BACTERIAL TOXINS

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BLACKWELL
SCIENTIFIC PUBLICATIONS
OXFORD

PREFACE

This monograph is based on a course of four public lectures given at University College, London, in November, 1949. Its purpose is to deal more fully with a subject that is treated only briefly in textbooks of pathology and bacteriology and has not yet found its way into textbooks of biochemistry. It is an attempt to present pathologists and bacteriologists with the point of view of a biochemist, and biochemists with a rich field of interesting problems.

I am greatly indebted to the Department of Biochemistry of University College for inviting me to give the lectures; to Dr E. P. Abraham, Sir Paul Fildes, Dr G. P. Gladstone, Dr W. T. J. Morgan, Professor E. T. C. Spooner, and my wife for reading the manuscript and for offering indispensable criticism and advice; to Dr Joan Gardner for reading the proofs; and to Mr L. T. Morton for preparing the index. I am grateful to Professor G. Payling Wright and Dr N. Ambache for many hours of interesting discussion on the pharmacology of botulinus and tetanus toxins, and I am sorry that the few lines I have given to this subject do not do justice to their patient explanations.

I thank Mr R. Christie and Dr J. J. Graydon for original prints of figs 7, 8, 9, 10 and 12; Dr W. A. Gillespie for fig. 6; and Dr C. Lamanna for fig. 2. I thank the Editors and Publishers of the following journals for permission to reproduce the figures indicated: *Australian Journal of Experimental Biology and Medical Science*, figs 7, 8, 9, 10 and 12; *British Medical Journal*, fig. 6; *Journal of Bacteriology* (The Williams & Wilkins Company), fig. 2; *Journal of Biological Chemistry*, fig. 3; *Journal of Experimental Medicine*, figs 4 and 5; *Journal of Pathology and Bacteriology* (Oliver & Boyd Ltd.), fig. 11. I thank Miss Mary Webb for producing neat typescript from my untidy manuscript.

Oxford. W. E. van Heyningen.

June 1950.

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INTRODUCTION

"The basis of all harmful effects of bacterial infection is quite certainly chemical; and only when the chemist has replaced the immunologist shall we be able to give an intellectually satisfying account of what happens when a particular parasite invades a particular host".

That is what Topley & Wilson (1936) said when they were considering why the presence of bacterial cells in the tissues should be harmful to the host that harbours them. Indirectly the proliferating organism might be harmful in a number of ways, for instance by interfering with the metabolism or with the detoxification mechanisms of the host, or by producing mechanical lesions, or by inducing the formation of toxic or inflammatory substances from the host's tissues. Generally it is difficult to define or trace these effects.

But the organism itself may be a source of substances that are directly toxic to the host. It has been known since the time of Roux & Yersin (1888) and Kitasato (1891) that certain bacterial species produce or contain antigenic poisonous substances of high molecular weight that give rise to more or less characteristic lesions or symptoms, including death, when injected into susceptible animals. These are the bacterial endotoxins and exotoxins. Many of them are sufficiently characteristic as antigens to be used in the identification and classification of the organisms from which they are derived. The toxins are not to be classed with the non-antigenic histamine-like substances of low molecular weight that are produced by some bacteria, nor should they be confused with certain antigenic products of bacteria, such as tuberculin, which are toxic only because they are antigenic. These substances are not toxic to the normal animal, but they may be highly toxic to an animal that has become sensitised to them by infection.

On the other hand, the exotoxins are closely related to, and sometimes indistinguishable from, a variety of other active antigenic substances produced by a number of bacteria. If these substances are not all lethal agents in the sense that the classical exotoxins are, they do at least contribute to the virulence and the pathogenicity of the parasite. From the biochemist's point of view they belong to the same class of compound as the lethal toxins and are equally interesting. Many of them appear to be hydrolytic enzymes, or kinases which catalyse the production of

active hydrolytic enzymes from inactive precursors in the body. They include (a) substances which attack the constituents of the blood, viz., the *haemolysins* which attack red cells, the *leucocidins* which attack white cells, the *coagulases* which clot plasma, and the *fibrinolysins* which dissolve fibrin clots, or prevent plasma from clotting; (b) the *hyaluronidases* or "spreading" or "diffusing" factors which increase tissue permeability; (c) the *proteolytic enzymes*. Perhaps lipases, nucleases and nucleotidases should added to this list, although evidence of their pathological importance, or indeed of their existence, is not always clear.

The part that is played by toxins in disease varies considerably in importance, as is illustrated by anthrax at the one extreme, and botulism at the other. When an animal is infected with *Bacillus anthracis* the spores germinate rapidly and the organism multiplies and invades the tissues. After death the capillaries are so loaded with emboli of the bacillus that it was sometimes thought that death is caused directly by mechanical blockage of the capillaries. On the other hand, the animal that dies of botulism need not have been infected by the causative organism, *Clostridium botulinum*. Indeed, the first instances of *Cl. botulinum* being recovered from naturally infected wounds have only recently been recorded (Hall, 1945). Death is usually the result of eating food on which the organism has grown and produced a highly potent toxin. In this respect botulism is comparable with ergotism. Botulism is a disease that is entirely due to bacterial toxins, and the same can be said, with the reservation that botulism is not really an infectious disease, of tetanus, diphtheria, gas gangrene and scarlet fever. Most infectious diseases fall between the extremes of anthrax and botulism.

The content of toxin, or the ability to produce it, is not necessarily a measure of the virulence or pathogenicity of the organism, even when toxin is the main cause of harm. The organism must be able to invade the host and establish a footing in a strategic situation where it can produce toxin and where the toxin can exert its harmful effect. The Park 8 strain of *Corynebacterium diphtheriae*, which is used for large-scale toxin production all over the world, was isolated from a relatively mild case of diphtheria. The neurotoxin of *Shigella shigae* is produced by avirulent as well as virulent strains. The complex endotoxins of the enteric group of organisms can be found in pathogenic and non-pathogenic bacteria.

Since the toxins are antigens it is often possible to immunise humans and animals actively or passively against their toxic effects,

so much so that tetanus has vanished as a military problem, and diphtheria is on the way to becoming a clinical curiosity. The antibodies to most of the endotoxins are not completely antitoxic, but they are antibacterial because most endotoxins are also the dominant somatic antigens of the organisms that contain them.

The bacterial toxins are conventionally classified as endotoxins and exotoxins, that is, according to whether they are found inside or outside the parent organism. A classification according to whether the toxins are derived from Gram-negative or Gram-positive organisms would be more significant, and less open to qualification. Such a classification would also roughly divide the toxins into endo- and exotoxins, as a consideration of the following points will show:

1. The "Gram-positive" toxins [1]) are found outside the parent cells. Such toxins are often called "true" toxins, or "soluble" toxins. A typical Gram-positive toxin is excreted during the phase of active growth of the organism and reaches its highest concentration at the time growth reaches its maximum, or soon after. Nevertheless, there are Gram-positive toxins, considered as typically extracellular toxins, that reach their maximum concentration long after, sometimes days after, the organism has reached its maximum growth. It is also true that some Gram-positive toxins (e.g. diphtheria, tetanus) can be recovered from washed cells, but in these cases more toxin is generally produced extracellularly than intracellularly.

The Gram-negative toxins appear to be structural components of the bacterial cells, but they are often found in cell-free autolysates. Many workers (e.g. Delafield, 1932; Olitzki, Avinery & Koch, 1942) have shown that the washed dead bodies of a number of Gram-negative organisms are toxic, whereas the bodies of Gram-positive organisms are not. In some cases, for instance *Brucella melitensis*, the lethal dose is the same whether the organisms are living or dead (Miles & Pirie, 1939 c). It has been stated that "thoroughly washed suspensions of most Gram-negative species, even non-pathogenic ones, are highly toxic." (MacLeod & Pappenheimer, 1948). Whether this generalisation can safely be applied to all Gram-negative organisms is a debatable point. Toxicity tests with bacteria are generally done in medical or near-medical laboratories where the organisms that are studied are derived from humans or animals. It is impossible to attach any precise meaning

[1]) Toxins will be called "Gram-positive" and "Gram-negative", not to indicate their own staining properties, but those of their parent organisms.

to the word "toxic", because any substance will be toxic if a large enough dose of it is introduced into an animal. When somebody injects a washed suspension of killed organisms into an animal he must presumably have some limit of weight or number of organisms in mind; if the lethal dose is above this limit the organism is not toxic. The limit is seldom stated; sometimes it is "half an agar slope", which does at least have a rough meaning. The most that can usefully be said on this point is that the lethal dose for mice of a typically toxic dead organism like *Shigella flexneri* type Z is 1.9 mg., and that of the purified endotoxin extracted from it is 0.23 mg. (Perlman & Goebel, 1946 a).

2. Antibodies to Gram-positive toxins are capable of completely neutralising their activity; that is, x units of toxin are neutralised by y units of antitoxin, and nx units of toxin are neutralised by ny units of antitoxin. On the other hand antibodies to Gram-negative toxins can only neutralise a fraction of their activities. That is, no matter how much antitoxin is added to the toxin, the toxicity of the mixture will not be reduced to less than about a fifth of that of the toxin alone.

3. The Gram-positive toxins are generally more toxic than the Gram-negative toxins. That this is true of typical purified, or partially purified, Gram-negative and Gram-positive toxins can be seen in Table 1. Often the toxicity of a particular toxin is confused with the amount of it that is produced by an organism. When the toxicity of a culture filtrate or of a bacterial extract is expressed as the volume that will kill an animal one has no knowledge of what weight of toxin is in that volume, and consequently no idea of the potency of the toxin. What is sometimes called a "feeble" toxin may be a very dilute solution of a potent toxin.

4. The Gram-positive toxins are generally less heat-stable than the Gram-negative toxins. But it is also true that some Gram-positive toxins are more heat stable than is generally thought. For example, a solution of *Clostridium welchii* Alpha toxin retains 75 % of its activity after being heated at 100° C. for 5 minutes, and 20 % after being heated at 100° C. for 30 minutes, (van Heyningen, 1942 b). Moreover, some exotoxins (e.g. Staphylococcus Alpha toxin (Fulton, 1943)) are inactivated by heat in an anomolous manner, that is, they are destroyed less by being heated at 100° C. (than they are by being heated at 65° C.

5. The Gram-positive toxins can be toxoided, that is, made non-toxic but still capable of stimulating the production of anti-

bodies that neutralise the toxins. Most Gram-negative toxins cannot be toxoided in the ordinary way.

6. The Gram-positive toxins that have been purified are simple proteins, whereas most Gram-negative toxins appear to be polymolecular phospholipid-polysaccharide-protein complexes. Earlier claims that tetanus toxin and the Streptococcus erythrogenic toxin are not proteins have been refuted.

7. The symptoms that are produced by most Gram-negative toxins are more or less the same, irrespective of the organisms they are derived from. On the other hand, the pharmacology of the Gram-positive toxins is generally more specific. The effects of botulinus intoxication, for example, are quite distinct from those produced by diphtheria toxin.

The generalizations about Gram-negative toxins have apparent exceptions in the cases of *Pasteurella pestis*, *Vibrio cholerae*, *Haemophillus pertussis*, and *Shigella shigae*. *Past. pestis* toxin appears to be a simple protein and it is more toxic than most Gram-negative toxins. *V. cholerae* is thought to produce a toxin of comparatively low molecular weight; *H. pertussis* and *Sh. shigae* produce heat-labile antigenic toxins which are toxoidable.

The toxicity of a number of poisonous substances for the most susceptible animals is recorded in Table I. Drugs of comparatively low molecular weight are compared with the polymolecular Gram-negative toxins, with the simple Gram-positive toxic proteins, with a toxic enzyme (urease), with a Gram-positive toxin that is known to be an enzyme (*Cl. welchii* Alpha toxin lecithinase), with a plant poison, and with a snake venom. The toxicities are expressed as the number of MLD's per unit weight and per micromole[1]). The molecular weight of the *Salmonella typhi* and the *Sh. shigae* toxins is taken as 500,000, which is about half way between the limits of 10^5 and 10^6 given by Miles & Pirie (1939 b) for a similar complex from *Br. melitensis*. The molecular weights of the *Cl. welchii* Alpha toxin, cobra venom neurotoxin, and tetanus toxin were guessed to be 100,000. Since there is practically nothing known about the nature of *Past. pestis* toxin no guess was made. The other molecular weights are reasonably authentic.

[1]) The MLD, or Minimal Lethal Dose, is the smallest amount of toxin that will kill all of the animals injected. It has not got as much meaning as the LD_{50}, the smallest amount of toxin that will kill 50 % of the animals injected. The MLD is larger than the LD_{50}.

