belonging to the *Euphorbiaceæ*, from which croton oil, the most powerful purgative known to us, is extracted.

STILLMARK (*loc. cit.*) was the first to prepare the toxic principle of these seeds, using methods analogous to those employed in the preparation of ricine.

A fuller investigation of crotine was made by ELFSTRAND.[1] He decorticated in the presence of alcohol and ether, and extracted them with water, a 10 per cent. solution of sodium chloride or glycerin, precipitated the active constituent by means of alcohol or ammonium sulphate, and purified it by dialysis.

It presents many analogies with the other toxines. In solution it is destroyed at 70° C., while the dry seeds are rendered non-poisonous at 110° C. Pepsin-hydrochloric acid is said to destroy it.

Toxic Effects of Crotine.—The lethal dose for frogs was found by ELFSTRAND to be about 0·23 grm. per kilo. The symptoms preceding the death of the animals were progressive paralysis and decrease in the reflex excitability and of the faradic excitability, affecting first the brain, then the spinal cord and nerves, and finally the muscles. Here again hyperæmia and ecchymoses of the intestinal membrane were also observed. Crotine has only a slight influence upon the heart, and the end-plates of the motor and sensory nerves are not affected.

Pike are killed by a dose of 0·04 to 0·1 grm. per kilo., the main symptoms being dyspnœa and paralysis.

In the case of *warm-blooded animals* (rabbits, dogs, cats, rats, hens, &c.), local inflammation and necroses are also produced by crotine, although only to a slight extent. It is far less poisonous than ricine or abrine (lethal dose, about 0·05 to 0·1 grm. per kilo.), and is also much less active when introduced *per os*.

The general symptoms are very similar to those produced by ricine, viz., convulsions, lowering of the pressure of the blood, diminution of temperature, respiratory paralysis, &c. A slight effect upon the eye can also be detected.

The appearance on *post-mortem* dissection is also very similar.

Action upon the Blood.—ELFSTRAND found that crotine also had an agglutinating action upon the blood of oxen, sheep, pigs, pike, and frogs, and a very much smaller effect upon that of cats,

[1] Elfstrand, "Ueber blutkörperchenagglutinierende Eiweisse," *Görbersdorfer Veröffentlichungen*, edited by R. Kobert, Stuttgart, 1898, 1.

while it had hardly any action upon human blood, and *none at all* upon the blood of dogs, guinea-pigs, rats, hens, geese, and pigeons.

Lau (*loc. cit.*) supplemented these results by others, in which he observed agglutination in the blood of the perch, but none in that of the cat and hedgehog. Neither investigator found any agglutination in the case of rabbits' blood, but there was a hæmolytic action. Crotine is stated not to possess the power of producing a precipitate with serum. It has an equal agglutinating action upon the washed erythrocytes and stroma. Oxygen is said to promote the action of crotine, while antitoxic substances that restrict its influence are present in the serum. It has no influence, or at least a very much slighter one than ricine, upon pus cells and other cells, but, on the other hand, it causes milk to coagulate. Ehrlich and Morgenroth,[1] and also Jacoby,[2] have studied the mode of action of crotine hæmolysine with the aid of the modern methods of investigating hæmolysines.

Morgenroth proved that crotine was a haptine by the fact that he was able to produce an immune serum in goats.

Jacoby found no support for the view that crotinolysine consisted of an amboceptor and complement, and was inclined to regard it as a haptine of the first class, like ricine.

By means of his experiments with partial saturation (*cf.* General Part), he has established that crotine must possess a complex constitution like that of diphtheria toxine, but it is noteworthy that very small doses of antitoxine increase the toxic action to a slight extent, since they eliminate the absolutely non-poisonous prototoxoids, which otherwise combine with part of the cell receptors, and thus reduce the toxic power. Then very rapidly, with the increase in the doses of antitoxine, the bulk of the poison is neutralised, and after that comes a wide zone with very slight affinity—*i.e.*, the *toxones*—which no longer effect complete hæmolysis, but with which Jacoby was yet able to produce immunity.

As is the case with other blood poisons (see, *e.g.*, Arachnolysine), insusceptibility and lack of power to combine with the poison also go hand-in-hand here. The blood-corpuscles of dogs and guinea-pigs do not combine with any trace of crotine.

A thermostable *anticrotine*, which apparently restricted the action of crotine in accordance with quantitative laws, was found by Jacoby in the extract of the mucous membrane of the stomach.

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[1] Ehrlich, "Verh. Ges. Charité-Aerzte, Feb. 1898," *Berl. klin. Woch.*, 1898, No. 12.

[2] Jacoby, "Ueber Crotin-Immunität," *Hofm. Beitr.*, iv., 212, 1903.

It was more fully investigated by LUST,[1] who found that it could be precipitated by alcohol, that it was "salted out" by ammonium sulphate (60 per cent. solution), and was not attacked by pepsin-hydrochloric acid.

ROBINE.

Robine, a fourth vegetable toxine, resembling ricine, was discovered by POWER and CAMBIER[2] in 1890, and described by them as a phytoalbumose. Its power of agglutinating the blood was also discovered by KOBERT. It is found in the bark of the so-called acacia, *Robinia pseudacacia*.

Its poisonous character had already been recognised as the cause of accidents to men and animals, several instances of which are cited by LAU (*loc. cit.*).

It acts upon the *blood* in a similar way to ricine, though its action is considerably weaker, and is absolutely lacking in the case of the blood of cats, dogs, and man.

In like manner its toxic power is disproportionately weaker than that of ricine and abrine. A dose of as much as 10 grms. per kilo. of body weight of robine freed from impurities by precipitation with potassium ferrocyanide and purified by re-precipitation with acetic acid (MERCK's commercial preparation) was required to kill a rabbit in four days. *Post-mortem* dissection showed nephritis, but otherwise nothing characteristic.

EHRLICH succeeded in producing immunity against *robine*, and discovered the fact that highly-immunised animals were also proof against ricine. Hence he is inclined to believe that *robine is a toxoid of ricine*. JACOBY has shown, as we have seen, that such ricine toxoids probably exist.

A closer investigation of robine is urgently needed, since this question is of the greatest theoretical importance.

HAY FEVER TOXINE.

Poisonous substances of a proteid nature have recently been regarded as the primary cause of hay fever and "autumnal cold." Toxines are present, according to DUNBAR and WEICHART,[3] both

[1] Lust, "Üb. einen Antikörper gegen Crotin im normalen Organismus," *Hofmeisters Beitr.*, vi., 132, 1904.

[2] Power and Cambier, *Pharmac. Journ.*, 1890, 711; *Pharm. Rdsch.*, Feb. 1890, p. 30.

[3] Dunbar, "Zur Frage betreffend die Aetiologie u. specif. Therapie des Heufiebers," *Berl. klin. Woch.*, 1903, 24-26; *id.*, "Z. Aetiol. des Herbstkatarrhes," *ibid.*, 1903, 28.

in the pollen of grasses which produce hay fever in the spring, and in that of *Ambrosiaceæ*, to which is attributed the "autumnal cold" of America.

It is sometimes possible to produce an antitoxine by immunising horses.

Further investigations are being made.

IV.—THE ANIMAL TOXINES (ZOOTOXINES).

SNAKE TOXINES.

Although venomous serpents have long been an object of fear and of interest to widely different races of man, yet the history of the *investigation* of their venom is quite recent. And this, one must admit, is remarkable, since surely nothing should have suggested itself more naturally to the scientific investigator than the application of recent results in toxicology, especially in connection with vegetable alkaloidal poisons, to the study of these poisons, which are as interesting to the investigator as they are important from the point of view of public hygiene. For, in India alone, more than 20,000 men perish annually from the bite of the cobra, *Naja tripudians*. And yet this branch of research remained almost completely untouched until the investigations into the nature of bacterial poisons, and especially those inaugurated by METSCHNIKOFF, ROUX, and YERSIN, compelled attention to be directed also towards these poisons, which have similar incredible toxic power. There were also external reasons for this neglect. The material for these investigations, at all events in the case of the most important venomous snakes, was hardly to be obtained in Europe. It was only when American medical science began to develop vigorously, and when the foundation of the modern Colonial Empires led to the study of tropical medicine, that the investigation of tropical venomous snakes also received special attention. We do not, of course, mean to assert that the poisonous characteristics of venomous snakes had not been frequently studied; this was notably the case with the South European vipers, and we shall give an outline of these earlier results in their proper place. But no systematic chemical and pharmacological investigation of the true poisonous substances was made until a relatively late period, when the study of bacterial poisons had opened up quite new avenues. Of the older researches mention may be made of those of FONTANA,[1] FAYRER and BRUNTON,[2] and WALL,[3] with whose results we shall deal presently.

[1] Fontana, *Trattado del veleno della vipera*, 1787.

[2] Fayrer and Brunton, "On the Nature of the Poison of Naja tripudians, &c.," *Proc. Roy. Soc.*, xxii., 68, 1874.

[3] Wall, "On the Poisons of Certain Species of Indian Snakes," *Proc. Roy. Soc.*, xxxii., 333, 1881.

Naturally, search was first made, as we find almost universally in the history of toxines, for alkaloidal substances comparable with the ptomaines. Thus GAUTIER, in 1881, isolated two alkaloidal substances, *naïn* and *elaphin*, from the venom of the *naja* and the *Trigonocephalus* (American fer-de-lance) respectively. He himself, however, had to admit that these substances were relatively harmless; and hence, in this case, science was spared the process of disillusion which otherwise followed almost universally the original over-valuation of the ptomaines.

GAUTIER, therefore, concluded that the "true active principle of snake poison contained nitrogen," but that it was "not of the nature of an alkaloid."

About the same time, WEIR MITCHELL and REICHERT,[1] in America, made a fuller investigation of snake poisons.

The venom of European vipers (*Pelias berus*) had already received somewhat more attention (FONTANA, VALENTIN,[2] and others). The true stage of development of this new branch of biology begins with these researches. Then follow the classical investigations of CALMETTE, to which, supplemented by those of MARTIN, FRASER and PHISALIX, and the quite recent work of FLEXNER, KYES and SACHS, our present knowledge is, in the main, due.

By a lucky chance, CALMETTE,[3] who was then head of the bacteriological institute at Saigon, obtained possession of the fresh poison glands of 22 cobras, and this formed the starting point for his classical investigations.

The *poison glands* of the serpents, which are similar to the salivary glands, are the true source of the poison; but CALMETTE[4] found that the *blood* of the cobra was also fairly poisonous. The intravenous injection of 2 c.c. killed a rabbit weighing 1,500 grms. in three minutes. On the other hand, the liver and gall are not poisonous. Even the blood of otherwise harmless serpents (*Tropidonotus*) was found to be poisonous by PHISALIX and BERTRAND.[5] This blood poison has certain peculiarities with which we shall deal presently.

The poison gland of the cobra yields on expression about 3 grms. of a transparent viscous fluid, which when exposed to

[1] Weir Mitchell and Reichert, "Researches upon the Venoms of Poisonous Serpents," *Smithsonian Contrib.*, No. 647, Philadelphia, 1885; Washington, 1886; quoted by Flexner, *loc. cit.*

[2] Valentin, "Einige Beobachtg. üb. d. Wirkg. des Viperngiftes," *Zeit. f. Biol.*, xiii., 80, 1877.

[3] Calmette, "Étude expérimentale du venin de *Naja tripudians*," *Ann. Past.*, vi., 160, 1892.

