Herter Lectures.[1]

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LECTURE I.

THE MUTUAL RELATIONS BETWEEN TOXIN AND ANTITOXIN.

Abstract.

INTRODUCTION.

The importance of practical and theoretical study of immunity. Influence of the side-chain theory. Cellular origin of antibodies. Relations between the constitution and distribution of chemical substances and their pharmacological action. Complexity of apparently simple functions.

Opposing views. Criticism of their experimental basis eliminates errors and reverses their significance. Importance of hypothesis to scientific advancement. Heuristic value of theory of immunity. Utility of succinct nomenclature and symbolic representation (algebra, chemistry).

HISTORY OF SIDE-CHAIN THEORY.

Studies upon the localization of various dyes and their relations to cellular chemistry (methylene-blue, neutral-red). Production of immunity with poisonous proteids; independently of Behring (ricin, abrin, robin). Immunity not only bacterial, but a general principle. Quantitative studies on augmenting immunity and determining its degree. Older obscure conceptions of the relations between antitoxin and toxin (union, destruction, cell-protection). Experiments in vitro eliminate obscurities of those on animals. Chemical nature of union demonstrated. Warmth and concentration hasten union.

TOXOIDS.

Difficulty in applying purely chemical conceptions in the case of diphtheria toxin. Law of equivalence apparently violated. Definitions of immune unit; technical toxin-values, lethal dose ("Dosis lethalis"), limit zero ("Limes null" = Lo), limit death ("Limes Tod" = L †). Different lots of diphtheria toxin though neutralized by a single immune unit contain different numbers of lethal doses. Investigation shows that upon standing the diphtheria toxin loses toxicity, but retains undiminished its affinity for antitoxin. The toxin molecule is quantitatively transformed into non-poisonous toxoid. Chemical illustration (anilin and sulphanilic acid). The simplest explanation of toxoid formation rests upon the assumption that the toxin-complex contains two groups of different functions: *a.* Haptophores, by which the complex enters into chemical unions; *b.* Toxophores, which occasion toxic action. Behring's objection, exemplified in hydrocyanic acid, not justified, because of the simple constitution of that poison. Complex organic compounds have repeatedly been shown to contain groups which confer definite properties upon the whole molecule. Anesthesiophore function of the benzoyl group in cocain; functions of the chromophore and auxochrome groups in dyes; color reactions of proteids due to definite groups (tyrosin, tryptophan, etc.). Evidence of this complex character of toxins also furnished by studies of hemolysins and bacteriolysins, which have their origin in the union of an amboceptor and complement and are analogous to toxins, the amboceptor representing the haptophore and the complement the toxophore group. Kyes' studies on cobralecithid.

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SIDE-CHAIN THEORY.

Haptophore Group. — The conception of the duplex nature of toxins, etc., forms the basis of the side-chain theory. Views regarding protoplasm. Fundamental nucleus and side-chains (receptors). These receptors subserve normal assimilative processes which are of synthetic character. Only those substances can be chemically incorporated with the protoplasm (assimilated), which possess a grouping most closely related to some constituent of the cell, fitting into its constitution as a key into a lock. The toxins represent, to a certain extent, toxic foodstuffs. It is mere chance whether they encounter suitable receptors in the cells of a particular animal species or not, as is shown by the irregularity in the action of given toxins in the animal kingdom (botulism). Elaboration of the side-chain theory. The antitoxins are receptors, produced in excess, and liberated from the cells. Appearing in the fluids they unite with the toxins for which they have specific affinity and thus protect the cells which have receptors of similar affinity from damage (analogy to lightning-rods). Not only toxins, but also toxoids, call forth antibodies. It is therefore chiefly the haptophore group which causes the cell-reaction, yielding antibodies by occupying the specific receptors. It is doubtful whether this cell reaction is purely regenerative. The great disproportion between the amount of toxin introduced and the quantity of antibodies obtained points to a concomitant cell-stimulation (Bindungsreiz of Wassermann). This is supported by the fact that a single immunization with suitable bacteria (*e. g.*, cholera) or toxin (*e. g.*, that of diphtheria) may induce an abundant production of antibodies.

EVIDENCE IN FAVOR OF THESE THEORETICAL CONCEPTIONS.

(1) The demonstration of specific receptors in organs susceptible to a particular poison. Wassermann's experiments: union of tetanotoxin with cerebral tissues. Disproof of opposing views of Behring and Besredka. Locking of native and experimental hemolysins by susceptible erythrocytes.

(2) The demonstration by Pfeiffer that during the latent period, before free antibodies appear in the serum, they may be obtained from the

bone-marrow with salt solution (loosely bound, partially liberated receptors).

