OBSERVATIONS UPON THE CONSTITUTION OF THE DIPHTHERIA TOXIN.[1]

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IMMUNITY is a reaction of the living organism towards a given class of poisons, in which are included the toxins, toxalbumins and ferments. The study of the antitoxin producing poisons consequently affords us the best possibility for elucidating this complicated and important phenomenon. There is not much to be expected at present from purely chemical methods of investigation, at least the history of the ferments, which are most closely related to the toxins, does not encourage one to place any implicit reliance upon research in this direction. Our primary duty, therefore, as medical investigators, is to search for a solution of such questions in the field that properly belongs to us—that of biological experiment. Progress was not to be hoped for so long as no clear views prevailed with regard to the essential nature of the antitoxin action and the opinions of the most

[1] Professor Ehrlich has been good enough to write the above paper in compliance with my suggestion that a general survey from his own pen of the results he has reached and which are recorded in many individual papers would be most helpful to his English-speaking colleagues. It is with his full concurrence that I undertook an English translation.—A. MACFADYEN.

I

competent inquirers differed widely, and so long as the problem was not decided whether the antitoxin acted chemically and directly on the toxin or indirectly in virtue of a species of cell immunisation. The first step which I was led to take in this direction consisted in excluding the animal experiments—so difficult to interpret—and in creating simple experimental methods, which would allow, if I might so express it, of work under purely chemical conditions. In this way the experiments *in vitro* originated, by which I was able to furnish the proof that in the case of ricin and abrin, poison and anti-poison act directly on each other. The significance of these investigations, which were speedily confirmed and expanded by Kossel, Camus and Gley, and Kanthack, is apparent, inasmuch as a direct chemical inter-reaction of the two components was proved and the vital theory, as upheld by Roux and Buchner, became untenable. At the same time the essential nature of the process, whether it consists in a destruction of the poison or a mutual combination of the two components, is not at all evident from these experiments. And the recent and valuable experiments of Martin and Cherry, in which mixtures of snake-venom and antivenene were filtered under pressure through gelatine membranes, only prove that snake-venom disappears in the mixture without giving any clue as to the action that takes place. I cannot, therefore, agree with Behring when he considers that these experiments demonstrate a chemical combination to have occurred.

At the time when the doctrine of immunity was in its initial stages it was assumed that the antitoxin destroyed the toxin, somewhat after the manner of a ferment. This point of view has, however, been gradually abandoned, and the suggestion that both components unite chemically now finds many supporters. There is, however, little to be gained by mere hypotheses, and it will be more satisfactory to consider the facts that favour a chemical explanation of the process.

Essentially the question to be proved is, whether neutral toxin-antitoxin mixtures (*i.e.*, such as have become innocuous to test animals) can once more, under given con-

ditions, develop toxic properties, a result which would be impossible if the toxin had been destroyed. In this connection, Buchner found that a mixture of tetanus toxin and tetanus antitoxin, barely innocuous to mice, remained toxic for guinea-pigs.

These results appear in a different light from our present standpoint, and the interpretation to be placed upon them leads me to anticipate a subsequent section of this paper. In such a mixture which approaches the Lo dose certain derivatives of the toxin are present in a free condition, viz. : toxoids and toxones—derivatives that exercise a toxic action on the one species of animals, but not on the other (mice). Similar effects have been noted by different observers in animals characterised as "hypersensitive." I refrain, however, from a discussion of this hypersensitive condition, as the subject still remains a most obscure one.

It would be rash to draw any conclusions of a general character from the uncertain, incomplete and confused observations that exist.

Now, oddly enough, it is just the investigations of Martin and Cherry which are considered the chief proof against the destruction theory, which originated with Roux, that make the matter appear doubtful. Roux and Calmette showed that whilst snake-venom is resistent to heat, the corresponding antitoxin is not. When a mixture of the two components, neutral to animals, was exposed to heat, they found that the mixture became toxic once more. The English authors have, however, shown that the effect as observed by Roux, only occurred when the mixture was heated shortly after its preparation—such a mixture is of itself toxic, and therefore contains unchanged toxin. If one allows the mixture to stand for about twenty minutes at room temperature, it becomes non-toxic and remains so, even after being subjected to heat. With this observation before us the hypothesis of Roux that both components exist unchanged in the neutral mixture becomes so untenable that even a supporter of the destruction theory might accept the same as valuable corroborative material. From this short summary it will appear that the reasons generally brought

forward in favour of a chemical combination of toxin and antitoxin are not in a position to withstand criticism. On the other hand, one can in another way—viz.: by the methods of quantitative analysis—readily furnish the proof that the processes occurring in the neutralisation of toxin and antitoxin are of a quantitative character, and that one is dealing with a process following the laws of definite proportion, which can be illustrated by the example of the formation of a simple salt.