[4] Calmette, "Sur la toxicité du sang de cobra," *Soc. Biol.*, xlvi., 11, 1894.

[5] Phisalix and Bertrand, "Sur le présence des glandes venimeuses chez les couleuvres," *Soc. Biol.*, xlvi., 8, 1899.

the air congeals into masses. CALMETTE[1] treated these glands with glycerin, with distilled water, and with a 10 per cent. solution of salt, and invariably obtained extremely poisonous extracts. The toxine acts most energetically when directly injected into the veins, and has less effect when injected subcutaneously or into the peritoneum and trachea; it is absolutely without action when introduced into the intestine.

The quantity of poisonous saliva secreted was found by CALMETTE (1895) to amount, on the average, to 0·135 grm., corresponding to about 30 to 45 mgrms. of dry substance, provided that from eight to fourteen days were allowed to elapse between the separate bites. On the other hand, after a lapse of two months each bite yielded about 0·22 grm. of saliva. The greatest amount that he was able to extract from the two poison glands of a dead snake was 1·136 grms. = 0·48 grm. of dry substance. Similar results were obtained in the investigation of other venomous snakes.

Thus, there is invariably a considerable increase in the amount excreted, and consequently in the danger from a bite when the snake has not bitten for a considerable time. The bite of hibernating snakes—*e.g.*, the European vipers—is thus the most dangerous in the spring.

Preparation of the Poisonous Principle.—Snake toxines have not been prepared in even an approximately pure state. The methods of concentration are exactly the same as those employed for all toxines and enzymes.

The poisonous principle can be extracted by water, salt solutions or glycerin, and purified by repeated precipitation, dialysis, &c.

MARTIN succeeded in eliminating part of the inactive substances present, by means of fractional coagulation, for on heating a solution of *holocephalus poison* in a 0·9 per cent. solution of sodium chloride, a foreign substance separated at 85° C., while the true poison remained still active at 90° C.

CALMETTE[2] subsequently used the following method in the preparation of a stable poison relatively free from proteids:—The solution of 1 grm. of cobra poison in 100 c.c. of water was filtered through sterilised filter paper, then heated in a hermetically closed glass tube for thirty minutes at 75° C., and then for twenty-four hours at 80° C., after which it was filtered through paper to remove the separated substances and finally dialysed.

[1] Calmette, "Étude expérimentale du venin de *Naja tripudians*," *Ann. Past.*, vi., 160, 1892.

[2] Calmette, "Sur le venin des serpents, &c.," *Ann. Past.*, xii., 214, 1897.

In this way he obtained 42 mgrms. of a dry residue, which still showed the biuret and Millon's reaction, but no other proteid reactions. The poison will pass readily through a Chamberland filter.

Chemical Nature of the Toxine.—After it had been found that the active agent was not an alkaloid there followed the usual period of *toxalbumins*, which to-day are practically discarded in the case of bacterial poisons. It is probable that snake poisons also are not proteids in the stricter sense of the word, and if that is so the attempts that have been made to investigate the proteids combined with them are, in the main, only interesting from the historical point of view.

WEIR MITCHELL found albumins in the poison of *Crotalus durissus* (rattlesnake), while WOLFENDEN[1] isolated various proteids (globulins, albumin, and albumoses), but no peptone from the venoms of the cobra and *Daboia*. KANTHACK[2] regarded the poison as a proto-albumose. MARTIN and SMITH[3] found a non-poisonous albumin, and two poisonous albumoses, a hetero- and protalbumose, but no peptone in the poison of *Pseudechis porphyriacus* and *Hoplocephalus curtus*.

As regards the constitution of the toxines themselves nothing is known.

Properties of the Toxine.—Snake poison shows all the properties characteristic of impure toxines as regards precipitability, &c. Cobra poison, however, according to CALMETTE, forms an exception in not being carried down by freshly precipitated calcium phosphate, which is otherwise a general characteristic of all these colloids. It is also not precipitated by magnesium sulphate, and thus contains no globulin.

It dialyses slowly, but appreciably. Viper venom is weakened by passage through a porcelain filter (PHISALIX[4]). It is much less affected by heat than other haptines. Cobra poison can resist a temperature of 90° C. for an hour, and of 38° C. for a day; it is only slightly injured by being kept at 97° C. for half an hour, but its activity is completely destroyed after exposure for the same length of time at 98° C. On the other hand, the *purified* poison (CALMETTE, 1890) is very susceptible to a temperature of 80° C., a solution in distilled water being more affected than in that containing salt or glycerin. This is also

[1] Wolfenden, "The Venom of the Indian Cobra," *Journ. of Physiol.*, vii., 327, 1886; *id.*, "The Venom of the Indian Viper (*Daboia*)," *ibid.*

[2] Kanthack, "The Nature of Cobra Poison," *Journ. of Physiol.*, xiii., 272, 1893.

[3] Martin and Smith, "The Venom of the Australian Black Snake," *Proc. Roy. Soc. New South Wales*, 1892, 240; *Malys Jb.*, 1894, 404.

[4] Phisalix, *Soc. Biol.*, xlviii., 233, 656, 1896.

characteristic of all haptines. It is an important point that the poison of the blood of the cobra is much more sensitive to heat than the saliva poison, since it is rendered inactive even after ten minutes at 68° C. (CALMETTE and DÉLÉARDE [1]).

Faradic currents have no influence upon it, but *continuous currents* have a destructive effect in a solution containing sodium chloride, owing to the electrolysis and production of chlorine. Viper venom, however, is said to be injured by currents of high intensity (PHISALIX).

The toxine appears to resist the action of dilute phenol, mercuric chloride (1 : 1000), copper sulphate, iodine, potassium iodide, alcohol, ether, chloroform, and essential oils. Ammonia, even in large doses, does not injure it until after a long time (KANTHACK). This highly-valued remedy has thus no action, at all events, upon the poison itself. The poison of *Vipera aspis* remained active for twenty years in a specimen of the snake preserved in spirit (MAISONNEUVE [2]).

A 1 per cent. solution of potassium permanganate, however, had a destructive effect upon the venom, and almost invariably saved the animal when injected into the same place immediately after the poisoning; but even after the lapse of a short time the injection had no effect, as was also the case when the permanganate was introduced at another place, even into the veins, or in the immediate vicinity of the point of inoculation. Calcium chloride, too, has an injurious influence on the poison (PHISALIX and BERTRAND [3]). Gold chloride has a still more pronounced action, but platinum chloride has no effect.

A 1 per cent. solution of gold chloride destroys the activity of the poison even when present in a very slight proportion. It also affords protection when introduced at other places, even against fairly large doses, and also for a short time after the poisoning. CALMETTE proposed to use this property of gold chloride for therapeutic purposes, but these results have been superseded by his own discoveries of an active immunisation and serum therapy.

A series of very interesting experiments showed that snake poison was greatly influenced by certain substances that were quite inert in themselves, to which PHISALIX,[4] in particular, has

[1] Calmette and Déléarde, "Sur les toxines non microbiennes," *Ann. Past.*, x., 675, 1896.

[2] Maisonneuve, "Longue conservation de la virulence du venin des Serpents," *Comptes Rend.*, cxxiii., 513, 1896.

[3] Phisalix and Bertrand, *Soc. Biol.*, xlvii., 443, 1895.

[4] Phisalix, "La tyrosine vaccine chimique du venin du vipère," *Comptes Rend.*, cxxvi., 431; *id.*, "Les sucs de champignons contre le venin du vipère," *ibid.*, cxxvii., 1036, 1898.

called attention in several publications. *Tyrosin* and *cholesterin* were found to possess this property.

Extracts of fungi with chloroform water are also stated to have a protective influence, and also when *injected beforehand* to produce immunity, especially against viper poison, the protection beginning twenty-four hours after the injection, and lasting until the twenty-fifth day.

The property possessed by these substances of rendering poisonous haptines inactive is due to a *combination* with the active principle, and has also been observed in the case of many other toxines, such as tetanus and botulotoxine.

Action of Extracts of Organs and Secretions.—Starting from the fact of the resistance offered by snakes, including harmless species, to the poison, search was made for antidotes to snake toxines in the extracts of different organs.

The bile, in particular, is *in vitro* an effective antitoxine. Snake poisons in general resemble bacterial poisons in being attacked by the bile. Whether the latter has only a simple destructive action—as, for example, in the case of diphtheria toxine—or whether it contains a definite antitoxine is not yet known with certainty; we shall return to this point presently. We must, however, assume that the action is purely chemical, for, according to CALMETTE,[1] the sodium salt of glycocholic acid has the same effect, so that there is good reason for attributing the action of the bile as a whole to that substance. The bile retains this property even after being heated to 100° C., but loses it at 120° C.

Having regard to the production of side-chain immunity in the case of tetanus, MYERS[2] endeavoured to detect an antitoxic function in the organs of the body, but only found it in the extract of the *suprarenal bodies*. But even in that case there was only an increase in the resistance *in vivo*, and not any specific antitoxic activity. CALMETTE, too, found that no combination took place between the toxine and the nerve substance or extract of the liver.

FLEXNER and NOGUCHI[3] have tested the neutralisation power of various organs upon three times the lethal dose of the poison of the copperhead snake, which killed a guinea-pig in forty-five

[1] Calmette, "Sur le mécanisme de l'immunisation contre les venins," *Ann. Past.*, xii., 343, 1898.

[2] Myers, "Cobra Poison in relation to Wassermann's New Theory of Immunity," *Lancet*, 1898, ii., 23.

[3] Flexner and Noguchi, "Snake Venom in relation to Hæmolysis, &c.," *J. of Exper. Med.*, vi., 277, 1902 (reprint).

minutes in a control experiment. The only organ that had an energetic protective action was the *brain*. It retarded the death of one animal for nineteen hours, while another survived twice the amount of a lethal dose which killed an animal in five hours in the control experiment. Extracts prepared from other organs did no more than retard the action to some extent. The hæmolysine did not enter into combination in the slightest degree.

Mode of Action of Snake Venoms.—Snake venoms, as we shall subsequently show more fully, contain, in addition to the two agents that act specifically upon the corpuscles of the blood, two poisonous constituents, *neurotoxine* and *hæmorrhagine*. Since the latter component manifests its activity chiefly in *crotalus* venom, and is almost entirely absent in the case of *cobra* venom, the following description deals primarily with the *neurotoxine* of the cobra and other snakes.

This poison is extraordinarily virulent. One drop of CALMETTE'S first glycerin extract killed rats and pigeons in less than an hour, and hens and rabbits in a somewhat longer time.

MARTIN [1] found the lethal dose of the poison of *Hoplocephalus curtus* (the tiger snake) for rabbits to be 0·03 mgrm. per kilo. This poison is stated to be the most active.

VALENTIN found the lethal dose of the poison of *Vipera aspis* for the frog to be 0·5 mgrm.

FLEXNER and NOGUCHI (*loc. cit.*) found that guinea-pigs were killed by a dose of 0·3 mgrm. of the venom of the copper-head snake (*Ancistrodon contortrix*).

CALMETTE [2] stated that in the case of a cobra that had not taken any food for eight months the virulence of the venom had considerably increased. While 0·7 mgrm. of the dry poison was originally required to kill a rabbit of 1,700 grms., 0·25 mgrm. was sufficient after two months, and 0·1 mgrm. after the death of the snake (for a rabbit of 2,000 grms.). Similar results were obtained in the case of another cobra during three months.