(3) Demonstration of the presence of receptors produced and fixed in definitely localized tissues. Increased abrin-locking by the immunized conjunctiva (Römer).

(4) Local production of specific precipitins for maja-albumin after intraocular injection (v. Dungern).

(5) All attempts to produce antibodies with alkaloids, antipyretics and antiseptics have failed. These substances do not enter into the composition of the protoplasm, they encounter no suitable receptors and therefore fail to call forth a specific antigenetic reaction.

TOXOPHORE GROUP.

The toxophore group need not necessarily be simple but may comprise various constituent groups which may be identical or different. Illustrations from alkaloidal chemistry; strychnine contains two oxygenated radicles both of which produce convulsions, and the drug owes its extraordinary toxicity to a combination of the two. Substances of the filicic acid series are active in proportion to the number of filicic butanon groups they contain. Ricin probably contains two toxophore groups, one agglutinating erythrocytes, the other causing its general toxicity. Three varieties of toxoids may be obtained from ricin, one non-toxic but agglutinating, one toxic but not agglutinating; both of these are incomplete toxoids. The third, complete, is neither toxic nor agglutinating (Tarobi). Tetanotoxin and diphtheria toxin also appear to contain various toxophore groups. In the former there may be a lethal toxophore group and a morbific group; and in the case of diphtheria we may have to distinguish between complexes which cause the chronic effects and those occasioning acute manifestations. It is possible that certain partial groups act more powerfully on certain animal species than on others, and this may explain the observations emphasized by Behring that modified toxins meet with a range of susceptibility in the animal kingdom different from that of the original, unmodified toxin. Of practical importance is the question whether for the rapid production of antitoxin it is more advantageous to use only toxoids, or whether the presence of partial components of the toxophore groups exert a more favorable cell-irritation.

The action of the toxophore groups is generally slow. Since the intravenous injection of toxins is rapidly followed by their locking in the tissues and disappearance from the blood, the incubation-period, which in the case of diphtheria *toxones* may last for weeks, can only be explained by the slow action of the toxophore complexes. This is further exemplified in the hemolysins. Other factors may enter into the production of a period of incubation (studies of Hans Meyer and of Roux on tetanus). It is difficult to express definite views regarding the mode of action of the toxophore groups. Hueppe's hypothesis; experimental evidence wanting.

RECEPTORS. SUSCEPTIBILITY TO TOXINS. RELATIONS TO METABOLISM.

The receptors of the cells are constituents of the protoplasm possessing a certain independent individuality, as is shown by the possibility of causing their augmented reproduction. In respect to the toxins they act as localizers or condensers and may from an infinitely dilute solution occasion a concentration of toxins in particular cells similar to the concentration of picric acid which takes place when wool is immersed in a dilute solution. Susceptibility to toxins may be divided into the following varieties:

(1) Great susceptibility. Toxophilic receptors exclusively in vital organs. Antitoxin production very difficult because of damage to cells, only possible through use of toxoids (guinea pig or mouse and tetanus).

(2) Slight susceptibility because of wide distribution of toxophilic receptors in indifferent tissues, *e. g.*, connective tissue, which lock the greater part of the toxins, and thereby protect more important tissues (fowl or rabbit and tetanotoxin).

(3) Insusceptibility, though toxophilic receptors be present, because toxophore groups do not act on protoplasm. Abundant production of antibodies. Rare condition (crocodile and tetanotoxin).

(4) Insusceptibility through lack of toxophilic receptors. Numerous examples clearly established (crotin, arachnin, specific hemolysins).

Definite receptor-complexes are associated with definite forms of metabolism. Uniform metabolic processes imply uniform receptor-complexes and therefore a uniform susceptibility to toxins (diphtheria toxins and guinea pigs; tetanotoxin and mice). Individually varying functions reveal themselves in extraordinarily variable susceptibilty (*e. g.*, crotin and rabbits). The embryonic erythrocytes of chicks possess no receptors for arachnolysin and are therefore not destroyed by this toxin. Immediately after hatching, the development of susceptible erythrocytes begins.

The study of the receptors has been of particular importance in elucidating the processes of assimilation. Former methods applied to damaged cells were not sufficiently refined. Isolated receptors obtained by immunization make an accurate study of assimilation and katabolism more approachable. The reproach brought forward by some controversialists that the theory of receptors should also explain regeneration is extravagant. It is an advance to be able to isolate certain wheels in the complex mechanism of the assimilation-processes and to ascertain their utility. The actual motive power is not revealed and the problem of regeneration of living matter will long remain unsolved.