TABLE 1. Toxicity of various drugs, toxins and toxic proteins

Poison	Toxicity per Kg Animal		Animal
	MLD/μg. poison	MLD/μMole poison	
Atropine	0.000,03	0.009	cat
Strophanthin	0.005	3.55	rabbit
Aconitin	0.077	60	rabbit
Salm. typhi toxin	0.000,2	100 ?	mouse
Sh. shigae toxin	0.000,2	100 ?	mouse
Past. pestis toxin	0.03	?	mouse
Urease	0.013	1,300	rabbit
Ricin	0.013	1,120	mouse
Cl. welchii Alpha toxin	0.4	40,000 ?	mouse
Cobra venom neurotoxin	0.9	90,000 ?	mouse
Diphtheria toxin	3.5	245,000	guinea pig
Tetanus toxin	1,200	120,000,000 ?	guinea pig
Botulinus type A toxin	1,200	1,200,000,000	guinea pig
Botulinus type B toxin	1,200	72,000,000	guinea pig

PART I

THE TOXINS OF GRAM-POSITIVE BACTERIA

CHAPTER I

GENERAL CHARACTERISTICS

I.i. Activity and Nature

Table 1 shows that the biological activity of the Gram-positive toxins is very high indeed. Aconitin is one of the most toxic drugs known, yet it is weight for weight less toxic than most of the Gram-positive toxins. The toxins have high molecular weights and therefore their activity per molecule is considerably greater than that of the drugs; but this difference would be less impressive if the toxins had more than one active centre per molecule, and it is conceivable that the toxins may in effect be aggregates of many active centres.

Those Gram-positive toxins that have been purified are proteins, and the behaviour of the other toxins suggests that they are also proteins, although this is only an assumption for the present. Sometimes a toxic preparation is reported that has the elementary analysis of a typical protein and it is assumed that the toxin is a protein, and that the preparation is in a highly purified condition. But such an elementary analysis could also hold for a preparation containing a small concentration of a non-protein toxin contaminated with a high proportion of protein — in a toxin preparation the most likely contaminants are proteins. On the other hand preparations of tetanus toxin have been described with properties different from proteins and it has been claimed that the toxin is not a protein. In fact tetanus toxin is a protein, and in the preparation concerned it was contaminated with an overwhelming proportion of non-protein material. The purified toxins appear to be quite ordinary proteins without prosthetic groups, their chemical nature offering no obvious clue to their mode of action. Many enzymes, of course, are simple proteins whose biological activity probably depends on the nature of their surfaces.

Pappenheimer (1947 b) and Bernheimer (1948) distinguish between slowly acting, highly toxic toxins and rapidly acting, less toxic toxins. They place botulinus, tetanus, and diphtheria toxins in the former category and *Cl. welchii* Alpha toxin and other haemolytic toxins in the latter. It is true that the former group of toxins acts

much more slowly than the latter. Wright & Hopkins (1946) have shown that an intradermal dose of *Cl. welchii* Alpha toxin is fixed in the skin within 20—30 minutes, whereas diphtheria toxin (Wright & Clark, 1944) is not completely fast after 8—12 hours. Although animals injected with minimal lethal doses of diphtheria toxin and the Alpha toxin take about the same time to die, it is possible to reduce the death time to less than an hour with a large enough dose of Alpha toxin, and impossible to reduce the death time with diphtheria toxin to less than about nine hours, no matter how large the dose. It is also commonly thought that the death times with botulinus and tetanus toxins cannot be reduced to less than about 8 hours, but Pillemer & Wartman (1947) have succeeded in killing mice within an hour by injecting as many as 500,000 lethal doses. The extreme toxicity of botulinus and tetanus toxins places them in a special category, but the difference between diphtheria toxin and *Cl. welchii* Alpha toxin is not so striking. The best preparation of Alpha toxin has 200 LD_{50}/mg. for 1 kg. of mice, and at least 50 % of this preparation is not toxin (van Heyningen & Bidwell, 1948); pure diphtheria toxin has 3500 MLD/mg. for 1 kg. of guinea pig (and only 3.5 MLD/mg. for 1 kg. of mice). As far as toxicity is concerned diphtheria toxin is more comparable with the Alpha toxin than it is with botulinus and tetanus toxins.

The susceptibility of different species of animals to a given toxin can vary considerably. For instance, diphtheria toxin is a thousand times more toxic for the guinea pig than it is for the mouse. Moreover, with crude preparations of toxin at any rate, the ratio of species susceptibility to a single toxin can also vary over wide limits. This point is discussed in a later chapter.

With the exception of the Alpha toxin of *Cl. welchii* we are not yet able to define the substrates of bacterial toxins in chemical terms (unless certain proteolytic enzymes are regarded as toxins). Sometimes the substrate can be defined anatomically (e.g. the nervous system, or the blood), but often the location of the substrate can only be described as "wide-spread". Moreover, the damage that toxins cause in animal cells, even if it results from an attack on a single substrate, can generally be seen in a number of different ways. *Cl. welchii* Alpha toxin is an enzyme that hydrolyses lecithin, and this action results in lethal, dermonecrotic, and haemolytic effects, besides those that can be observed *in vitro*. It is interesting that the neurotoxins are not dermonecrotic. This probably means

that their substrates are not wide-spread. The dermonecrotic action is specific in that it is not caused by toxoid and is inhibited by antitoxin; since it is shown by many diverse toxins it follows that the skin contains the substrates of many toxins. It would be remarkable if these substrates were confined to the skin and it is probable that they are found in most of the other tissues of the body. If a toxin is not dermonecrotic it can be assumed that it attacks a specialised tissue.

Because the Gram-positive toxins are probably proteins, and are active in very low concentrations, they resemble enzymes. The Alpha toxin of *Cl. welchii* is known to be an enzyme, and it is likely that there are more toxins that are enzymes. It is also possible that toxins may be very intimately concerned with enzyme action, for instance by being direct enzyme inhibitors like the protein trypsin-inhibitor of the Soya bean, or by being kinases that convert inactive zymogens to active enzymes. But it does not follow that all toxins are enzymes, or that they are all directly concerned with enzyme action. To say that their action must in some way affect the enzymes of the host is to state an unhelpful truism. Recent work on the active polypeptide fractions of protein hormones (see Li, 1949) hints at another possibility for the mode of action of toxins. The mode of action of biologically active proteins is one of the most interesting problems of biochemistry to-day and research on the bacterial toxins offers inviting and rewarding prospects in this field.

I.ii. Production and Purification

A typical Gram-positive toxin, like the Alpha or the Theta toxin of *Cl. welchii*, is produced and excreted into the medium during the phase of active growth of the organism, and it reaches its highest concentration soon after the growth of the parent cells has reached its maximum. This is shown in figure 1.

On the other hand, the classical toxin of *Cl. tetani* reaches its maximum concentration in the culture filtrate long after the culture has attained maximal growth, and considerable further amounts of toxin can be obtained by allowing the fully grown organisms to autolyse.

Sometimes a toxin may be excreted in an inactive form during the early stages of growth and become active later. Turner & Rodwell (1943) found that the Epsilon toxin of *Cl. welchii* is excreted as a non-toxic antigenic precursor that is slowly converted into

active toxin by proteolytic enzymes that are also produced by the organism. A phenomenon that is reminiscent of this was reported of pneumococcal haemolysin by Cohen, Halbert & Perkins (1942). This toxin remains within the cells, provided the pH is not allowed to fall, and reaches its maximum after 12 hours, at the same time as maximal growth is reached. But on storage at 5°—10° C. for a further 15 hours "maturation" takes place and the total yield of haemolysin increases 4-fold or more.

A growth medium from which purified toxin is to be prepared should be as free as possible of substances that are difficult to separate from the toxin. A synthetic medium composed of amino acids and other growth factors is obviously the ideal, but a synthetic medium is expensive to use on a large scale, and although it may allow the growth of the organism it will not always support luxuriant growth. Even if it supports growth it will not necessarily favour the production of high yields of toxin. Since the toxins are proteins, or at any rate not dialysable, it may suffice to use a medium containing ill-defined but dialysable constituents that can readily be removed from the toxic filtrate by dialysis. Often it is impossible to obtain very high yields of toxin except on complex media containing non-dialysable constituents. This does not necessarily mean that non-dialysable constituents are necessary for toxin production, but rather that unidentified toxigenic factors have not been separated from such constituents. Whether very high yields of toxin should always be sacrificed for comparative simplicity of medium is a debatable point. The chemistry of toxin purification is largely the chemistry of the substances that contaminate the toxin, and it may be easier and more profitable to remove these substances from the toxic culture filtrate than from the medium before inoculation. The value of potent filtrates, obtained in this instance by culture selection rather than choice of media, is illustrated by work with tetanus toxin. Eaton & Gronau (1938) had produced toxic filtrates from a culture of *Cl. tetani* grown on a veal infusion broth containing peptone. On purifying the toxin by cadmium and ammonium sulphate precipitation they obtained a preparation that was 125 times as active per unit of dry weight as the crude filtrate. Pickett, Hoeprich & Germain (1945) used a similar medium, but a much more toxigenic strain of *Cl. tetani* that produced a hundred times as much toxin. They purified the toxin in the same way as Eaton & Gronau and although their overall purification was only 3 times as efficient, their final product was a hundred times as

active as Eaton & Gronau's, and 70 % as active as the crystalline toxin that was later isolated by Pillemer (see below). It is also worth noting that Pillemer obtained pure tetanus toxin from culture filtrates of the organism grown on a complex medium.