A comparative determination of the toxicity of different snake poisons gave the following values :—

[1] Martin and Cherry, "The Nature of the Antagonism between Toxines and Antitoxines," *Proc. Roy. Soc.*, lxiii., 420, 1898 ; Martin, "Relation of the Toxine and Antitoxine of Snake Venom," *ibid.*, lxiv., 88, 1899.

[2] Calmette, "Contrib. à l'étude des venins," *Ann. Past.*, ix., 225, 1895.

	Lethal Dose for Rabbits of 1,600 to 2,000 grms. in 3 to 4 hours.	Lethal Dose for Guinea-pigs of 450 to 550 grms. in 3 to 4 hours.
Naja tripudians (1 to 3), .	0·3 to 0·6 mgrm.	0·05 mgrm.
Naja haje (4 to 6), . .	0·3 ,, 0·7 ,,	0·07 ,,
Cerastes (horned viper), .	1·5 ,, 2·0 mgrms.	0·1 ,,
Crotalus, ...	3·5 ,,	0·3 ,,
Trigonocephalus, . . .	2·5 ,,	0·2 ,,
Hoplocephalus, . . .	2·5 ,,	...
Acanthophis, . . .	1·0 ,,	0·08 mgrm.

Elliot, Sillar, and Carmichael[1] found the lethal dose per kilo. of body weight of the venom of *Bungarus cæruleus* to be as follows:—Frogs, 0·5 mgrm.; rats, 1·0 mgrm.; and rabbits, 0·08 mgrm.

According to Fraser and Elliot[2] the lethal dose per kilo. of body weight of the venom of the sea-snake (*Enhydrina*) is 0·09 mgrm. for rats, 0·06 mgrm. for rabbits, and 0·2 mgrm. for cats.

Guinea-pigs are thus twice as susceptible as rabbits. The dog is still less susceptible. The pig, hedgehog, and the mongoose (*Herpestes*), a small carnivorous mammal, are almost refractory. It required not less than 8 mgrms. of cobra poison to kill a mongoose. The hedgehog is but little affected by the bite of, at all events, the viper. According to Phisalix and Bertrand[1] forty times the lethal dose for a guinea-pig is required. The blood of the hedgehog itself is then poisonous, but this toxicity disappears on heating.

Snakes themselves, whether poisonous or harmless, are almost immune, though not absolutely so, as, for example, the ring-adder, for which the lethal dose is 0·03 grm. (Fraser,[3] Phisalix and Bertrand[4]).

Fishes, lizards, and worms are also not completely immune.

Most snake poisons also produce, like other toxines, severe local effects, such as violent inflammation, œdema, hæmorrhage, and even necroses.

These local inflammatory effects, however, do not appear to be

[1] Elliot, Sillar, and Carmichael, "Action of the Venom of the *Bungarus cæruleus*," *Lancet*, 1904, ii., 142.

[2] Fraser and Elliot, *Lancet*, 1904, 141.

[3] Fraser, "Immunity against Snake Poison," *Brit. Med. Journ.*, 1895, i., 1309.

[4] Phisalix and Bertrand, "Glandes venimeuses chez les couleuvres," *Soc. Biol.*, xlvi., 8, 1894; xlvii., 639, 1895.

an integral part of the general action of snake toxine, which is quite analogous to what has been observed in the case of other toxines. Thus, according to CALMETTE (1895), the local action is greatly weakened by heating the poison to 80° C., while the general toxic power is unaffected. KAUFMANN[1] states that chromic acid and permanganate have an exactly similar effect.

The local effects also vary very considerably in intensity with snake poisons of different origin. They are only produced to a slight extent by cobra venom, whereas they are very pronounced in the case of crotalus venom. According to the conclusions arrived at by WEIR MITCHELL and REICHERT, and confirmed by later researches (*vide infra*), these pyrogenic substances must be sharply differentiated from the true *neurotoxic principle*. But their action is undoubtedly connected with that of the second main constituent, the *hæmorrhagine* (*vide infra*).

The absorption of the venom is extremely rapid. A rat inoculated at the tip of the tail cannot be saved after the lapse of a minute by amputation (CALMETTE), and dies five minutes later than the animal used in the control experiment. The poisoning is also exceedingly rapid in the case of man.

The bitten limb swells up, and this is followed by contraction of the mouth, clenching of the teeth, swooning, and death in the deepest coma.

The mortality fluctuates between 25 and 45 per cent. It largely depends upon the amount of poison introduced. If the snake has bitten shortly beforehand, or if the clothes have afforded some protection, the bite is relatively free from danger; but it is extremely dangerous when it is in a spot that contains numerous vessels. Injection into the veins is almost invariably fatal.

The general symptoms begin with weakness, vomiting, shortness of breath, and ptosis. There is loss of faradic excitability of the muscles, and the immediate cause of death is the stoppage of the respiration. *Frogs*, however, which are able to survive the loss of pulmonary respiration for a longer period, live for some time (up to thirty hours).

The action of the salivary poison of the vipers (*Vipera Redii*, &c.) is very similar to that of the cobra. A. Mosso[2] found that the intravenous injection of 0·0077 grm. per kilo. into a dog

[1] Kaufmann, "Sur le venin de la vipère," *Soc. Biol.*, xlvi., 113, 1894.

[2] A. Mosso, "Die giftige Wirkung des Serum des Mureniden," *Arch. f. exper. Path.*, xxv., 111, 1888.

produced accelerated respiration, followed in a short time (fifteen minutes) by paralysis of the respiratory centre. There is diminution in the action of the heart, which continues to beat after breathing has stopped. By means of artificial respiration life can be prolonged for about two hours; spontaneous breathing even begins again; but eventually it stops once more, and the animal dies quietly after slight convulsions.

According to PHISALIX and BERTRAND,[1] 0·3 mgrm. of the venom of *Vipera aspis* will kill a guinea-pig, the symptoms being hypothermia, dilatation of the blood-vessels, and areas of hæmorrhage.

VALENTIN observed a diminution in the amount of oxygen absorbed.

The heart is not *directly* affected. As far back as 1873 it was proved by PANCERI and GASCO[2] that the isolated heart of an axolotl continued beating as before in their preparation of the venom of *Naja egiziana*. This venom resembles cobra poison in its action, but is weaker.

According to ELLIOT,[3] cobra venom in very dilute solutions (1:10 millions) has a stimulative action upon the isolated heart of the frog, while a solution of 1 : 500,000 causes paralysis. In the case of mammals the heart is only brought to a standstill by very large doses, and the poison acts primarily upon the respiratory centre.

Enhydrina venom does not act upon the isolated heart and the smallest vessels, but upon the respiratory centre and the peripheral nerves.

ROGERS[4] found that the venom of enhydrina produced paralysis of the respiratory centre and of the motor nerves. It is thus analogous to cobra venom.

The pressure of the blood does not change during artificial respiration. Apart from this, the initial increase in the pressure is, of course, followed by a decrease, as was shown by ALBERTONI.[5] KAUFMANN,[6] too, observed a diminution in the case of the venom of *Pelias berus*.

[1] Phisalix and Bertrand, "Toxicité du sang de la vipère," *Comptes Rend.*, cxvii., 1099, 1893.

[2] Panceri and Gasco, "Agli effetti del veleno della *Naja egiziana*," *Atti Acad. Reale Napoli*, 1873, 73, quoted by Mosso, *loc. cit.*

[3] Elliot, "Action of Cobra Poison," *Proc. Roy. Soc.*, lxxiii., 183, 1904.

[4] Rogers, "On the Physiological Action of the Poison of the Hydrophidæ," *Proc. Roy. Soc.*, lxxii., 305, 1903.

[5] Albertoni, "Sull'azione del veleno della vipera," *Lo Sperimentale*, 1879, quoted by Mosso.

[6] Kaufmann, *Soc. Biol.*, xlviii., 860, 1896.

VALENTIN[1] found that there was a manifest want of excitability in the muscles and nerves of a frog after five hours. The central nervous system was deprived of its excitability sooner than the sciatic nerve endings.

Thus, general poisoning by snake toxines is due primarily to their action upon the central nervous system, and, above all, upon the motor centres of the medulla. On the other hand, the peripheral nerves are not affected, at all events in the case of the frog (CALMETTE). In this action the *neurotoxic component* predominates. We shall see presently that this is not the case with all snake poisons, and notably crotalus venom, in which the chief effects are produced by the component possessing hæmorrhagic powers. The hæmolytic component in that venom need not be taken into account here, and will be dealt with separately.

The fact that neurotoxine is no more active on intercerebral injection than when otherwise introduced shows that it is a specific poison for the central nervous system, and does not combine with cells elsewhere, being *monotropic*, to use Ehrlich's terminology.[2]

Cobra poison which was free from hæmolysine and hæmorrhagine was injected into the cerebellum by FLEXNER and NOGUCHI (*loc. cit.*), and it was found that the lethal dose was not less than that required in subcutaneous injection.

The behaviour of crotalus poison is quite different in this respect, for the lethal dose was twenty times less on intercerebral injection. This venom contains but little neurotoxine, and, in the main, it enters into combination in another way. The great activity of crotalus poison on intercerebral injection is, in fact, due solely to its hæmorrhagic function, and therefore disappears on heating the solution to 75° C., at which temperature the hæmorrhagine is destroyed. An intermediate position is occupied by the venoms of the mocassin snake and of the ancistrodon, since they contain a large proportion of both components.

Fresh cobra poison has a very violent action, resembling that of abrine, upon the conjunctiva. The poison can be deprived of this property, however, by being heated to 90° C., which does not materially affect its toxicity.

No absorption appears to take place thence, such as has been observed in the case of ricine. The same result was obtained by VALENTIN in his experiments with viper venom.

[1] Valentin, "Einige Beobachtungen über die Wirkungen des Viperngiftes," *Z. f. Biol.*, xiii., 80, 1877.

[2] Ehrlich, "Ueb. d. Bezieh. von chemischer Konstitution, Verteilg. u. pharm. Wirkg.," *Festschr. f. Leyden*, 1902 (reprint).

The differences observed in the action of different snake toxines can for the most part be attributed to the variations in the proportions of the separate components, sometimes the neurotoxine and sometimes the hæmorrhagine predominating. Occasionally, too, the hæmolytic principle doubtless has some toxigenic effect.

The venoms of the crotalus, trigonocephalus, and cerastes are distinguished from cobra poison by their much greater activity, especially as regards the local effects (œdema, necroses, &c.).

Moreover, while, according to CALMETTE, cobra poison can be entirely deprived of its local irritant action by heat, the toxicity of crotalus venom is stated by M'FARLAND[1] to be almost completely destroyed by the same treatment. Owing to the terrible injuries produced, M'FARLAND was absolutely unable to produce immunity by means of subcutaneous injection, and only succeeded in his purpose by the use of intravenous injections.

As was found by FLEXNER and NOGUCHI, the characteristic poisonous constituent in the venom of *crotalidæ* is *exclusively the hæmorrhagine*, while the neurotoxic component, which predominates in cobra venom, has here only a very slight share in the effects. The venoms of the mocassin snake and copper-head snake (*Ancistrodon*) contain both components.

This explains why the venom of the crotalus and of other allied species of snakes (*Pseudechis*, &c.) produce areas of hæmorrhages that are not caused by cobra venom; this characteristic was closely studied by WEIR MITCHELL and REICHERT. The poisonous constituent that produces the hæmorrhage is destroyed at 75° C., while the venom simultaneously loses *part* of its toxicity, so that not less than ten to twenty times the original lethal dose is required to cause death; the symptoms resemble those produced by cobra venom, and must therefore be attributed to the neurotoxine.