The demonstration that erythrocytes possess extraordinarily numerous and various receptors is particularly important. It indicates that these corpuscles, in addition to their function as oxygen-carriers, play an important rôle in metabolism in storing intermediary bodies.

Investigation has been greatly aided by a knowledge of the receptors, since they can be used in the analysis of animal and vegetable poisons to separate them into partial-toxins having different haptophore groups. Thus it is possible by specific locking with specific antibodies to analyze snake poison into neurotoxic, endotheliotoxic and two hemotoxic components. Of the latter, one agglutinates, and the other, through the formation of lecithid, hemolyses the blood (Meyer, Flexner, Kyes). Similar analyses of tetanotoxin and diphtheria toxin are possible. The method is a great advance in a most difficult field of research.

CONCLUSION AND PRACTICAL FORECAST.

The immunization industry which has for its aim the production of sera of highest potency, has made marked progress. Sera of much higher antitoxic value than have hitherto been obtained will be the result. The knowledge that the immunity reaction is reduced by too highly toxic treatment of the animals is spreading. An effort is being made to restrict this immune reaction to tissues of slight vital importance (connective tissue) and to protect vital organs from injury. In this direction are the methods of immunization with toxin and antitoxin injected separately or after admixture. Thus an apparently neutralized toxin may cause antitoxin production (Park). This is due, as will be explained in the next lecture, to the presence of ultra-toxoids.

Progress in the production of curative sera is to be expected only when it becomes possible to obtain antitoxins of greater avidity than those now procured. That this is theoretically possible is shown by the investigations of Kyes who demonstrated that through the immunization of rabbits with cobra-lecithid it is possible to obtain an antibody of considerably higher avidity than that obtained by Calmette with cobra-venom in horses (antivenin). The selection of particularly favorable animal species and the use of toxins of least avidity or ultra-toxones will, most probably, bring this problem much nearer its final solution.

LECTURE II.

PHYSICAL CHEMISTRY *V.* BIOLOGY IN THE DOCTRINES OF IMMUNITY.

Abstract.

INTRODUCTION.

PHYSICO-CHEMICAL investigations of Arrhenius and Madsen concerning the application of physical chemistry to the study of toxins and antitoxins. Their belief that tetanolysin is a uniform substance possessing only a weak affinity to its antitoxin and that its neutralization occurs in accordance with the law of Guldberg and Waage. Attack upon my doctrine of plurality of toxic constituents. Favorable reception of these opposing views in certain scientific circles lacking special competence in sero-therapeutical questions. Protest against the attempt of these authors to claim exclusive priority in the development of these questions. The path of biological investigation — the method of partial neutralization of toxins — followed by them was first opened and by long years of work developed by me. The contention that my investigations represent merely an application of Thomsen's principles in his study of affinities between strong acids and bases is incorrect.

Equally unwarranted is the reproach made by these authors that my investigations are inaccurate and have been conducted without sufficient consideration of sources of error. The most important of my results have been confirmed by them, especially as regards the existence of toxoids and their occurrence with varying degrees of avidity, as "prototoxoids," "syntoxoids." On the contrary, the researches of myself and collaborators have been conducted with the utmost precision and care without consideration of cost or time. Nor can I admit that I have violated the principle of choosing the simplest possible explanations. Such explanations received always my first consideration and, as in the case of diphtheria-toxin, gave place to more complex ones only when the experimental results made this necessary.

POINTS OF DEPARTURE. METHODS AND RESULTS OF PARTIAL NEUTRALIZATION.

It was to be determined whether in the production of toxoids a change in avidity for antitoxin occurred. The only available method for this was that of partial saturation or neutralization. A prerequisite of this method is the exact determination of the test-constants, viz., the simple fatal dose, and the Lo and the L† doses, which have reference to the immunity unit (I. E.), and each of which may be the starting point of the investigation. It is then determined by how many fatal doses the toxicity of a given quantity of toxin, as for example the Lo dose, is lessened by its mixture with a fraction, say one tenth, of the immunity unit of antitoxin. Then with another mixture containing a larger amount of antitoxin a second determination is made, and so on. If the toxin were a single substance of uniform composition and the antitoxin possessed for it a strong affinity, like that between hydrochloric acid and potassium, the quantities of toxin which disappear or are neutralized in these mixtures would be in direct ratio to the quantities of added antitoxin. In fact, however, the process of neutralization takes places after a quite different scheme, as is shown by a glance at the exhibited charts.

It is advantageous to present these experimental results in graphic form, and for this different methods are available: