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TRANSLATION

THE "TOXIN-ENZYLE" HYPOTHESIS AND THE ROLE OF BACTERIAL ENZYLES IN THE PATHOGENESIS OF INFECTIONS

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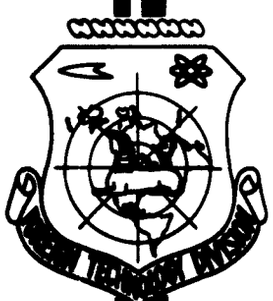

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THE "TOXIN-ENZYME" HYPOTHESIS AND THE ROLE OF BACTERIAL ENZYMES IN THE PATHOGENESIS OF INFECTIONS

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THE "TOXIN-ENZYME" HYPOTHESIS AND THE ROLE OF BACTERIAL ENZYMES IN THE PATHOGENESIS OF INFECTIONS

I. V. Domaradskiy

In recent years many works have appeared in which the authors attempted to identify bacterial toxins* with enzymes, and toxicosis is considered the consequence of the action of these enzymes on appropriate substrates. It is assumed that death of an organism ensues either as a result of the destruction of cell structures or under the effect of toxic products which are formed secondarily (Konikov, 1950; Gubarev, 1952, 1956; Galaev, 1956; Gubarev and Ivanovskiy, 1958; Matveyev, 1959; Zaplatina, 1959; Poverennyy, 1961 et al.).

1.

As is known the "toxin-enzyme" hypothesis arose as a result of studying gram-positive bacteria. It is precisely in these bacteria that toxins having catalytic properties were found. And this is not by chance.

All species leading a parasitic mode of life are found vastly more rarely among gram-positive bacteria than in the group of

* In this article we will deal mainly with the so-called exotoxins.

gram-negative bacteria. Many of them, especially the sporiferous (Bac. botulinus, Bac. tetani, Bac. anthracis, Bac. sordellii, Bac. perfringens and other causitive agents of gas gangrene) are frequent (or permanent) inhabitants of the external environment, including the soil (Mishustin and Pertsovskaya, 1954; Matveyev, 1959, 1960). In view of this the collection of enzymes of pathogenic gram-positive bacteria is distinguished by a great wealth and diversity. In particular they are widely represented by hydrolases, enzymes which accomplish the first steps in the breakdown of complex organic substances which cannot be per se assimilated by cells. These enzymes are apparently excreted into the ambient medium. Other enzymes participating in intermediate metabolisms are usually not excreted to the external environment or pass to it only after cell necrosis. Those that are called toxins are also excreted to the external environment and are among the gram-positive bacteria. Pathogenic gram-negative bacteria, with the exception of Bact. dysenteriae Shiga, which by virtue of their paracitic mode of life mainly have intracellular enzymes, do not produce true toxins.

Finally, judging by the data in the literature, all toxins or factors of aggression having catalytic properties are hydrolases.*

Therefore, there is nothing surprising that toxins-enzymes are found only in representatives of gram-positive microbes and only in those whose toxin production is directly associated with processes of cell nutrition.

From our point of view it would be rash to apply the data on the identity of enzymes with toxins obtained in the study of this narrow group of bacteria to all microorganisms.

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* The exception is only the water-soluble oxidase of 1-amino acid found in snake venom (Zeller, Maritz, 1944).

A great similarity does exist between toxins and enzymes and this is noted when there were still no clear concepts of the chemical nature of the latter (Wells, 1929). One of the features of this similarity is the toxicity inherent to certain enzymes upon parenteral administration. A classical example confirming this is crystalline urease which causes breakdown of blood urea and, as a consequence, of this ammonia poisoning of the organism (Kirk, Sumner, 1931); birds whose urea is not the final product of nitrogen metabolism, are resistant to urease (Howell, 1932). Another example is the toxicity of crystalline trypsin and chymotrypsin for rabbits, rats, guinea pigs, cats, and dogs (Rocha e Silva, 1931a, b; 1940). Both these enzymes cause myocardial damage to animals (Hendley et al., 1956). Cathepsin, formed by streptococci of group A, has a similar effect on rabbit myocardium (Kellner, Robertson, 1954a, b). It is possible to give other examples indicating the toxicity of enzymes.

The features of similarity between enzymes and toxins also include their ability to cause the formation of corresponding antibodies. True, enzymes are rather weak antigens (Dormarabskiy, 1961).

Under the effect of formaldehyde and heating, certain enzymes can be changed to anaenzymes. This is proved by the example of papain and gelatinase of the tetanus bacillus (Ramon, 1957) which under the indicated effects lose their enzymic activity but retain the antigen properties. Serum of animals immunized with "anapapain" or "anagelatinase" has the ability to suppress the activity of the corresponding enzymes not treated with formalin and not subjected to heating. We can conclude from this that the active centers of enzymes are apparently not identical to the determinative groups causing antigen properties. The similarity of enzymes with toxins is also manifested in this.

Finally, enzymes like toxins are proteins; most of them are thermolabile, although in certain cases they can withstand the effect of high temperatures without a decrease in specific activities (Thompson et al., 1958).

However, the similarities between toxins and enzymes is also insufficient for their identity since there are no indisputable proofs of the presence of catalytic properties for the overwhelming majority of toxins.

In spite of all this there is a tendency to enlarge the frameworks of the "toxin-enzyme" hypothesis and to convert it to a universal theory of the action of toxins. The presence of catalytic properties is even postulated for such toxins as diphtheria, botulinus and tetanus (Gubarev, 1952). The facts testifying against this hypothesis are not sufficiently taken into account.

The latent period in the action of toxins and their unusual activity force us to assume that they act not like enzymes but like compounds suppressing the transformation and synthesis of certain of the most important cell components (Dubos, 1957). In particular, the available data indicate that diphtheria toxin in sensitive animals block the synthesis of cytochrome b or one of the enzymes close to it (Pappenheimer, Hendee, 1947; Pappenheimer, Williams, 1952; Pappenheimer, 1947a, b). Moreover diphtheria toxin noticeably inhibits the synthesis of protein of human carcinoma. In this respect its action differs from the action of cyanide and dinitrophenol (Strauss, Hendee, 1959). Another toxin produced by the fungus Fusarium lycoperici and causing wilting and leafroll of tomatoes affects metabolism because by its structure it is very similar to the growth factor streptogenin. In this case between the toxin, which is a peptide derivative of asparagin, glycine, and serine, and streptogenin whose composition includes

glutamic acid and glycine, there is an antagonism of a noncompetitive nature (for details see Wooley, 1954).

The so-called endotoxins of gram-negative bacteria, which in most cases are lipo-polysacchride-protein complexes completely devoid of any catalytic properties, evidently act according to the type of inhibitors. A characteristic feature of the action of endotoxins is that regardless of their origin they cause a similar picture of intoxication and similar biochemical shifts in an organism (Zil'ber, 1958; Dubos, 1957; Domaradskiy et al., 1961; Delaunay, Lebrun, 1947 et al.). According to the opinion of Heyningen (1950), this feature should underlie the differentiation of toxins of gram-negative and gram-positive bacteria since each toxin of the latter has its own specificity of action.

Unfortunately we still do not know the points of application of the action of most toxins. We can only say that in the case of the toxins of the causative agents of tetanus and botulism, cholinesterase of the main ganglia of the central nervous system is not such a point (Isibasi, 1959).*

The instability of the condition of the adherents of the "toxin-enzyme" hypothesis, from our point of view, is easily demonstrated by the following examples.

First, the investigators most frequently have to deal with unpurified toxic fractions of microbes in which other biologically active substances are present together with the toxins proper. Many of

* Buehler et al. (1947) considers that when explaining the high toxicity of botulinus toxin it is necessary to take into account: 1) the polar nature of the compound, 2) the arrangement of amino acids within the molecule and the geometric configuration of protein as a whole, 3) the possibility of an atypical peptide bond between the aspartic and glutamic acids, 4) the possibility of the presence of toxic groups in unanalyzable amounts, 5) the high molecular weight of protein.

...em have a catalytic activity. For example staphylococcal enterotoxin preparation contained apyrase wherein it is still impossible to determine where the action of enterotoxin ends and the action of apyrase begins (Sugiyama, Dack, 1955). The same can be said about o-streptolysin whose leukotoxicity and ability to inhibit respiration of mitochondria are associated with the presence of diphosphopyridine nucleotide (Carlson et al., 1956, 1957), or about staphylocoagulases containing lipase (Drummond, Tager, 1959a, b), etc. An underestimation of this circumstance can lead to serious errors.

Second, it is difficult to explain why one and the same enzymes excreted by various microbes sharply differ in their toxicity. For example, little understood is the reason for the approximately similar toxicity of the lecithinases of the pathogenic microbe Bac. perfringens and the nonpathogenic microbe Bac. cereus in spite of their dissimilarities as antigens (Chu, 1949) and the absence of toxicity for lecithinase of the anthrax causative agent (Costlow, 1958). An explanation of the relatively lesser toxicity of the lecithinase of Bac. cereus in comparison with that of the lecithinase of Bac. perfringens is that the former is suppressed by normal serum which apparently is not applicable to lecithinase of any origin since the nontoxic lecithinase of Bac. bifermentans is not suppressed by normal serum (Miles, Miles, 1950).

Third, since it was established that the participation of active centers of an enzyme in the actions of an enzyme with an antibody is not obligatory (Kirk, 1933; Kirk, Sumner, 1934; Haas, 1940; Campbell, Fourt, 1939), we cannot yet give an answer to two questions: 1) does the toxicity of enzymes depend only on their active group and 2) what explains the mechanism of the therapeutic actions of corresponding antiserums.

Fourth, acceptance that enzymes have a direct toxic action on tissue, cells, or metabolism does not explain (or does not always explain) the cause of their over-all action on an organism. It is not precluded that in addition to enzymic action, toxins-enzymes have other properties no less important for the origination of disease (Dubos, 1957; Heyningen, 1958).

Finally, different enzymes and substances not having a catalytic activity cannot cause similar symptoms of a disease or a similar reaction with respect to isolated organs. For example, the permeability of capillaries is increased not only by hyaluronidase, but also by trypsin, chymotrypsin, plasmin and proteinase of the skin (Paskhina, 1955). Erythrocytes are dissolved by saponins and also by the α-toxin of Bac. perfringins and the proteinase of staphylococcus (Robinson et al., 1960). A picture of beri-beri can be caused by B_1 avitaminosis of an exogenous origin, by infection of the organ with Bac. thyaminolyticus and by protein deficiency. Common symptoms are noted in tetanus and strychnism.

In all these cases (as in many others) we deal with the same consequence but with a different mechanism of action.

It follows from what has been stated that when solving the problem of the mode of action of toxins we must show extreme care, and a particular conclusion obtained on the basis of studying the toxins of individual species of bacteria cannot unconditionally be applied to other toxins. The method of analysis, no matter how attractive it seems, is not applicable in this case.

2.

However, while we object to those who consider all toxins as enzymes we do not deny the role of enzymes of bacteria in the pathogenesis

corresponding disease in some cases. Conversely, we consider that too little attention has been devoted to this aspect of the problem.

The abilility to become adapted in a susceptible host, which is an "external environment" for a pathogenic microbe, to a considerable extent depends on the biochemical activity of the microbe. In contrast to microbiologists who judge the biochemical activity only by the behavior of bacteria on "variegated" series, we enclose a broader meaning in this concept. Biological activity is the intensity of metabolism, the intensity of all reactions occurring in a cell and causing its vital activity. From the indicated position not a single pathogenic microbe can be considered as biochemically inert. We will recall that the evolution of microbes mainly concern those processes which pertain to energy metabolism (fermentation, oxidation) and to a lesser extent affect anabolism. Anabolism of various organisms, including biochemically "active" or "inert" (from the point of view of microbiologists) faces one problem, "the building of body substances-protoplasts-, which in different representatives of life, according to present-day concepts, differ only in a slight degree" (Shaposhnikov, 1960).

A disease can be caused only by that pathogenic microbe which, all other things being equal (susceptibility of the macroorganism, state of the histohematic barriers, etc.), has a high biochemical activity sufficient to provide its source requirements of nutrition and energy. The deciding role in the development and outcome of a disease therefore belong to these bacterial enzymes.

There are many examples that bacterial enzymes can play the role of the pathogenic agents. One of them is the participation of thyaminase of Bac. thyaminolyticus in the development of B_1 avitaminosis

(Kleeberg, 1958). Another example is urease of Proteus mirabilis which causes the development of experimental pyelonephritis. In contrast to Proteus, Escherichia coli, deprived of urease activity, does not cause lesions of the renal epithelium either in vivo or in vitro (Braude, Siemienski, 1960).

The pathogenic significance of staphylococcal enzymes does not evoke doubts. Among them a particular place belongs to coagulase (Smith, Johnstone, 1958; Blobel, Berman, 1961), which, in the opinion of most investigators, is an enzyme (Lieb, 1960; Fusillo, Jaffurs, 1955). Fusillo and Jaffurs, (1955) consider it an adaptive enzyme.

Coagulase has antigen properties (Duthie, Lorenz, 1952; Blobel et al., 1960); animals actively immunized with coagulase are more resistant to infection by coagulase-positive strains of staphylococci (Boake, 1956).

Less is known about the pathogenic role of other enzymes in toxic filtrates of staphylococci. We can only note that lipase is inactivated by human blood gamma-globulins (Richou et al., 1960, 1961) and has no relation to blood clotting by coagulase (Drummond, Tager, 1959b) whereas protease, purified to a considerable extent of concommitant substances, has properties of α-hemolysin (Robinson et al., 1960).

However, most typical is the example of the participation of the enzymes of Bac. perfringins and other bacteria in the pathogenesis of gas gangrene (for a detailed review see Kaplanskiy, 1954 and Chistovich, 1951). This was first proved by Macfarlane and Knight (1941) who identify α-toxin of Bac. perfringins with the enzyme lecithinase (lecithinase C). The result of the action of this enzyme is the destruction of the protective layer of lecithin on particles of protein and fat and the destruction of cell structures, in particular

decomposition of the membranes of erythrocytes (Macfarlane, Oakley Anderson, 1941).

Lecithinase activity of α-toxin is very great. For example, a single lethal dose of it can split all lecithin contained in mouse blood. The activity of lecithinase C is paralyzed by a specific antitoxin wherein competition for lecithinase exists between the antitoxin and lecithin (Zamecnik, Lipmann, 1947).

Macfarlane and MacLenan (1945), Oakley et al., (1946) and others detected another enzyme, collagenase, which dissolves collagen of connective tissue, in the composition of the toxin of Bac. perfringens.* This enzyme, or kappa-toxin, is also neutralized by a specific antitoxin. In addition to collagenase, the filtrates of Bac. perfringens cultures contain other proteolytic enzymes, in particular lambda-enzyme. The latter was originally detected in a filtrate of a culture of Bac. perfringens type B and, according to the data of Oakley (1948), it can split proteins obtained from the skin of animals and also native collagen but does not act on animal muscles and denatured collagen. The problem as to what proteins are decomposed by collagenase and what ones by other proteolytic enzymes, including the lambda-enzyme, is in need of further investigation.

Nothing is yet known about the role of deoxyribonuclease of Bac. perfringens (Robb-Smith, 1945) in the pathogenesis of gas gangrene.

Among the bacterial enzymes we can presently consider only the lecithinase of certain clostridia and possibly the proteinase of staphylococcus as toxins (Robinson et al., 1960). Other enzymes in the sense in which the word "toxin" is usually used, are not toxins

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* It was recently established that collagenase of another causative agent of gas gangrene, Bac. histolyticus, has together with a proteolytic activity a trans-cis-propyl (hydroxyproline)-isomerase activity (Shpikiter, 1961).

because there is no specificity in their action on an organism, they do not kill tissue cells on which they act, and many of them (e.g., hyaluronidase, collagenase, deoxyribonuclease, certain fibrinolysins) decompose apparently exclusively extracellular substrates. These enzymes create only the necessary conditions for the development of microorganisms, the background, on which evolves the specific picture of the corresponding infectious process. Once again we repeat: this aspect of the activity of bacterial enzymes must not be underestimated.

In individual cases the changes in an organism caused by enzymes can be expressed very vigorously and in themselves serve as a cause of disease or prolong its course. It was established, for example, that the bacterial enzymes of dysentery caused the formation of different amines which in turn promote the formation of fatty degeneration of the liver in this infection (Takatori, 1955). The formation of amines and ammonia by intestinal bacteria also plays a role in the development of hepatic coma (Phear, Ruebner, 1956). Glutamine accumulates in brain tissue in pneumococcal infection of rats (Vyshepan and Porfir'eva, 1958). In the pathogenesis of cholera an important role is attributed to mucinase and lactic acid excreted by the cholera vibrion into the intestine. It is assumed that mucinase decomposes the epithelium and lactic acid increases the permeability of the intestines, thus fostering loss of liquids by the organism (Jenkin, Rowley, 1959). Enzymes of many bacteria decompose the group-specific substances of the blood (Iseki et al., 1959), cause intravascular coagulation (Smith, Johnstone, 1958; Blobel, Berman, 1961), intravital lysis and agglutination of arithrocytes (Keppie et al., 1955), cause pulmonary hemorrhages (Howes, Mandl, Zaffuto, 1960) and other phenomena.

The question arises in connection with the role of enzymes in the pathogenesis of infection: what special features of the chemical composition or metabolism distinguish a pathogenic microbe from a nonpathogenic microbe?

As far as we know no substantial differences have been established in the chemical composition of pathogenic and nonpathogenic microbes.*

The same amino acids are found, for example, in the proteins of pathogenic and nonpathogenic staphylococci (Sasaki, 1958). Moreover, the amino acid composition of the proteins of nonpathogenic cocci mainly coincides with that of the cholera and choleralike vibrios, differing from them only by the absence of an unidentified amino acid and aminobutyric acid. However, the spots of these amino acids are slightly demonstrated on certain chromatograms, which gives us grounds to consider this difference as being only quantitative (Samarina et al., 1950). The data on the identity of the amino acid composition of cholera and paracholera vibrios were confirmed by L. F. Pustovalova (1961).

True, many pathogenic and nonpathogenic microbes differ from one another with respect to the antigen structure, including with respect to polysacchrides. However, this is usually established by immunological and, very rarely, chemical methods. Proof of the relative ineffectiveness of serological methods of differentiation of pathogenic microbes from nonpathogenic is the fact of finding common antigens

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* As Tracey indicates (1954), "the chemical composition of the majority of organisms that have been studied is approximately the same. Glucose is the dominant sugar in potatoes and teredo, in the elephant and in bacteria. Many other sugars and about twenty amino acids within proteins are universal for all living organisms." A discussion of this question is in the works of Lise (1958) and Shaposhnikov (1960).

even in phylogenetically unrelated species of microorganisms, for example in pneumococcus and Friedlander's bacillus (Avery et al., 1925), in pneumococcus and various strains of yeasts (Neill, 1939).

It is even more difficult to establish the differences in the chemical composition of virulent and avirulent strains of pathogenic bacteria.

The V_1 antigen is not an exclusive attribute of virulent strains of the typhoid bacillus, but is found in a number of other nonpathogenic species (Almon, 1943; Luippold, 1942, 1944; Stuart et al., 1943; Petrosyan and Zvenigorodskaya, 1957 etc.). Similarly the presence of M-protein in streptococci of group A does not always serve as an index of their virulence (Todd, Lancefield, 1928).

The virulent strains of Escherichia coli are similar to avirulent strains with respect to chemical composition, in particular the content of the toxic lipopolysacchrides (Harvey, Phillida, 1960). According to the data of Bennett (1959), the virulent strains of Bac. anthracis contain appreciably more phosphorous of DNA than the avirulent microorganism. The content of RNA was the same both for the virulent and avirulent strains. However, for three avirulent strains of the anthrax microbe sensitive to penicillin, the ratio of RNA and DNA was the same as for the virulent strains.

Apparently, a reliable difference in the chemical composition of virulent and avirulent strains could be established only for mycobacteria. In contrast to the avirulent strains, the virulent strains of mycobacteria contained a special lipoid on the cell surface which causes their growth as coillike bands. A single injection of this lipoid (cord-factor), which per se does not cause morbid symptoms, accelerates the course of both acute and chronic tuberculous infection (cited from Model', 1958).

We can note further that not one of the autotrophic microorganisms, i.e., microbes which do not use organic substances as a primary energy source (Lise, 1958), has pathogenicity for man and animals. All pathogenic microbes belong to the number of heterotrophic organisms. However, the degree of heterotrophy of pathogenic microbes varies within wide limits. Some of them are parasites, do not grow at all or are cultivated with difficulty on artificial nutrient media, others can lead a saprophytic mode of life and can be satisfied with a negligible collection of organic substances. The latter group enables us to distinguish, in addition to zoonoses, anthroponoses, and anthropozoonoses, still another group of diseases, namely sapronoses (Terskikh, 1958). Of the number of diseases caused by microbes, botulism and also, probably, proteism and melioidosis, can be referred to sapronoses (Fournier, Chambon, 1958).

The genus Pasteurella is an interesting example of a gradual increase in the demand for organic substances (Berkman, 1942).

The requirement for sources of energy (and nutrition) varied not only in different species of pathogenic microbes but also in individual strains of one and the same species of microbe (Kuzin, 1946; Stefenson, 1951; Domaradskiy, 1955, 1958; Domaradskiy, Ivanov, 1957).

Attempts to establish a relation between the pathogenicity or virulence of a microbe and the characteristics of its metabolism yields contradictory results. Possibly this is because the concept of "virulence" is still far from sufficiently refined.

We find the most distinct definition of virulence in the work of L. A. Zil'per (1958) who considers that "virulence in contrast to pathogenicity is not a species characteristic. It is a quality inherent to a given strain of microbes, it is an individual characteristic and not a species characteristic. We could say that virulence

is the quality or degree of pathogenicity in a given strain of an infectious agent."

From this position if we are concerned with microbes whose main characteristic as pathogenic agents is their ability to cause toxemia and not an infection proper (Bac. botulinus, Bac. tetani, Bac. perfringens etc.), we can place an equality between virulence and toxigenicity. In addition, we perceive a difference between toxigenicity and toxicity. Toxigenicity is the property of the pathogenicity of a definite group of microbes producing toxins which are easily excreted to the environment in a soluble form and have a characteristic pharmacologic activity. Toxicity is inherent to most bacteria and is associated with decomposition products and cell metabolism. Even cells of nonpathogenic species of microorganisms can have toxicity. This explains in particular the absence of correlation between virulence and "toxigenicity" of the plague microbe (Englesberg et al., 1954) and the multiple rise of the toxicity of its decomposition products when treating a culture with glycocoll (Korobkova, 1957) or surface-active substances (Pannell, 1955; Goodner et al., 1955).

The ineffectiveness or contradictions of the attempts to establish a relation between virulence and the characteristics of metabolism can be demonstrated by the following examples.

As is known Huddleson (1942) and Merz (1938) were the first to show that virulent strains of brucellae has a higher catalase activity than avirulent strains. Later these observations were confirmed by a number of authors, including Schierholz and Jeder (1956). However, according to the data of Guerra (1959), the catalase reaction cannot be used either for differentiation of types of brucellae or for determining the degree of their pathogenicity. Geissler (1959a) arrived at the same conclusion.

The determination of urease activity can serve only to distinguish Brucella suis from Brucella abortus but is not suitable for determining the degree of pathogenicity of brucellae (Geissler, 1959b).

According to Rockenmacher (1949) a direct relationship exists between the activity of catalase and the virulence of the plague microbe. However, M. N. Dzhaparidze (1953) denies this. It was also established that the virulence of the plague microbe is not associated with fibrinolysin, hyaluronidase, the capacity to coagulate plasma of various species of animals, the oxidation under aerobic and anaerobic conditions of a number of substrates, including amino acids purine and pyrimidine bases, the activity of transaminase, aldolase, phosphatase and a number of other enzymes (Sagar et al., 1956a, b; Saxena et al., 1957; Srikantan et al., 1957a, b; 1958; Misra et al., 1960).

The capacity of strains of Salmonella typhi to oxidize glucose tyrosine, and glutamic acid does not depend on the presence in them of V_1-antigen (Baron, 1955; Costa, Villela, 1959). Judging by the data of Galayev and team (1961) the correlation between pathogenicity and decarboxylase activity of Escherichia coli cannot be considered as proved.

Edwards (1960) could not establish a relationship between toxin formation and the activity of catalase for the use of maltose in cultures of Corynebacterium diphtheriae.

Fleming and Foshay (1955, 1956) made an interesting attempt to reveal the biochemical nature of the virulence of tularemia bacteria. Judging by the data which can presently be considered only as preliminary, virulent strains of tularemia microbes in contrast to avirulent strains have the capacity to split citrullin and a higher activity than enzymes oxidizing glutamic acid. Conversely, the avirulent strains are distinguished by high activity of the system

aspartate-alanine-transaminase. The oxidation rate of serine or amination of pyruvate is the same for both groups of strains.

On the other hand, in individual cases there is such an expressed dependence between pathogenicity and some aspect of microbial metabolism that the degree of its pathogenicity (i.e., virulence as meant by Zil'ber or toxigenicity) can be judged only on the base of results of in vitro experiments without resorting to experiments on animals. Unfortunately, such a dependence is far from always the exclusive cause of microbial pathogenicity.

In recent years titration methods for α-toxin of Bac. perfringens (or antitoxin) have been widely introduced in practice; these methods following Nagler's principle are based on the decomposition by toxin of the lecithin walls of serum fat particles or of egg lecithovitellin (Nagler, 1941; Heyningen, 1941; Tsekhnovitser et al., 1943; Anan'yeva, 1942; Dotsenko, 1948 and others). However, more accurate methods are those methods in which the lecithin activity of the toxin is dependent by the amount of phosphorus of the split-off phosphorylcholine or by the amount of carbon dioxide displaced by the product of lecithin splitting.

To distinguish pathogenic strains of mycobacteria from nonpathogenic, it is especially important to determine dehydrogenase and catalase activity.

In the presence of glucose, nonpathogenic strains of mycobacteria bleach methylene blue for 1-3 min, whereas strains of avirulent (attenuated) Mycobacterium tuberculosis or BCG, for 3-15 min. In contrast to this, suspensions of young virulent Mycobacterium tuberculosis do not bleach methylene blue during the course of an hour (Bloch, 1950).

Virulent strains of mycobacteria in comparison with avirulent strains and saprophytic mycobacteria have a higher catalase activity. Inactivation of the catalase of virulent strains occurs at 64-68° and of avirulent strains at 58-60° (Schweiger et al., 1958).

Presently the determination of catalase activity is most frequently used when studying the virulence of drug-resistant strains.

Viallier and Cayré (1958), who established a dissimilar sensitivity to hydrogen peroxide of strains of mycobacteria having similar catalase activity, propose to determine the degree of pathogenicity by the survival rate of cells of these strains in contact with 0.15% hydrogen peroxide for one hour.

Other data on the relation between the enzymic activity of mycobacteria and their virulence can be found in the works of Model' (1958), Segal and Bloch (1956), Kolsut (1959), and others.

A no less striking example is the relation between the characteristics of metabolism and the pathogenicity of staphylococci.

The main criterion for distinguishing in vitro pathogenic strains of staphylococci from nonpathogenic is coagulase (Vygodchikov, 1959; Munch-Petersen, 1961).

In addition to coagulase, phosphatase is an index of staphylococcal pathogenicity (Marinelli et al., 1958). Tests for phosphatase and coagulase coincided in 98.7% of the cases when checking 209 strains of Staphylococcus aureus (170 coagulase-positive and 39 coagulase-negative). However upon multiple subculturing the strains sometimes lose phosphatase activity (Pérez-Miravete, Gallardo, 1959).

It was also established that the pathogenicity of staphylococci in the overwhelming majority is correlated with the presence of arginase in it. For instance, out of 70 pathogenic strains, 69 contained arginase, and out of 25 strains considered nonpathogenic only 4 had

arginase (Soru et al., 1957). In addition, the ability only after 2-3 hr growth to form extracellular deoxyribonuclease is characteristic of pathogenic strains of staphylococci; strains of nonpathogenic species of staphylococci also form this enzyme but it can be detected only at considerably later periods, after one or two days (Redaelli, Rosaschino, 1958).

Another test with ammonium molybdenate was proposed for determining the degree of staphylococcal pathogenicity (Myers, 1959).

4.

Each new work confirms the thought first expressed about 100 years ago by Roux: "Death in infectious diseases occurs as a result of intoxication." Therefore it is just for this reason that it is so important to know why under certain conditions the products of the vital activity of microbes are toxic for an organism and how the toxins act. Dubo (1948) said, "Unfortunately, our ignorance in these problems is truly frightening." Possibly Dubo somewhat exaggerated, but there is unconditionally a portion of truth in his words. Our data, which in no way pretends to be complete, show how far we still are from solving these problems.

The main task now is to study the factors causing the pathogenicity of bacteria. At the same time we must stop attempting to identify all toxins with enzymes and devote more attention to the "nonspecific" pathogenetic role of the latter.

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