It would be conceivable that the general toxicity in this case might be due to the hæmolysine.

But the hæmolysine can be eliminated, as we shall presently show, by making it combine with susceptible erythrocytes, without destroying the general toxic power.

Hence, it follows that the poison that causes the hæmorrhage is not identical with the true hæmolysine any more than it is with the neurotoxine, but that a third individual poison is present, to which FLEXNER and NOGUCHI have given the name *hæmorrhagine*.

[1] M'Farland, "Immunisation of Animals to Rattlesnake Venom," abstr., *Centralbl. f. Bakt.*, xxix., 496, 1901.

This poison is also present in cobra venom though its amount is ten times less than in the venom of the mocassin snake and one hundred times less than in that of the rattlesnake.

Owing to the great variation in the proportions of the three components, neurotoxine, hæmolysine, and hæmorrhagine, the ratio between the lethal dose and the dose of hæmorrhagine, just capable of detection, is also very variable. Thus, in the case of cobra venom the lethal dose (0·1 mgrm.) corresponds to *one* hæmorrhagic dose, that of the venom of the mocassin snake (0·2 mgrm.) to 20, that of copper-head venom to 60, and that of rattlesnake venom (1·0 mgrm.) to 1,000 doses.

The histological changes produced by hæmorrhagine in the blood-vessels have been more closely studied by Flexner and Noguchi. The effect is not one of diapedesis but of rents in the walls of the blood-vessels, in which perforations are then formed. Stases occur in the vessels and also giant cells which block the small vessels. The red and white blood-corpuscles equally escape.

They attribute this perforation of the walls of the vessels to a cytolysine having a specific action upon the endothelium of the walls.

On the other hand, Wall (*loc. cit.*) observed very marked differences between the venoms of the colubrine cobra and the viperine *Daboia Russeli*, which cannot be accounted for solely by the difference in the proportions of the separate components.

Daboia venom very rapidly produces violent convulsions, in the course of which death frequently ensues, and these are followed by paralysis which does not, however, as in the case of cobra venom, specially affect the breathing apparatus. Nor is the respiration in general acted upon so rapidly by daboia venom. It invariably produces mydriasis, but there is none of the salivation that is characteristic of cobra venom. Albuminuria is invariably produced by daboia venom, but never by cobra venom. The former is a very powerful blood poison. Hence, those infected with the venom are still in great danger even after surviving the first stage of convulsions and paralysis. Whereas in the case of cobra poisoning the question of life or death is decided within a few hours, those bitten by the daboia may die as late as the end of the second week.

In fact, Lamb[1] was able to show that the hæmolysine of the daboia possesses an *absolutely different amboceptor* from that of cobra venom (*vide infra*).

Toxoids of Snake Toxine.—The existence in snake toxine of non-poisonous or only slightly poisonous toxoids with an immunising power has not been definitely determined, but is probable.

[1] Quoted by Kyes, *Berl. klin. Woch.*, 1903, No. 43.

Phisalix and Bertrand[1] found that the poisonous serum of the common viper or adder lost its toxic, but not its immunising power when heated for fifteen minutes at 58° C., and that the venom of *Vipera aspis* behaved in a similar way when heated for some minutes at 75° to 90° C. The same investigators found that poison weakened by high-tension currents (*vide supra*) could still produce immunity.

By the aid of more accurate quantitative experiments on the lines devised by Ehrlich, Myers[2] has detected toxoids in cobra hæmolysine.

Flexner and Noguchi (*loc. cit.*) observed an unmistakable formation of toxoids in cobra venom that had been allowed to stand for three weeks. The lethal dose rose from 0·1 to 0·4 mgrm., while there was no appreciable decrease in the dose of antitoxine required for neutralisation, four lethal doses being used as the standard poison. Hence, protoxoids are formed. The same process occurred still more rapidly in an incubating oven, the lethal dose increasing tenfold in nineteen days, although, in addition to the formation of toxoids, there was also a partial decomposition. On the other hand, pepsin and papaïn completely destroyed the poison without any formation of toxoids.

The Hæmolysine of Snake Poisons.—The analogy between snake poisons and vegetable toxines, and in particular the poison of *eels' blood*, also extends to the activity *in vitro* of their hæmolytic function.

Many snake poisons also act hæmolytically *in vivo*, for it was observed long ago by Fontana that the intravenous injection of vipers' venom into rabbits produced coagulations, &c.; while, on the other hand, the blood of animals that have died from the poison becomes incoagulable, as was recorded by Fayrer and Lauder-Brunton,[3] Albertoni (*loc. cit.*), and others, and confirmed by Mosso (*loc. cit.*), as regards the venom of the viper.

In the case of the latter there is also a formation of methæmoglobin as a secondary product due to the action of an oxydase; this does not occur in the case of cobra hæmolysine (Phisalix[4]).

[1] Phisalix and Bertrand, "Atténuation du venin de vipère par la chaleur," *Comptes Rend.*, cxviii., 288, 1894.

[2] Myers, "The Interaction of Toxine and Antitoxine," *Journ. of Pathol.*, vi., 415, 1900.

[3] Fayrer and Lauder-Brunton, "On the Nature of the Poison of *Naja tripudians*, &c.," *Proc. Roy. Soc.*, xxi., 371, 1873; xxii., 68, 1874.

[4] Phisalix, "Action du venin de vipère," *Soc. Biol.*, liv., No. 27, 1902.

The action of snake venoms upon the blood was subsequently more closely studied by FLEXNER and NOGUCHI.[1]

For this purpose they used the venoms of *Naja tripudians*, *Crotalus adamanteus* (rattlesnake), *Ancistrodon piscivorus* (mocassin snake), and *Ancistrodon contortrix*, all of which only showed slight differences.

The action of these was tried upon the blood of the dog, rabbit, guinea-pig, sheep, ox, pig, necturus, and frog. The blood-corpuscles were washed before the experiment, and then only simple agglutination without hæmolysis took place. The blood of the rabbit was the most susceptible to this influence, and then that of the guinea-pig, dog, sheep, pig, and ox.

The amount of hæmolysis to be observed in defibrinated blood stood in no constant relationship to the agglutination of the washed blood-corpuscles. The separate action of the hæmolysis and agglutination could be observed at 0° C., since the latter was not affected by the temperature.

Cobra venom was the strongest hæmolytic agent, and rattlesnake venom the weakest. Dogs' blood was the most susceptible, and ox blood the least, leaving out of the question frogs' blood, which was almost completely refractory.

The hæmolysines are very resistant to the influence of heat. They were not affected at all by temperatures of 70° to 80° C., and were only slightly injured even after fifteen minutes at 100° C. In this respect they resemble the heat-resisting bacterial lysines; the agglutinines, however, are destroyed in thirty minutes at 75° to 80° C. The hæmolysine can also withstand a temperature of 100° C. for thirty minutes. On the other hand, the lysine is destroyed by the same chemical agents as the toxic components (KYES and SACHS, *vide infra*).

The hæmolytic principle is absolutely distinct from the true nerve poison. A solution of the poison that has been freed from toxine by treatment with brain substance still retains all its lytic properties, and is therefore still poisonous. But if it is then subjected to further treatment with blood-corpuscles, it is, in the case of cobra venom, which contains hardly any hæmorrhagine, rendered almost completely innocuous. In immunisation, however, both antitoxine and antilysine are formed, so that anti-snake venom serum also prevents hæmolysis.

Hæmolysis occurs *only* in the presence of fresh serum, and this has been shown by PHISALIX[2] to be also the case with the

[1] Flexner and Noguchi, "Snake Venom in Relation to Hæmolysis," *Journ. of Exper. Med.*, vi., 277, 1902 (reprint).

[2] Phisalix, "Action du venin de vipère, &c.," *Soc. Biol.*, liv., No. 27, 1902.

hæmolysine of *vipers' venom*. The serum contains a *complement*, and the snake venom a (heat-resisting) *amboceptor*. Snake hæmolysine is thus not a simple lysine, like ricine, staphylotoxine, &c., but a *haptine of the second class*.

It contains a series of different amboceptors, which, when tested by Ehrlich's method, combine with different blood-corpuscles, although the poison is *never* entirely spent in the process. These amboceptors, again, vary in their affinity for different normal complements, so that all kinds of combinations are produced, some of which are fully active, while others have only slight activity, or none at all.

If the blood be first agglutinated by means of ricine, snake venom has still a hæmolytic action upon it, although the colourless stroma remains agglutinated.

The poison has also the property of preventing the bactericidal function of normal sera when added in the proportion of $\frac{1}{20}$ mgrm. to 1 c.c. of the serum. Only in the case of necturus serum is this action inconstant, for it depends upon a fixation of the *complements*, which in that serum do not invariably enter into combination.

The study of snake hæmolysine was continued by Flexner and Noguchi[1] in a later investigation. *Fresh* snake venom itself was found to contain no complement, and thus to cause only agglutination and never hæmolysis in washed blood-corpuscles.

Yet *snake sera* themselves may, undoubtedly, *sometimes* contain complements capable of entering into combination. *Snake venoms* contain amboceptors of different kinds, with sometimes more affinity for the complement of their own sera, and sometimes more for that of foreign sera. They are related to, but not *identical* with, the amboceptors of snake sera, the latter being *inter alia* invariably "*iso-complementophile*."

Thus, while other poisons only produce hæmolysis with the aid of the serum complements, partial hæmolysis is *invariably* caused by *cobra venom*, even after ever so thorough a washing of the blood-corpuscles. This circumstance, coupled with the fact that the action of cobra venom is also promoted by serum that has been heated, and is thus free from complements, has led to the assumption that *endo-complements* for cobra venom are present in the blood-corpuscles themselves. This conclusion was also arrived at, about the same time, by Kyes[2] and Kyes

[1] Flexner and Noguchi, "The Constitution of Snake Venom and Snake Sera," *Univ. of Pennsylv. Med. Bull.*, 1902, [Nor.] (reprint).

[2] Kyes, "Ueb. d. Wirkungsweise des Cobragiftes," *Berl. klin. Woch.*, 1902, Nos. 38, 39 (reprint).

and SACHS,[1] in Erhlich's Institute, and new and extremely interesting discoveries were also made with regard to the relationship between snake venom and the chemical substances in the corpuscles of the blood. In the first place, it was found by KYES that there were two sorts of blood-corpuscles—viz., those dissolved by cobra venom (such as, *e.g.*, those of the guinea-pig, dog, man, rabbit, and horse), and others that were only dissolved with the aid of a complement—*e.g.*, those of the ox, sheep, and goat. KYES next found suitable complements for these, and was thus able to confirm FLEXNER'S conclusion as to the complex structure of cobra lysine.

In the case of those that were soluble by themselves, KYES was able to disprove the assumption of a simple lysine of the type of ricine by the fact that hæmolysis occurred with dilute, but not with concentrated, solutions of the poison, which would obviously be out of the question in the case of simple poisons. Such a decrease in the action of poisons added in excess is only explicable on the assumption of a *diversion of the complement by the excess of amboceptors*, as was first demonstrated by NEISSER and WECHSBERG.[2]

It was found that the *blood-corpuscles themselves contained a complement*, which could be fixed and diverted by the excess of amboceptors. This *endo-complement* passed into solution on treatment of the blood-corpuscles with water, and then the originally insoluble blood-corpuscles also became susceptible to the action of the cobra poison.