If ten equivalents of an acid are mixed with ten equivalents of a base (or 100 with 100), a neutral mixture is obtained in each instance. We find exactly the same relationship to exist in the interaction of toxin and antitoxin. If a given quantity of diphtheria antitoxin (*e.g.*, the immunising unit used as a test basis) is mixed with varying quantities of a given toxin, an amount of the toxin which is exactly neutralised by the I.U. can always be determined. To this dose of toxin I apply the term Lo dose (Limes O). Through the mixture of all the toxic groups with antitoxin, prepared by adding one Lo dose to one I.U., a neutral mixture results which has no injurious effect on test animals.

It has thus been proved that corresponding to the laws of definite proportion, by means of one such single estimation upon any given amount of serum, the corresponding Lo dose of the toxin can be determined by a simple process of multiplication.

I found, for example, on using $\frac{1}{10}$ I.U. the Lo dose of a given toxin to be 0·24. On making an analogous determination with 1 I.U. the Lo dose was found to be 2·4—exactly tenfold. Similar figures confirming the above results are to be found in the papers of Kanthack and Madsen.

A second important test result, however, behaves quite differently in this respect, viz., that which I have termed the L+ dose (Limes, death). The L+ dose is that amount of a given diphtheria toxin which is not completely neutralised by a given amount of diphtheria antitoxin (I generally use one I.U.), to the extent that *exactly* one toxin dose, sufficient to produce the death of a guinea-pig of 250 grm. weight, remains unneutralised. This toxin dose may be

termed one lethal guinea-pig equivalent. To give an example in the case of a given toxin : I found on using $\frac{1}{10}$ I.U. an L+ dose of 0·037, but on using one I.U. only the value 0·26 was obtained. We see, therefore, that the postulated law regarding multiplication of doses is only admissible for the Lo dose, but *not* for the L+ dose. An example will make clear the reason for this difference. If ten equivalents of HCl are mixed with eleven equivalents of KOH, one equivalent of KOH remains unneutralised. If, however, the same amount of alkali is to remain free on using 100 equivalents of acid, one must add *not* 10 × 11, but only 101 equivalents of acid. In the above mentioned example in which I mixed $\frac{1}{10}$ I.U. with 0·037 toxin there remained exactly one lethal guinea-pig toxin equivalent free. On mixing 1 I.U. with 0·37 toxin the resultant mixture contained ten free lethal guinea-pig equivalents, *i.e.*, a fatal dose for ten guinea-pigs. Therefore the value 0·37 for the L+ dose obtained by multiplication, but experimentally found to be 0·26, is much too large, inasmuch as the L+ dose on neutralisation with the corresponding amount of serum leaves only one lethal guinea-pig equivalent free.

However simple it may be to determine the questions affecting the Lo and L+ dose on one and the same toxin, the matter assumed quite a different aspect when, proceeding from the above data, I undertook a comparative examination of different toxins. Instead of finding the postulated uniformity, in accordance with the law of equivalents, there occurred such differences and variations that I gave up, for a long time, any hope of finding the right clue. The great importance pertaining to these questions leads me to give a brief outline of the method by which I was able to reach the goal. The diphtheria toxin contains, besides the specific secretory products of the bacillus, a large number of different substances (salts, meat extractives, peptone, &c.). These substances are, however, without any significance with respect to the specific action of the bacilli, inasmuch as they can neither produce the specific intoxication nor combine with the antitoxin. We may therefore, in our considerations, ignore completely these associated bodies, and, in a physio-

logical sense, regard the diphtheria poison as a solution of the specific toxin in an indifferent fluid.

The problem resolves itself into this :—Can we regard the toxin-broth as a solution of a *pure* substance, and as containing solely a body characterised by its toxic and combining properties? The method for deciding this question is a simple one, and consists in a comparative examination of different toxins. Suppose, in the case of a given weak poison, the fatal dose for a guinea-pig is 0·5 c.c., in the case of a second 0·03, and in the case of a third 0·002. Granting that the toxin solutions were pure, in the above-defined sense, it follows that in these different quantities exactly the same amount of toxin must be present, that is to say, one lethal guinea-pig toxin equivalent—on this assumption, the same amount of antitoxin ought to suffice to render each of the three doses innocuous. But this is not what is found to occur, as a matter of fact, inasmuch as each of these doses may require a differing amount of antitoxin for neutralisation.

On the assumption of a pure and definite toxin, a uniform amount of antitoxin (*e.g.*, 1 I.U.) ought, in the case of different toxin-broths, to neutralise such amounts that by means of the same, an equal number of guinea-pigs are always killed ; or, in other words, the Lo dose of different poisons, as ascertained with 1 I.U., ought to possess exactly the same toxicity. I have carried out such investigations with great exactitude upon about twenty different toxins, and have found the most marked variations, so that in one case the Lo dose (reckoned for 1 I.U.) corresponded to sixteen, and in another extreme instance, to 136 lethal guinea-pig doses.

It follows from these facts that the toxic broth does not represent a pure toxin solution, in the above-defined sense, but that in addition to the poison it must contain other bodies, which, whilst capable of combining with antitoxin, do not possess any marked toxicity. At the same time these " non-poisons " (ungifte) are in so far specific, that each one only occurs in that nutrient soil in which the given micro-organism has vegetated.

The observations upon the so-called spontaneous attenuation of the toxin furnish a certain explanation of the origin and significance of such bodies. It is well known that a toxin-broth, in course of time, gradually diminishes in toxicity. A freshly prepared toxin-broth, having a lethal dose for guinea-pigs of 0·2, may, after some months, require several such doses to produce the lethal effect.

I have now definitely proved (and this has been confirmed by Madsen's excellent researches) that in the course of this attenuation the neutralisation power does not necessarily undergo the slightest modification, *i.e.*, the Lo dose of the freshly prepared toxin is exactly the same as that of the attenuated.

How is this phenomenon to be explained? If a toxin broth, when freshly made, possesses the same Lo dose (in relation to 1 I.U.) as it does after attenuation, it follows that in both phases it must contain exactly the same amount of groups capable of combination, groups which may be represented by toxin or by the combining " non-poison " (ungift).

When, therefore, we find in the case of a given poison that the Lo dose remains the same in both phases, but that whilst at first it represented fifty, and finally only twenty-five lethal doses, it necessarily follows that these several twenty-five toxin equivalents have become transformed quantitatively into twenty-five equivalents of a " non-poison " capable of combination. This quantitative change points to the combining " non-poison " being a derivative of the toxin. Corresponding to this genesis I designate as toxoids such species of combining " non-poisons."

But it is specially to be noted that other species of combining " non-poisons " also occur in the toxin broth, which are primary secretory products of the diphtheria bacillus, and which, therefore, I distinguish by the special name of toxones, in order to distinguish them clearly from the toxoids.

The toxones possess less affinity for the antitoxin than toxins and toxoids, so that in the course of the partial neutralisation of a poison, the latter become neutralised

before the former. Through this circumstance it is easy to estimate with approximate accuracy the amount of the toxone, as I have already shown in my earlier researches upon the standardisation of the diphtheria toxin. I may mention that the amount of toxone present can be readily estimated by the difference between the Lo and L+ doses —the greater this is the greater the amount of toxone present. On testing a number of fresh toxins in which the toxone was still unimpaired, I found that the L+ dose was double that of the Lo dose. In these cases the poison contained exactly the same amount of toxone as toxin, so that the diphtheria bacillus had simultaneously produced with each part of toxin an equal amount of toxone.

As regards the properties of the toxone I will only mention here that it does not possess the property of killing test animals acutely, but it possess a certain power of producing indurations at the seat of injection, which are, however, less marked than those due to the toxin. On the other hand— and this would allot to the toxone a most important *rôle*— it appears to produce the slowly developing diphtheritic paralyses, as my own observations and those of Madsen and Woodhead tend to show. The circumstance that not only in the diphtheria patient, but also in animals infected with living bacilli such paralyses occur, favours the view that the toxone represents a primary secretory product of the diphtheria bacillus.

As a result of these various observations the question suggested itself whether the toxoids represent uniform substances (Einheitliche Stoffe), or whether they also consist of several groups. I was able to prove that in old diphtheria toxin-fluids at least three different species of toxoids occur which are differentiated through their affinity to antitoxin, and which I distinguish as proto-deutero- and trito-toxoid. Amongst these the proto-toxoid has the maximum affinity to antitoxin, and becomes neutralised in the first instance and before the other toxoids by antitoxin. I cannot now refer in detail to the lines I was led to follow, and I must therefore refer the reader to my publication upon the constitution of the diphtheria toxin and to the very detailed and clear ex-

perimental researches of Madsen, which fully confirm my observations. The guiding principle which led me to the determination of the actual constitution of the diphtheria poison and its varying composition of toxin and toxoid is a relatively simple one, and depends upon the *partial* neutralisation of a constant toxin amount with varying amounts of antitoxin. With this object in view suppose that one has determined (*e.g.*, with one I.U.) the Lo dose of a poison which is represented by x c.c., and which contains, say, 100 toxin doses—this toxin amount x is mixed with fractions of 1 I.U., say, $\frac{1}{5}$, $\frac{2}{5}$, $\frac{3}{5}$, $\frac{4}{5}$, I.U., and for each of these mixtures the number of lethal doses that still remain is determined.

In the first mixture, prepared with the smallest amount of antitoxin, only those portions of the poison (toxin and toxoid) become neutralised which possess the greatest affinity to antitoxin, whilst corresponding to the increasing additions made of antitoxin, the weaker and the weakest components eventually become neutralised, and of these the toxones, possessing as they do the least affinity, combine last of all. If we represent such a process of neutralisation in a simple schematic fashion, and assume that the broth contains only one kind of poison and one kind of toxoid, in exactly the same amounts, the experiment may assume three phases, which are dependent on the toxoid becoming neutralised—(1) simultaneously, or (2) subsequently to the toxin. The following table, in which the lethal capacity of the individual mixtures is given, will illustrate this point :—

Lo (= 100 toxin doses).

	I.	II.	III.
$\frac{1}{5}$ I.U. ...	100 ...	80 ...	60
$\frac{2}{5}$,, ...	100 ...	60 ...	20
$\frac{3}{5}$,, ...	80 ...	40 ...	0
$\frac{4}{5}$,, ...	40 ...	20 ...	0
$\frac{5}{5}$,, ...	0 ...	0 ...	0

It will be evident that, on this simple supposition, a single estimation carried out on any given mixture gives one a full insight into the constitution of a toxin. If we find, on mixing with $\frac{2}{5}$ I.U., the value 60, we are un-

doubtedly dealing with a toxoid of the same affinity as the toxin.

In the case of the poisons of more complex constitution, obviously one analysis is not enough; four to six are generally necessary for the adequate analysis of these toxins. I refrain from discussing the numerous analyses of individual toxins I have carried out, or the method of graphically illustrating the results in the form of the " toxin-spectrum." I will only touch on a few of the results of these prolonged investigations. It was found that the immunising unit used as a basis for official testing purposes contains 200 combining units, the combining unit being represented by that amount of antitoxin which is exactly sufficient to neutralise the lethal dose for a 250 grm. guinea-pig, i.e., one lethal guinea-pig toxin equivalent.

The same equivalence with respect to the antitoxin can also be exercised by the *non*-toxic constituents of the broth, i.e., the toxoids and toxones. If one ever succeeded by chemical methods in obtaining a diphtheria toxin, free from toxoids and toxones, its constitution would be such that (tested with 1 I.U.) the Lo dose would be sufficient to kill exactly 200 guinea-pigs.

Such an ideal toxin, according to my observations, is not capable of existence, as the bacilli always produce toxone along with the toxin, and consequently the number of lethal doses contained in the Lo dose is always considerably less than 200. On the other hand I have succeeded, and Madsen likewise, in finding two toxins which partially contain such a pure poison. In the case of the toxin I investigated, if one added to the Lo dose (which *per se* kills eighty-four guinea-pigs) $\frac{100}{200} = \frac{1}{2}$ I.U., the toxicity of the mixture was found to be 23. But if one added to the Lo dose a larger amount of antitoxin, viz., $\frac{115}{200}$ I.U., the mixture then possessed only a toxicity of 7. The further addition of $\frac{15}{200}$ I.U. (corresponding to fifteen combining units) had thus lessened the toxicity from 23 to 7, i.e., it was diminished sixteen times. Consequently, through the addition of fifteen combining units of antitoxin, sixteen lethal guinea-pig doses had become neutralised, i.e., each com-

bining unit had almost exactly neutralised *one* lethal guinea-pig dose. Thus the original mixture of the Lo dose $+ \frac{1}{2}$ I.U. was so constituted, that of the portions remaining unneutralised the pure toxin possessed the maximum affinity and became neutralised in the first instance.