It is an interesting fact that iron affects the production of at least three Gram-positive toxins, — *Cl. welchii* Alpha toxin, tetanus toxin, diphtheria toxin — and one Gram-negative toxin that resembles the Gram-positive toxins — *Sh. shigae* neurotoxin. The highest yields of toxin are obtained when the iron content of the medium is very low, in some cases when it is lower than that necessary for maximal growth of the organism. It is unlikely that the mechanism by which iron inhibits toxin formation is the same in all these cases.

The concentration of toxins in bacterial filtrates, in terms of weight of toxin per unit volume, is very low even in the most potent filtrates. It is probably of the order of 5—20 mg. protein per litre at the most, and losses during purification must be anticipated. Therefore, if the purification of toxins is intended, it is necessary to obtain large volumes of culture filtrate. It is doubtful if success can reasonably be expected unless volumes of the order of 100 litres or more are used.

I.iii. Other Toxic Proteins

A review of the bacterial toxins would be incomplete without at least a passing reference to those similar toxic proteins that are produced by organisms other than the bacteria. Abrin, crotin and ricin are toxic haemagglutinating and haemolytic proteins found in the seeds of *Abrus precatorius*, *Croton tiglium* and *Ricinus communis*. The mode of action of these toxins is not yet understood, but the recent isolation and crystallisation of ricin is a step in the right direction. (Kabat, Heidelberger & Bezer, 1947; Kunitz & McDonald, 1948). The snake venoms contain a number of toxic proteins, including haemorrhagins, haemagglutinins, haemolysins, neurotoxins, lecithinases, lysolecithinases, and various other enzymes such as phosphoesterases, cholinesterases, amino oxidases and proteases[1]). Crotoxin, a neurotoxin from the rattle snake *Crotalus terrificus*, has been crystallised by Slotta & Fraenkel-Courat (1938), and De (1944) has crystallised lecithinases from the haemolytic fractions of the venoms of *Naja tripudians* and *Bungarus fasciatus*. An interesting

[1]) See Macfarlane, 1937; Ghosh, De & Chaudhuri, 1941; Githens, 1935; Fairbairn, 1945; Bovet & Bovet, 1943; Zeller, Kocher & Maritz, 1944; Gulland & Jackson, 1938.

aspect of the activity of snake venoms has been discovered by Chain (1937, 1938, 1939; Chain & Goldsworthy, 1938). The venoms of the cobra and the black tiger snake contain proteins which in high dilution inhibit fermentation and glycolysis by yeast extracts. The inhibition is specifically counteracted by antivenin. Apparently the active protein is a nucleotidase because it hydrolyses the phosphoric acid linkages in co-enzyme I and thus inhibits all those dehydrogenases (lactic, malic, β-hydroxybutyric and amino acid) that need the co-enzyme for their activation.

Various Egyptian scorpions also produce toxic proteins, but little is known of their nature or mode of action (see Mohammed, 1944).

There are one or two enzymes that are toxic in low concentrations. Urease, obtainable from a number of sources, including bacteria, is toxic because it produces toxic concentrations of ammonia from the blood urea of the animal injected. It is not toxic to the chicken, whose blood does not contain urea. Glucose oxidase (notatin) is toxic because it catalyses the production of toxic concentrations of hydrogen peroxide. Although its toxicity has not yet been determined, it is likely that histidine decarboxylase is toxic because it catalyses the production of histamine.

I.iv. Antitoxins and Anti-enzymes

For a long time it was thought that enzymes were not antigenic because nobody had succeeded in producing antibodies against them that would inhibit their activity. Consequently there was a tendency to place enzymes and toxins in separate categories (see, for example, Wells, 1929). Actually some enzymes, for example, the lecithinase (Alpha toxin) of *Cl. welchii*, do stimulate the production of neutralising antibodies, and in fact most, if not all, enzymes are antigenic in that they stimulate the production of antibodies, but these antibodies do not always neutralise the activity of the enzyme. Urease-antiurease floccules retain most of the activity of the urease in the floccules, catalase is practically unimpaired when mixed with anticatalase, and tyrosinase is not inhibited by antityrosinase.

The fact is that the biological or catalytic activity of a protein is not necessarily concerned with its antigenicity. Inactive proteins, or toxoided toxins, can still be antigens — a point that seems to have escaped Sevag (1945), who suggests that those functions of proteins that are responsible for their biological activity are also responsible for their antigenic activity. The antibody to an enzyme