This endo-complement is destroyed by exposure to a temperature of 62° C. for thirty minutes. Sometimes, too, it can be washed almost completely out of the blood-corpuscles by means of physiological salt solution. The discovery of such complements in *red* blood-corpuscles is very interesting, as bearing upon the views of the French school that the leucocytes are invariably the source of the complements.

KYES also endeavoured to explain why *heated* serum is still able to bring about the action of the poison, in which case there can be no question of complements; even boiling the serum for an hour does not injure this power. In his opinion, *the substance possessing this stimulative power is lecithin*, which also acts as a "complement" for cobra venom when dissolved in methyl alcohol. The two enter into so firm a combination

[1] Kyes and Sachs, "Zur Kenntn. d. Cobragift aktivierenden Subst.," *Berl. klin. Woch.*, 1903, Nos. 2-4 (reprint).

[2] Neisser and Wechsberg, "Ueb. d. Wirkungsart baktericider Sera," *Münch. med. Woch.*, 1901, No. 18 (reprint).

that on the addition of ether practically no lecithin is dissolved. This compound has an intense hæmolytic action, even at 0° C. The lecithin of the sera is more or less firmly combined with albumin, so that it is necessary to heat it for varying periods in order to obtain active *free* lecithin. The lecithin, as KYES and SACHS have fully demonstrated, has no connection with the true complement of sera, which is destroyed by heat.

They found, for instance, that the true complement could be destroyed by papaïn and ether; moreover, active sera containing the complement had a restrictive action upon lecithin. But it would seem that the so-called *endo-complements* are nothing more than *lecithin*, whose sensitiveness to heat in the blood-corpuscles is a deceptive phenomenon due to its being combined with the hæmoglobin. Aqueous extracts of the stroma freed from hæmoglobin do not show this sensitiveness to heat.

Owing to their containing lecithin, bile and heated milk, and also the similarly constituted *cephalin*, have this stimulative effect. These substances have in themselves only a very slight hæmolytic action. It is probable that the fatty acid group in the lecithin is the ultimate active hæmolytic factor.

Cholesterin has been found an antidote to this stimulative effect of lecithin, and it has also a protective action in normal sera, and, as was mentioned above, has an influence upon the toxic components of snake venom (PHISALIX). It has a similar antihæmolytic effect upon *saponine* (RANSOM [1]).

On the other hand, cholesterin has no action upon the true complements of sera that stimulate activity.

The amboceptors of cobra venom combine with lecithin in accordance with quantitative laws.

The fact mentioned by FLEXNER and NOGUCHI that all the washed blood-corpuscles are not dissolved appears to be due to the removal of the lecithin by too liberal washing.

KYES[2] even succeeded in isolating these "*lecithides*" of cobra amboceptors. The 1 per cent. solution of cobra venom was shaken for two hours with a solution of the purest lecithin in chloroform. On now separating the chloroform layer by rapid "centrifuging," and treating it with ether, the cobra venom lecithide was precipitated, while the excess of lecithin remained dissolved in the ether.

[1] Ransom, "Saponin und sein Gegengift," *Deutsch. med. Woch.*, 1901, 194.

[2] Kyes, "Ueber die Isolierung von Schlangengiftlecithiden," *Berl. klin. Woch.*, 1903, Nos. 42, 43.

The hæmolytic function of the cobra venom was attached quantitatively to this lecithide, whereas the neurotoxic function remained absolutely unaffected by the lecithin. The lecithide possessed only hæmolytic powers, while the neurotoxic function was retained *exclusively* by the residual poison.

The lecithide is insoluble in ether and acetone, but dissolves in chloroform, alcohol, and toluene, and is readily soluble in water. Its properties are thus quite distinct from those of its two components. On standing in aqueous solution it becomes gradually insoluble without losing its hæmolytic power. It does not separate out from a hot solution. It does not give the biuret reaction. It dissolves all blood-corpuscles equally, and in contradistinction to this function of the poison itself, does so *without a period of incubation.* The lecithide is hardly affected by a temperature of 100° C. Its action, like that of the fresh venom, is prevented by cholesterin. Closely analogous lecithides have been obtained from all the other hæmolytic snake poisons examined, including those of *Bothrops*, *Naja haje*, *Bungarus*, *Trimeresurus*, and *Crotalus.* Thus the same "*lecithinophile*" group is invariably present, even though the amboceptors may differ in other respects.

Leucocidine of Snake Venoms.—Sterile exudations containing 20 to 25 per cent. of lymphocytes were obtained by the intra-pleural injection of the dead cells of *B. megatherium* (Flexner and Noguchi). Cobra venom in solutions containing 0·002 per cent. acted upon these, the action of the other venoms being weaker. The movements of the leucocytes cease first; then follows the decomposition of the cells, and, finally, that of the lymphocytes. In the case of washed leucocytes little more than agglutination occurred. Flexner and Noguchi concluded from their experiments on the modes of combination that the agglutinines were identical with, but the lysines different from, the corresponding agents of the red blood-corpuscles. The leucolysine was also of complex structure.

Summary.—We have thus in snake venoms four distinct active principles, the proportions of which show great variations.

1. *Hæmagglutinines.*—These are destroyed by a 0·2 per cent. solution of hydrochloric acid in twenty-four hours, and in a short time by heating them to 75° C.

2. *Hæmorrhagine* (principally in crotalus venom).—This is only destroyed after about two days by hydrochloric acid (2 per cent.) and pepsin-hydrochloric acid, and can resist the temperature of an incubating oven.

3. *Hæmolysine*, which is very slowly destroyed by hydrochloric

acid (up to 3 per cent.), but rapidly destroyed by pepsin-hydrochloric acid. Exposure to an incubating temperature destroys it to the extent of about 80 per cent.

4. *Neurotoxine.*—This is fairly resistant to the action of hydrochloric acid (up to 3 per cent.) and to pepsin and papaïn. It loses about 90 per cent. of its toxicity by being allowed to stand for nineteen days.

The hæmagglutinine and hæmolysine attack the blood-corpuscles exclusively, while the hæmorrhagine attacks the endothelium of the walls of the vessels, and the neurotoxine the cells of the central nervous system.

IMMUNISATION AGAINST SNAKE TOXINE.

Snake Antitoxine.

The close relationship between snake toxines and true toxines is shown, above all, by their power of producing an antitoxine. The first attempts to produce immunity against snake venom were those of SEWALL,[1] who used crotalus venom in his experiments.

CALMETTE succeeded in showing that, even after a single injection of half a lethal dose, the serum of the animal treated had an unmistakable antitoxic action *in vitro*. FRASER,[2] too, was able to produce antitoxine to the venoms of the cobra, crotalus, diemenia (South Australia), and sepedon (Africa). He obtained preparations capable of resisting as much as fifty times the lethal dose.

Calmette's method is essentially as follows:—About $\frac{1}{20}$ of the lethal dose is first introduced, and this is followed every two or three days by very gradually increasing doses (up to $\frac{1}{10}$ of the lethal dose). The same result can be obtained with poisons chemically weakened by means of gold chloride or calcium chloride (CALMETTE[3]).

After four or five weeks the animals can resist twice the lethal dose. They can then be treated every eight or ten days with larger doses, until a very high degree of immunity can be pro-

[1] Sewall, "Exper. on the Preventive Inoculation of Rattlesnake Venom," *Journ. of Physiol.*, viii., 203, 1887.

[2] Fraser, "Immunity against Snake Poison," *Brit. Med. Journ.*, i., 1309, 1895.

[3] Calmette, "Propriétés du serum des animaux immunisés contre le venin des serpents," *Comptes Rend.*, cxviii., 120, 1004, 1894.

duced. CALMETTE rendered a rabbit so immune in one year that it was able to receive eighty times the lethal dose of cobra venom (40 mgrms.) without showing any reaction. The serum of this animal was so rich in antitoxine that 5 drops (about 0·25 c.c.) neutralised 1 mgrm. of cobra venom. An ass received 0·2 grm. of cobra venom in three months, and another 0·16 grm. in two months. Half a c.c. of the serum then neutralised 1 mgrm. of the venom.

The injection of 4 c.c. of this serum four hours previously afforded protection against twice the lethal dose. According to PHISALIX and BERTRAND,[1] however, the antitoxine to viper poison is not effective until thirty-six to forty-eight hours after its introduction into the body. When an absolutely fatal amount of venom is injected, and then, after the lapse of an hour, 4 to 5 c.c. of this serum, the animal usually survives, but an hour and a half is the maximum time if there is to be any reasonable certainty of a cure.

This anti-cobra serum *also affords protection against the neurotoxic components of other snake poisons*, and also against *scorpion venom* and *eels' blood*, and to some extent against *abrine*, but has no effect upon ricine or upon diphtheria virus, or tetanus poison.

The serum has just the same sort of *antitoxic* action as the true antitoxines, but, on the other hand, CHATENAY (quoted by CALMETTE, *loc. cit.*) observed hyperleucocytosis after the introduction of the poison into immunised animals, whereas hypoleucocytosis could be observed in the case of the control animals.

These observations were confirmed by CALMETTE and DÉLÉARDE. They found that animal charcoal impregnated with abrine and introduced into the peritoneal cavity of immunised animals was absorbed to a large extent by the leucocytes, while there was practically no absorption in the case of the control animals. They came to the conclusion that the leucocytes produced and stored up antitoxine.

According to PHISALIX and BERTRAND[2] normal antisera are produced by the guinea-pig, horse, and hedgehog, as well as by the rabbit, but not by the hen.

Passive immunity soon disappears, but in the case of active immunity the higher its degree the longer it persists. Immunity can be inherited.

Each of the three components of the poison (hæmolysine, neurotoxine, and hæmorrhagine) produces its specific anti-body

[1] Phisalix and Bertrand, "Sur la propriété antitoxique du sang des animaux vaccinés contre le venin de vipère," *Comptes Rend.*, cxviii., 356, 1894.

[2] Phisalix and Bertrand, *Soc. Biol.*, xlviii., 396, 1896.

in the immunisation process. Hence the different antisera differ very greatly in their action. It was shown by STEPHENS and MYERS[1] that there were antisera to hæmolysine, or that the ordinary antisera also contained antihæmolysine; yet even in this case, according to FLEXNER and NOGUCHI, the antisera vary in their activity, since the hæmolysines, again, possess amboceptors of different kinds, and the antidotes act by means of anti-amboceptors.

The anti-cobra venom contains an antitoxine not only to cobra neurotoxine, but also to that of the most widely differing snake venoms (M'FARLAND[2]), and also an antitoxine to the hæmolysine, but is completely lacking in the anti-body to the *hæmorrhagine* of *crotalus venom;* and since this is the chief active constituent in crotalus venom (*vide supra*), Calmette's "antivenine" is powerless against that poison.

In like manner, anticrotalus serum contains chiefly antihæmolysine and antihæmorrhagine, but not antineurotoxine; hence, it neutralises the hæmolytic but not the neurotoxic function—*i.e.*, the general toxicity—of cobra venom.

FLEXNER and NOGUCHI[3] obtained a serviceable antiserum to rattlesnake venom on removing the substances that produced the necroses by treating the toxine with dilute hydrochloric acid or iodine trichloride. The serum had no action upon the venoms of the cobra, daboia, and mocassin snake.

In the case of those venoms which contain both the main poisonous components, such as those of the mocassin and copperhead snakes, the antisera naturally contain both specific antibodies.

The action of anti-snake-venom serum, according to all that is known about it, is apparently quite analogous to that of the other antitoxines—*i.e.*, it combines with and neutralises the poison without destroying it. A very interesting proof in support of this view was furnished by Calmette's experiment (1895), of which an outline was given in the General Part. He found that on heating a physiologically neutral mixture of snake toxine and antitoxine to 68° C. the antitoxine could be eliminated, so that the original toxic action again appeared. After the heating the mixture behaved exactly like the toxine, either if it had contained antiserum or normal serum. In like

[1] Stephens and Myers, *Proc. Path. Soc.*, *Lancet*, 1898, i., 644.

[2] M'Farland, "Some Investigations upon Antivenine," *Journ. Amer. Med. Assoc.*, Dec. 1901; abstract in *Centralbl. f. Bakt.*, xxxi., 792.

[3] Flexner and Noguchi, "Upon the Production and Properties of Anticrotalus Serum," *J. of Med. Research*, xi., 363, 1904.

manner, the toxine could be removed from the mixture by precipitation with calcium chloride, so that the previously neutral serum again had a protective action (1896). These results, which are extremely important from the theoretical point of view, have been conditionally confirmed by MARTIN and CHERRY,[3] who were able, under certain conditions, to effect a separation of the poison of *Hoplocephalus curtus* from the antitoxine by heating the mixture to 68° C.

Thus, when the components of the mixture have only been allowed to act upon each other for a short time, or when a relatively large amount of poison has been used, CALMETTE'S statement is correct; the antitoxine is destroyed and the toxic activity restored by heating the mixture. After fifteen minutes, however, the combination is so firm that it can no longer be broken up. Again, it is impossible to effect a separation by filtration through a gelatin filter under pressure, which otherwise allows the free toxine but not the antitoxine to pass, since no part of the mixture passes through the filter.

The conclusion that there is a simple combination is in no way affected by the fact that, according to MARTIN, the dose that combines with a definite quantity of snake venom *in vitro* is much less than that required for previous immunisation when injected *subcutaneously*. The quantity is considerably greater and may even amount to a thousand times as much. On the other hand, only about the same quantity is required if *intravenous* injection be used.

MARTIN attributed this to a much slower diffusion of the antitoxine than of the toxine on subcutaneous introduction, the toxine being also very rapidly distributed through the body by way of the anastomosing lymph tracts.

It is also worthy of note that CALMETTE (1895) found that tetanus antitoxine and antiabrine had also a certain action upon snake toxine, so that the serum would thus seem to be not absolutely specific. On the other hand, the sera of animals that had been treated with strychnine, curare, and various bacteria proved as absolutely powerless against the venom as normal human serum.

Anti-snake-venom serum only becomes inactive when the temperature reaches 68° C. Calcium chloride and gold chloride do not affect its protective power. It can also be kept for a long period without the addition of phenol.

[3] Martin and Cherry, "The Nature of Antagonism between Toxines and Antitoxines," *Proc. Roy. Soc.*, lxiii.; Martin, "Relation of the Toxine and Antitoxine of Snake Venom," *ibid.*, lxiv., 88, 1899.

CALMETTE[1] has devised the following method for the valuation of snake serum :—

The poison is dried and dissolved in distilled water and the lethal dose for 1 kilo. of rabbit determined. A rabbit weighing 2 kilos. is then treated with increasing doses of the serum under examination and the protective dose against a single lethal dose determined. A serum, 1 c.c. of which protects 1 grm. of animal against the single lethal dose, is taken as unity. Thus, if 1 c.c. of the serum under examination protects 2 kilos. of animal, the strength of the serum is two thousand-fold. According to CALMETTE, the minimum strength must be one thousand-fold; sera of, at least, four thousand-fold strength are used in the tropics.

This true antitoxic power of the immune serum has nothing to do with the property possessed by tyrosin, cholesterin, &c., of rendering the toxine innocuous, for all these substances act just as they do in the case of tetanus toxine, only combining with the poison *in vitro*, and never *producing immunity*. Their action is thus quite distinct from that of the true *antitoxines*.

The question whether or no the serum and bile of venomous snakes contain *antitoxine* stands upon a somewhat different footing. As was found by FRASER,[2] not only is the bile of the *cobra* particularly active, but also that of the rattlesnake, &c., possesses incomparably greater powers of destroying the poison than the bile of other animals; and even that of non-venomous snakes has a greater protective power than theirs. This protective function is also retained by alcoholic precipitates of these biles. On the other hand, it must not be lost sight of that, as FRASER[3] himself found, the antivenom is not definitely specific, but that it also acts upon bacterial toxines.

At the same time, we frequently find that the bile merely has a *destructive* action upon toxines similar to that of other digestive fluids, so that the question whether or no the bile contains a true antitoxine has not yet been decided.

Hence, one is hardly justified in attributing the resistance of snakes to this function alone. It is, indeed, more conceivable that the natural immunity of these animals is due to a continual new formation of large quantities of antitoxine, rather than to a

[1] Calmette, "Sur le venin des serpents," *Ann. Past.*, xi., 214, 1897.

[2] Fraser, "The Treatment of Snake Poison with Antivenene," *Brit. Med. Journ.*, ii., 417, 1895; "Antivenomous Properties of the Bile of Serpents," *ibid.*, ii., 125, 1897.

[3] Fraser, "Antitoxic Qualities of the Bile of Serpents," *Brit. Med. Journ.*, ii., 595, 1897.

fixed power of reacting upon any poison absorbed. But surely this condition must depend, in the main, upon an innate want of receptors; for, as we have seen, even the blood of venomous snakes is poisonous.

In the case of the *hedgehog*, too, which is to some extent refractory, the want of susceptibility appears to be principally due to its possessing few receptors; after the introduction of the venom its blood also is *poisonous*. The effect of the venom upon the hedgehog is similar to that of tetanus toxine upon the alligator; for the hedgehog, although but slightly susceptible, yet produces fairly considerable amounts of antitoxine. It thus appears to possess receptors, but these are, for the most part, in the organs less vitally important. On the other hand, CALMETTE (1895) found the serum of the *pig* and *mongoose* to contain extremely little antitoxine, although both animals are almost completely immune to snake venom.

Doubtless this is also the case with snakes themselves. The main cause of their insusceptibility may be a congenital want of receptors, or to the existing receptors being too far apart; but at the same time it is, of course, not improbable that they also produce *antitoxine* and excrete it with the bile.

Toad Toxine (Phrynolysine).

Certain toads contain in their skin and blood, in addition to the better-known alkaloidal poisons (bufotaline, &c.) an apparently true toxine possessing hæmolytic powers.

PHISALIX and BERTRAND[1] appear to have been the first to discover the existence of a second poison, and its hæmolytic action was recorded by PUGLIESE.[2]

"*Phrynolysine*" was then investigated more closely by PRÖSCHER.[3]

In his experiments PRÖSCHER chiefly employed extracts of the skin of the fire toad (*Bombinator igneus*), though the skin of the garden toad (*Bufo cinereus*) was also used.

Phrynolysine has all the characteristics of toxines, and particularly their great sensitiveness to external influences. It is non-dialysable. It becomes inactive fairly rapidly.

It produces hæmolysis equally well in a neutral or slightly

[1] Phisalix and Bertrand, "Recherch. s. la toxicité du sang du crapaud commun," *Arch. d. Phys.*, xxv., 517, 1893.

[2] Pugliese, *Arch. d. Farm.*, 1898, quoted by Pröscher (*loc. cit.*).

[3] Pröscher, "Zur Kenntnis des Krötengiftes," *Hofm. Beitr.*, i., 575, 1901.

acid solution. Sheep's blood is the most susceptible, and then comes the blood of the goat, rabbit, dog, ox, hen, and guinea-pig, while that of the pigeon, frog, and toad is hardly affected. In the case of sheep's blood, about 0·3 mgrm. is sufficient to dissolve a litre completely.

There is as yet no reason for concluding that phrynolysine has a complex structure.

Normal sera do not contain any anti-body. By immunisation, however, it is possible to produce an antilysine, which in a dose of 0·025 c.c. affords protection against a dose of the toxine sufficient to hæmolyse 1 c.c. of a 5 per cent. emulsion of sheep's blood.

Salamander Poison.

A poisonous substance that formed an antitoxine was discovered, by PHISALIX,[1] in the skin of the back of the Japanese salamander (*Sieboldia maxima*).

The poison is soluble in water and glycerin, and possesses little stability. It is completely destroyed by twenty minutes' exposure to a temperature of 60° C., and also by alcohol.

It produces œdema and areas of hæmorrhage in the frog, and in warm-blooded animals necroses also. Paralysis also occurs, and the excitability of the nerves is gradually lost; death results from the paralysis of the respiratory system.

The poison is weakened by being heated to 50° C., but still retains its immunising power. Animals thus treated can then resist much larger doses, not only of this poison but also of viper venom and eel's-blood poison (PHISALIX[2]), whence we may conclude that it has a certain degree of relationship with these toxines.

Spider Venom.

Poisonous spiders occupy an important position in the popular imagination. Very many spiders, and notably the *tarantula*, have been accused of possessing toxic properties. Little, however, was known scientifically about spider poisons until the appearance of KOBERT'S[3] comprehensive monograph.

[1] Phisalix, "Act. phys. venin du Salamandre," *Soc. Biol.*, xlix., 723, 1897.

[2] Phisalix, "Propr. immunisantes du venin du Salamandre," *Soc. Biol.*, xlix., 823, 1897.

[3] Kobert, *Beitr. z. Kenntn. d. Giftspinnen*, Stuttgart, 1901 (gives the older literature from the earliest times); *id.*, "Giebt es für den Menschen gefährliche Spinnen?" *Die Med. Woche*, 1902, 154.

KOBERT was able to show that there was no specific poison in those species of spiders most accused of containing it, and in particular the *tarantulas*. In fact, real poisons were only found in two genera—viz., *Lathrodectes* and *Aranea diademata* (*Epeira diadema*, the garden spider).

The *Lathrodectes* are distributed over the whole globe. The most important species are those of Italy (*L. tredecimguttatus*, Malmignatte) and of South Russia (*L. erebus*, Karakurte), with those of New Zealand (*L. scelio* and *Hasseltii*, Katipo) and of South America (*L. mactans*). As far back as 1765 VALMONT DE BOMBARE gave a description of the poisonous properties of the malmignatte, and since then they have frequently been studied. Experiments on animals have also been made.

A whole series of reports has been collected by KOBERT with regard to the Russian spider, and from these it appears that the *Lathrodectes* not only inflicts great injury upon cattle, horses, and camels, but even kills men. The effects are very severe: violent pains, priapism, sleeplessness, great prostration, cold sweating, fever, and dyspnœa. The general symptoms closely resemble those caused by poisoning with bacterial toxines. No striking alterations can be observed in the vicinity of the bite. Convalescence is very tedious, but fatal cases are very rare.

KOBERT has made experiments on his own account with aqueous extracts of these Crimean karakurtes, some of the extracts being prepared from the fresh animals, and others from those that had been partially dried.

These extracts were found to be very poisonous, producing exactly the same results as those observed in men bitten by the spider. The intravenous injection of a few mgrms. per kilo. of organic substance into dogs and cats produced dyspnœa, convulsions, and paralysis of the respiratory system and heart, speedily ending in death. Rabbits, rats, and birds were also susceptible to the poison, but the hedgehog offered somewhat more resistance. Frogs and leeches could also be poisoned. There was practically no difference in the action of extracts obtained from the front and from the back portion of the spider. New-born spiders were more venomous than full-grown ones, and even the eggs were poisonous. The poison had an injurious effect upon the *isolated heart of the frog*, even in the proportion of 1 : 100,000.