If the symbol T represent toxin, there became neutralised under these conditions—

$$T\ T\ T\ T\ T$$

Such pure toxin " zones " in the toxin spectrum are very rare and are only met with in the case of freshly prepared toxins. In contrast to this, one finds in the case of all toxins another form of combination represented by less extended " zones " of hemitoxin. One can with every toxin without exception prepare mixtures with antitoxin, which are so constituted that the addition of each additional combining unit ($\frac{1}{200}$ I.U.) reduces the toxicity by $\frac{1}{2}$ (in contradistinction to 1 in the case of pure toxin). There are always exactly the same portions of toxin and toxoid simultaneously neutralised corresponding to the following figure, in which T = toxin and I = toxoid, viz. :—

$$T\ I\ T\ I\ T\ I\ T$$

It requires no further demonstration that this occurrence can only be due to the affinity of the given toxin in question being the same as that of the toxoid. The question forces itself upon one, through what primary conditions is the toxoid formation brought about? The following observations will help towards an answer.

The toxin is defined through two attributes, of which the one is represented by the specific toxicity, the other by the specific combining power with antitoxin. I have already in the investigation upon the standardisation of toxins touched upon the question whether these two functions are bound up in the *same* complex of atoms, or whether the toxin molecule contains two independent groups, of which the one (the toxophore) conditions the toxicity, and the other (the haptophore) the combining property. I had at that time to leave the question undecided, but as the result of my further investigations upon toxoids I am now in a position to decide this question in the sense of duality.

The evidence in favour of the complete separation of the two functions rests especially upon the discovery of the hemitoxins, which, as mentioned above, are to be met with in all toxin broths to a greater or less extent. In the case of hemitoxin, there are always simultaneously neutralised equal parts of toxin and toxoid. Both these bodies must, therefore, possess exactly the same affinity to antitoxin. If we consider that the hemitoxin is derived from pure toxin through the half of the toxin molecule changing into the toxoid molecule, it follows that in the course of the metamorphosis of toxin into toxoid the affinity to antitoxin does not undergo the slightest modification but remains exactly the same. This is only comprehensible on the supposition of the existence of two different groups which are completely independent of one another.

We must, therefore, thus represent the action of the poison that with the help of the haptophore groups, the toxin molecule becomes "anchored" to the cell, and that it comes in this way within the sphere of action of the toxophore group. The poisoning is an effect of the toxophore, the antitoxin production an effect of the haptophore group.

The physiological significance of these two groups is of very different character, as will be seen from what has just been said. The biological rank of the two groups of the toxin molecule does not appear to be equivalent. The toxophore group, with functions of a ferment-like character, must as regards constitution be more complicated than the haptophore group, whose simple functions might well correspond to a simple structure.

This point of view would explain the ready disintegration of the toxophore group and the relative stability of the haptophore group. We thus see that the supposition of two different groupings explains, in the easiest manner, the phenomena observed in the attenuation of the diphtheria toxin, and especially the fact that the combining power of the broth remains intact, whilst the toxicity markedly sinks. From this point of view some phenomena observed, in the case of certain toxins, may be explained in an unconstrained fashion. The singular fact has been noted by Courmont

that frogs, treated with large doses of tetanus toxin, will remain for an indefinite period without toxic symptoms, if they are kept in a cool atmosphere. If, however, the frogs are placed in an incubator, a quickly fatal tetanus intoxication immediately ensues. Dr. Morgenroth proved, in my Institute, that the healthy animals, under cool conditions, had already fixed the tetanus poison in their central nervous system. If he gave to the frogs a sufficient quantity of tetanus poison and injected, after they had remained some time in a cool place, an amount of tetanus serum more than sufficient to neutralise the entire toxin dose, supposing the toxin to be still harboured in the blood, there yet occurred, despite the serum treatment, a typical and quickly fatal tetanus so soon as the animals were placed in the incubator.