KOBERT also succeeded in producing immunity by cautiously inoculating experimental animals, so that karakurte poison appears to be a true toxine. Boiling renders the poison completely inactive, and alcohol has the same effect. Introduced

per os the poison has no effect. It has also a *hæmolytic* action, and promotes coagulation.

Hence the lathrodectes contains a *true toxine which in many cases has first an irritant action upon the heart and central nervous system, but, eventually, invariably causes paralysis.*

Arachnolysine.

Of the German spiders *Chiracanthium nutrix* appears to contain a poison which has not yet been investigated.

KOBERT found, however, in the common garden spider, *Aranea diademata* (*Epeira diadema*), a toxine completely analogous to lathrodectes poison; it, too, was very poisonous, and it was possible to produce immunity against it. It was somewhat less active, but more stable, than lathrodectes poison.

The extracts of other German spiders were inert. The poison of the garden spider was also found by KOBERT to possess hæmolytic powers, which were subsequently more closely investigated by HANS SACHS,[1] with the aid of Ehrlich's method.

Arachnolysine has a very rapid and intense solvent action upon the blood-corpuscles, but there is great variation in the resistance offered by the corpuscles of different species of animals. The blood-corpuscles of the rat and of the rabbit are the most susceptible, 0·028 mgrm. completely dissolving 0·05 cm. of the blood. On the other hand, the blood of the guinea-pig, horse, sheep, and ox is *absolutely* refractory.

The blood-corpuscles of chickens are quite insusceptible, according to SACHS,[2] in consequence of a total lack of receptors. It is only when these first blood-corpuscles have gradually disappeared that the lysine has any action, and it is not until after two to four weeks that the normal susceptibility of hen's blood is attained.

The toxine is not very sensitive to the action of heat, and can resist a temperature of 70° C. for forty minutes.

Insusceptible blood-corpuscles do not combine with the poison, so that the behaviour of this lysine is quite in accord with that of other haptines. A further point of agreement is that arachnolysine enters into combination with the *stroma* of *susceptible* blood-corpuscles.

SACHS, by immunising guinea-pigs and rabbits, succeeded in

[1] Sachs, "Zur Kenntnis des Kreuzspinnengiftes," *Hofm. Beitr.*, ii., 125, 1902.

[2] Sachs, "Ueber Differenzen der Blutbeschaffenheit in verschied. Lebensaltern," *Centralbl. f. Bakt.*, xxxiv., 686, 1903 (reprint).

producing a highly active antitoxic serum, which, when mixed with the poison, also prevented the hæmolysis. Now, inasmuch as the blood of the guinea-pig is insusceptible, and therefore contains no receptors, the antiarachnolysine must have been formed from other receptors. Since, however, it has also an antilytic action, it is probable that the same conclusions may be drawn as to its constitution as in the case of *ricine* (*q.v.*), to which it offers many points of resemblance.

Scorpion Venom.

Valentin,[1] in 1874, investigated the effects of the venom in the sting of the tail of a Tunisian scorpion (*Androctonus occitanus*, Claus).

Small frogs were usually killed by the sting, but not larger ones. It caused tetanic convulsions, and also twitchings of the fibrillæ, while the reflex excitability gradually disappeared from behind forwards.

Very similar results were also observed later by Bert[2] and others.

According to Wilson[3] scorpion venom causes convulsions and death from asphyxia in the guinea-pig.

The poison resembles veratrine in acting directly upon the muscles, while the nerves are not affected. Certain animals that live in the desert (jerboa, gerbillus) offer great resistance to scorpion venom, an immunity probably acquired from frequent stings.

Calmette found (1895) a poison in the tail segment of *Scorpio afer*, and isolated it by extracting the crushed bodies with water and drying the extract in a vacuum.

The poison killed mice in doses of 0·05 grm., and guinea-pigs in doses of 0·5 grm., the symptoms closely resembling those produced by snake venom.

It also behaves in every other respect, and especially in its relation towards the antitoxine, so exactly like snake toxine, as to justify the conclusion that scorpion venom differs no more from snake venoms than do these from each other, and also that, like them, it contains the neurotoxine of the snakes in admixture with small proportions of foreign bodies. This conclusion is also supported by the fact that Kyes (*loc. cit.*) was able to isolate

[1] Valentin, "Ueber d. Giftw. d. nordafrik. Scorpiones," *Zeit. für Biol.*, xii., 170, 1876.

[2] Bert, *Soc. Biol.*, xxxvii., 574, 1885.

[3] Wilson, "Action of Scorpion Venom," *Journ. of Physiol.*, 1904, 31.

from scorpion venom a lecithide possessing an immediate blood-solvent action, and exactly analogous to that obtained from cobra venom. The *sting* of the scorpion is not very dangerous to man, but only because the amount of venom injected in a single sting is too small.

Fish Venoms.

An apparently true toxine was isolated by BRIOT[1] from the poison glands of the *Trachinus draco* (greater weever), though its existence had already been known. He extracted the poison glands by means of glycerin containing chloroform, and filtered the neutral solution.

The poison causes convulsions and paralysis in frogs, and death results from prostration (GRESSIN, BOTTARD). The heart is also directly affected (POHL[2]).

It has a very similar energetic action upon guinea-pigs, especially on intraperitoneal injection (and this was also found to be the case by PHISALIX with *Trachinus vipera*), but has less effect upon rabbits.

A characteristic symptom is the rapid paralysis of the extremity where the poison was injected. Death results almost instantaneously after intravenous injection; but the animal recovers very rapidly from the effects of smaller doses. The poison also causes severe local injuries on subcutaneous injection.

The toxine is destroyed when heated for thirty minutes at 100° C., and also by calcium chloride and gold chloride.

The poison also possesses hæmolytic powers. The *normal serum* of the horse contains an antihæmolysine against trachinus lysine, just as it does against snake hæmolysine. This is destroyed at 50° C., but the lysine can resist a temperature of 100° C. for a short time (twenty minutes).

Trachinus poison is quite distinct from snake venom, since its action is different, and an anti-snake-venom serum has no restrictive effect either upon its toxic or its lytic function.

Rabbits can best be rendered immune by the cautious injec-

[1] Briot, "Études sur le venin de la vive (*Trachinusdraco*)," *Journ. de Phys. et Pathol.*, 1903, 271 (reprint). Briot cites the following works on poisonous fish:—Gressin, *Contrib. à l'étude de l'appareil à venin chez les poissons du genre vive*, Thesis, Paris, 1884; Bottard, *Les poissons vénimeux*, Thesis, Paris, 1884; Phisalix, *Bull. du Museum d'Histoire Natur.*, 1899; Contière, *Les poissons vénimeux*, Thesis, Paris, 1899. See also Kobert, *loc. cit.*

[2] Pohl, *Prager Med. Woch.*, 1893, 31.

tion of a mixture of the poison with the serum of an animal already immune.

The serum contains a specific antitoxine, which, however, does not invariably afford protection against the local effects.

Certain other fishes also appear to contain poisons of the nature of toxines, which have not yet been investigated, such as, *e.g.*, the lamprey (*Petromyzon*), *Scorpæna*, *Pterois*, *Serranus*, *Plotosus*, *Synanceia*, &c. (KOBERT [1]).

Further investigation is required to determine whether the poisoning in these cases is due to true toxines.

In the case of certain other poisonous fish—*e.g.*, the Japanese species of *Tetrodon* (*fugu*)—poisonous protamines appear to play the chief part. It does not come within the scope of this book to give further details of fish poisons in general.[2]

The Poison of Eels' Blood (Ichthyotoxine).

An exceptional position is occupied by the poisonous substance which occurs in the serum of the eel and certain allied fish (*Muræna*, Conger). By its occurrence as a normal product of animal life it is related, on the one hand, to the snake venoms, which are also found in the blood of poisonous snakes; while, on the other hand, in its hæmolytic function it recalls the agglutinating toxines of the vegetable kingdom; and yet again shows a certain relationship, as regards this property, with the hæmolysines of the normal sera of different animals, which, according to EHRLICH, are also not simple "alexines," but receptors of the second class provided with amboceptor and complement.

In what group, therefore, ichthyotoxine should eventually be placed cannot be decided off-hand. It is convenient, however, for reasons apart from its internal constitution, to describe it provisionally after the other zootoxines.

A similar poison appears to be produced in the *poison glands* of the muræna, but little is known about it.[3]

The toxicity of eels' blood was discovered by A. Mosso,[4] who also studied its toxic effects. He found that the blood of poisoned animals lost its power of coagulating, and this was confirmed by

[1] Kobert, "Ueber Giftfische u. Fischgifte. Vortrag i. Rostocker Fischereiverein," *Die Med. Woche*, 1902.

[2] Further details are also given by Vaughan and Novy, *loc. cit.*, 188, *et seq.*

[3] Anatomical and toxicological data are given by Kobert, *loc. cit.*

[4] A. Mosso, "Die giftige Wirkung des Serums der Mureniden," *Arch. f. Exper. Path.*, xxv., 111, 1889.

Delezenne,[1] who compared its action with that of propeptones. U. Mosso[2] also investigated the properties of this poison.

Then came the notable work of Kossel[3] and Camus and Gley,[4] who discovered the solvent action of eels' blood poison upon the blood, and showed that immunity could be produced against it, and that it formed an *antitoxine*.

Preparation and Properties of the Poison.—Camus and Gley obtained the poisonous serum by taking the blood by means of a sterilised pipette from the aorta of the eel, placing it in a sterilised vessel, and either allowing the serum to separate spontaneously or with the aid of centrifugal force. They thus obtained about 0·6 c.c. from 100 grms. of the fish.

The serum has a slight green colour, frequently of a yellowish shade. It can be kept for a long time unaltered if protected from light.

No attempt has yet been made to prepare the toxic principle of the serum in a pure form.

U. Mosso showed that it possessed exactly the same physical and chemical properties as all toxines; that it was destroyed by heat, acids, alkalies, &c.; that it could resist drying *in vacuo*; and that it was not dialysable. It was insoluble in alcohol of 90 per cent. strength.

Action of Eels' Blood.—The serum of different eels frequently varies considerably in its toxic effects, the season of the year having an influence as well as the origin of the fish, as has also been observed in the case of snake venoms (Wehrmann[5]).

The river eel of the Baltic coast contains, according to Springfeld,[6] a very much weaker toxic serum.

Different animals also vary in their susceptibility when the poison is introduced in the same way (intravenously). Dogs

[1] Delezenne, "Action du sérum d'anguille sur la Coagul. du Sang," *Arch. d. Phys.*, xxix., 646, 1897.

[2] U. Mosso, "Recherches sur le nature du venin, qui se trouve dans le sang de l'anguille," *Arch. Ital. d. Biol.*, xii., 229, 1889.

[3] Kossel, "Zur Kenntnis der Antitoxinwirkung," *Berl. klin. Woch.*, 1898, 7.

[4] Camus and Gley, notably "Recherches sur l'action physiolog. de sérum d'anguille," *Arch. Intérnat. de Pharmacodyn.*, v., 247, 1898 (reprint); also "De la toxicité du sérum d'anguille pour des animaux des espèces differents," *Soc. Biol.*, 1898, 129; "Immunis. contre l'action globulicide, &c.," *Comptes Rend.*, cxxvi., 428, 1898; and "Nouvelles recherches sur l'immunité contre le serum d'anguille," *Ann. Past.*, xiii., 779, 1899.

[5] Wehrmann, "Sur les propr. toxiques du sang, &c.," *Ann. Past.*, xi., 810, 1897.

[6] Springfeld, "Ueb. d. gift. Wirkung des Blutserums des Flussaals," Dissert. Greifswald, 1889.

appear to be the most susceptible (lethal dose, according to Mosso, 0·02 c.c. per kilo.), while the hedgehog is almost refractory. A. Mosso states that it has no action when introduced into the stomach, though it is poisonous when injected into the small intestine. On the other hand, a serious case of poisoning in man after eating eels' blood is reported by PENNAVARIA.[1] Subcutaneous injection produces necroses and abscesses. The course of the poisoning takes two distinct forms, according to the dose. Rabbits die in convulsions a few minutes after receiving 0·1 c.c. per kilo. of a very active serum, while sometimes there is also a flow of saliva and blood into the urine. Myosis is hardly ever absent, and sometimes exophthalmus occurs.

The effects are attributed by Mosso to paralyses of the vagus, preceded by stimulation.

With small doses or weak poisons, however, conditions of paralysis are produced, together with fine tremors, areas of local anæsthesia, dyspnœa, flow of saliva, cries, &c., which, after a great loss in weight, end in death, though only after some hours, or even days (KOSSEL).

Similar results have been observed with guinea-pigs. When very large doses have been given death occurs so rapidly that sometimes even the convulsions fail to appear.

In the first case, therefore, the bulbar symptoms, especially the rapid paralysis of the respiratory centre, predominate; in the second case, the spinal symptoms. The peripheral nerves of the respiratory centre do not lose their capacity for being stimulated.

In the case of the frog A. Mosso was able to prove that the excitability of the nerves and muscles rapidly decreased; sensation, especially in the hind legs, disappeared before the power of motion, probably through the destruction of the tracts from the spinal cord to the brain. The *isolated heart of a frog* was not affected.

In warm-blooded animals the *pressure of the blood* rises immediately after the injection, and then sinks. *The action of the heart* slackens and becomes irregular, but the heart still continues to beat after death (BARDIER[2]). After very large doses, however, it was found by A. Mosso that the animal died very rapidly from paralysis of the heart, while breathing still continued for a minute. The animals (dogs) also died from paralysis

[1] Pennavaria, *Farmacista Italiano*, xii., 328, 1888 (quoted by Kobert, *loc. cit.*).

[2] Bardier, "Action cardiaque du sérum d'anguille," *Soc. Biol.*, l., 548, 1898.

of the heart when artificial respiration was employed. The autopsy showed congestion of the intestinal tract, lungs, and suprarenal bodies; severe lesions of the kidney, even when death was rapid (PETTIT[1]); hyaline degeneration; and swelling of the cells. Blood was present in the urine in the bladder.

Serious alterations in the nervous system, resembling those produced by tetanus, were detected by WESTPHAL[2] by means of Nissl's method.

Thus we see that the effects produced by the poison are closely similar to those caused by *ricine*, snake venom, &c.

Heated serum (58° C.) has still a slight action in very large doses (100 times the ordinary lethal amount) upon the animals, which lose considerably in weight.

Action upon the Blood.—Eels' blood has an energetic hæmolytic action upon the blood even in the body (CAMUS and GLEY). The iris becomes tinged with red; areas of hæmorrhage occur and exudations of blood into the peritoneum, while erythrocytes and hæmoglobin are found in the urine. The arterial blood contains hæmoglobin. As a rule, the resistance of the erythrocytes is so weakened by the addition of $\frac{1}{1000}$ to $\frac{1}{10000}$ part of eels' serum, that they give up their hæmoglobin even to a 0·7 per cent. solution of sodium chloride, whereas the normal corpuscles of rabbits' blood do not part with it until the dilution of the salt solution reaches 0·48 to 0·5 per cent. The blood-corpuscles of the guinea-pig behave in a similar manner, whereas the erythrocytes of the hedgehog have been found refractory, as is also the case with those of hens, pigeons, tortoises, frogs, toads, and bats. It is particularly interesting that, according to H. SACHS, new-born rabbits have a relatively high power of resistance, and do not acquire suitable receptors until a later period (*cf. Arachnolysine*). The hæmolytic function of eels' serum is not affected by cautious neutralisation with hydrochloric acid.

The addition of other sera has also no influence upon it, but it is destroyed by heating it to 55° C. The solvent action does not take place at 0° C., but is very energetic at 23° C.

According to WENDELSTADT,[3] the addition of small quantities of glycogen has an influence upon the hæmolysis.

[1] Pettit, "Altérations rénales consécutives à l'injection du sérum d'anguille," *Soc. Biol.*, l., 320, 1898.

[2] Quoted by Kossel (*loc. cit.*).

[3] Wendelstadt, "Einw. v. Glykogen a. hämolyt Vorgänge, *Central. f. Bakt.*, xxxiv., 831, 1903.

Immunisation against Eels' Blood.—The poison of eels' blood also shows itself to be a true toxine by the fact that it is possible by means of it to produce immunity in susceptible animals.

According to KOSSEL, CAMUS and GLEY, and WEHRMANN and TCHISTOVITCH,[1] rabbits are the best animals to use for the purpose. From 0·05 to 1 c.c. is first introduced, either by subcutaneous or intravenous injection; the animals usually stand this dose well, and can then readily be immunised to a higher degree. It is very difficult to immunise guinea-pigs since they usually succumb; dogs bear the treatment well, but yield only weak antisera. Goats, on the other hand, appear to be suitable animals. Hens and pigeons yield only traces of antitoxine, and that, too, only against the blood-solvent action *in vitro*. Pigeons are very susceptible to the poison, although their erythrocytes are hardly attacked at all by eels' serum. The formation of antitoxine is very rapid, and even after three or four injections a serum is obtained of about $\frac{1}{10}$ to $\frac{1}{20}$ the neutralising strength—*i.e.*, from 10 to 20 c.c. of serum are required to neutralise 1 c.c. of eels' serum.

TCHISTOVITCH determined the strength of his serum as follows:—Five drops of eels' blood (1 : 10 of a 0·7 per cent. solution of sodium chloride) were treated with increasing doses of antitoxine. The reagent for testing both the hæmolytic and the toxic action, consisted of a few c.c. of rabbits' blood diluted to 20 c.c. Parallel tests were made in each case, and some remarkable results were obtained. Thus, while the resistance offered by the rabbits themselves showed a continual increase, the proportion of antitoxine in their serum did not increase in the same manner; but, on the contrary, became continually *less*.

At the same time, the erythrocytes of these animals also showed special characteristics. KOSSEL and CAMUS and GLEY had simultaneously discovered that the erythrocytes of immunised animals were in themselves (*i.e.*, thoroughly freed from serum) under certain conditions *refractory* to the hæmolytic action of eels' blood. TCHISTOVITCH now discovered that the blood-corpuscles as such dissolved very readily when the proportion of antitoxine was *high*; but that they were more or less refractory when the amount of antitoxine in the serum decreased.

There is here a certain parallelism in the phenomena, which, assuming the correctness of the facts, appears to point to a *disappearance* of the receptors for eel toxine, both in the body

[1] Tchistovitch, "Études sur l'immunisation contre le sérum d'anguille," *Ann. Past.*, xiii., 406, 1899.

cells and in the erythrocytes. If we assume that the toxic and hæmolytic principle of eels' serum are identical, we must also assume that receptors adapted to it are present in the body cells as well as in the erythrocytes; a cessation of the formation of receptors under the influence of the immunisation process would then be sufficient to account for both the immunity to the action of the poison notwithstanding the diminished production of antitoxine and also the want of susceptibility of the erythrocytes.

It is, of course, open to question whether both principles are really identical. Here we meet with the same difficulty as in the case of ricine, in which, too, the action upon the blood can be easily prevented without destroying its toxic power; and there, also, the protective influence upon the erythrocytes affords, under normal conditions, a measure of the antitoxic power. And yet, as we have seen above, the question whether or no ricine contains two active substances has not yet been settled, although there is much to be said in favour of JACOBY'S view (*q.v.*) that we may here be dealing with a double-branched receptor.

In the case of eels' blood, however, the conditions are somewhat different.

Here the question is whether the blood-solvent function of the serum ought not to be separated entirely from the toxic function, inasmuch as there may here be hæmolytic processes exactly analogous to those produced by several other *normal sera* acting upon foreign erythrocytes. As EHRLICH and MORGENROTH have shown, in numerous researches, the action in those cases is to be attributed to series of peculiar *haptines* with different specific amboceptors and complements.

It has, of course, not yet been proved that there are not also here, as JACOBY assumes for ricine, two *separate ergophore groups* on one amboceptor, one of which has a hæmolytic and the other a toxic action.

This is also not irreconcilable with the results obtained by TCHISTOVITCH, who found that the hæmolytic function was destroyed by heating the toxine to 55° C. (which, however, is absolutely denied by CAMUS and GLEY), and that the serum thus rendered partially inactive produced *antitoxine* just as before. All these facts could be explained just as well by a partial formation of toxoid as by assuming the existence of two specific haptines with different haptophore groups.

In any case, a definite conclusion can only be obtained by exact combination experiments, on the lines devised by EHRLICH. An attempt must be made to determine whether or no eels'

serum still retains its toxic power after removal by means of specific combinations of the receptors adapted to the corpuscles of the blood.

Toxine of Fatigue.

An apparently true toxine has been separated from the muscles of over-fatigued mammalia by WEICHART,[1] who prepared an extract from them under the strictest aseptic precautions. In small doses the toxine has an immunising effect, but in large doses is fatal. It produces a true antitoxine on immunisation.

[1] Weichart, "Über Ermüdungstoxin, I. and II.," *Münch. med. Woch.*, 1904, No. 1, *et seq.*

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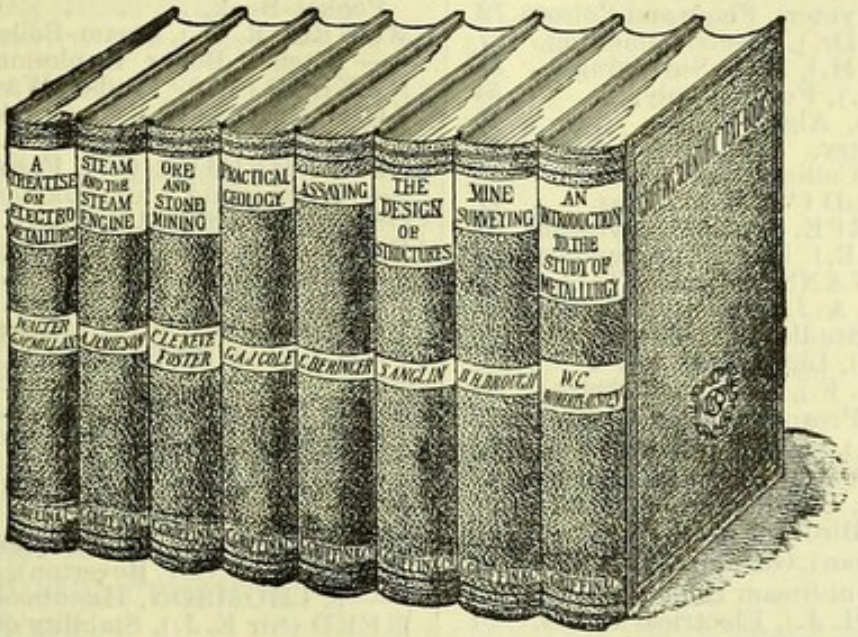

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