This was, of course, only done after some days in order to allow sufficient time for the absorption of the serum. In the case of the cold frogs a portion of the tetanus poison must have left the blood and passed into the tissues, as only under this supposition can the absence of the serum action be understood. The experiments made by Dr. Billinger, and confirmed by Professor Dönitz, are probably to be explained in the same way, that marmots during their winter sleep are not affected by tetanus toxin, and first succumb to its action when they wake out of the same. We must, therefore, suppose that the *haptophore* groups of the tetanus toxin can become fixed by the nervous system at the lower temperature, but that the toxophore groups first become active above certain temperature limits. Such a thermic limitation to the activity of the toxophore group may at first appear remarkable. But numerous analogies are to be found amongst a class of bodies closely related to the toxins —the ferments. For example, any given quantity of rennet ferment when kept at zero produces no casein precipitation, but this immediately occurs when the milk in question is placed at a higher temperature. Apparently we have here a complete analogy to the frog experiments.

A second important question can be readily explained on the supposition of the existence of two groups, viz.: the question of the so-called incubation period observed in the

case of most toxins with very few exceptions (snake venom). When on the injection of a single fatal dose of tetanus toxin the first symptoms appear twenty-four, forty-eight, or fifty-two hours, the question arises whether we are here dealing with a function of the toxophore or haptophore group. This point has been decided for diphtheria and tetanus toxin by Prof. Dönitz by the intravenous injection of a given quantity of toxin and the estimation of the amount of serum necessary, when directly administered, to completely neutralise the toxin effect. This dose was so conditioned that it completely neutralised the toxin present in the blood. If, however, a little time was allowed to elapse between the injection of toxin and antitoxin (six minutes) it was found that the single neutralising dose was no longer adequate to prevent death. A portion of the toxin must therefore have left the vascular system and a dose sufficient to produce death been fixed by the central nervous system. It follows that the union between poison and tissue, which is a function of the haptophore group, occurs quickly. Further, the long incubation period must be referred to a slow functionating of the toxophore groups. Whilst in the case of the ordinary bacterial poisons the latent period does not generally last longer than twenty-four hours, there are substances which show a much longer latent period, e.g., the toxones which produce the diphtheritic paralyses in test animals after two or three weeks.

The dual groups found in the case of the toxins occur also in the case of ferments. Anti-ferments can be produced in the body which completely neutralise the action of the given ferment. Dr. Morgenroth by successive injections of rennet ferment produced a serum which neutralised large quantities of the rennet ferment in vitro.

As already stated, the haptophore group of the toxin is relatively stable, the toxophore or ferment-like group is unstable, and readily undergoes disintegration. With these facts before us the formation of hemitoxin can be readily explained. Taking the above material and collective observations as a basis, we may reconstruct the origin and fate of the hemitoxins in the following manner:—The process usually

occurs in two phases, the hemitoxin is derived from pure toxin, and the toxoid from hemitoxin, e.g. :—

T T T T T T

T I T I T I

I I I I I I

The toxin is thus not a uniform entity, but contains two modifications which are present in exactly the same amount. The one modification readily disintegrates and forms hemitoxin, the other modification on disintegration forms pure toxoid. We may confidently state that the difference between the two modifications does not depend upon the haptophore group, as these possess the same combining power in toxoids as in toxin. The destruction of the toxin action points more emphatically to the toxophore group, and we must assume that the toxin contains two different toxophore groups, and that these two varieties are always simultaneously produced. The recent investigations of E. Fischer help towards an understanding of this phenomenon. These investigations show that as regards the enzymes and their objects of attack a similarity in molecular configuration must exist if a reaction is to occur. Fischer has rendered it most probable that the ferment group, analogous in many respects to the toxophore group, possess an asymmetrical constitution. If this is so a priori two possibilities exist—either the diphtheria bacilli produce one single group, or they produce simultaneously two. When one cell simultaneously produces two asymmetrical components, it frequently occurs that the two components are formed in exactly the same amount, e.g., inactive oxalic acid. On such a supposition the formation of hemitoxin is capable of an unconstrained explanation. In many instances with the aid of micro-organisms an optically active half of a racemic compound has been split off, and one and the same organism may at one time attack the levo- and at another the dextro-rotatory modification of the compound.

E. Fischer has noted similar differences in the action of ferments.

The above considerations might help one to understand how in the toxophore group the one modification is first

attacked on storage of the broth and its original toxicity
becomes reduced by one half (hemitoxin). It is difficult to
decide what special influences control this change. It may
be due to the presence of slowly-acting enzymes, which
gradually develop their activity under certain conditions that
arise in the conserved broth.