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ATTEMPTS TO OBTAIN BETTER RESULTS WITH THE BACTERIAL ANTIGEN ("VACCINE") THERAPY OF LOW GRADE CHRONIC ("FOCAL") INFECTION

I. POSSIBLE ERRORS OF USUAL METHODS

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DURING the past seventeen years we and our associates have been making a careful study of the problem of low grade chronic ("focal") infection in an effort to determine its significance. Such a study included investigation of the field of bacterial antigen ("vaccine") therapy and has resulted in the development of diagnostic and therapeutic bacteriologic methods which we believe are superior to others we have tried. This series of reports presents a discussion of the progress achieved so far.

Although most physicians believe that there is little merit in the "vaccine" therapy of low grade chronic infections, its continued use by some suggests that it must have value. Differences of opinion as to its value might be based on differences in the technique of preparing and administering the "vaccines." That there may be room for improvement in manufacture, even in standardized vaccines that have been used extensively for a long time, is indicated by the recent radical change in the type of organism used in the preparation of typhoid vaccine.[1] Our findings indicate that the failure to secure results in bacterial antigen therapy of chronic low grade infections may be caused by: selecting cultures that are incapable of inciting the production of suitable antibodies or of suitable quantities of them; improperly treating the cultures; failing to provide adequate surgical and supportive measures; and using unsatisfactory dosage with respect to both the interval and dose. More specifically, the following factors may have a profound effect on the course of such treatment.

POSSIBLE BACTERIOLOGIC ERRORS

a. *Culture of unsuitable foci.*—In the search for possible foci of infection a significant focus may be overlooked and cultures may be made of other foci. For example, in a patient with chronic prostatitis and rheumatism the prostate was considered the obvious focus, but only nonpathogenic organisms were found in the prostatic fluid. Highly pathogenic organisms were found in the nasal and

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oral cavities, which were the seat of long-standing but almost asymptomatic infection. Quite often special attention is paid to one particular focus, while others may be ignored.

If, as seems reasonable on the basis of available data,[2] "*Streptococcus viridans*" is the common denominator of low grade chronic infections, then knowledge of the incidence of pathogenic types of this organism should prove useful in indicating their most likely sources. Ruggier and Chapman[3] found resistant (i.e., probably pathogenic) streptococci to comprise 72 per cent of streptococci from periapical pockets, 54 per cent of those from extracted teeth, 44 per cent of those from the pharynx, 35 per cent of those from the nasopharynx, and 28 per cent of those found in the feces ("enterococci" being ignored). By use of an improved technique pathogenic types of streptococci were found in the pharynx in *every* person with low grade infection. For this reason the pharynx (and any periapical pockets or extracted teeth), should be cultured in every case. (The most common source of pathogenic staphylococci is the nasal cavity, which likewise should be cultured routinely.) Additional cultures should be taken from other sources whenever there is suspicion of active infection. For example, in one patient there were only 200,000 pathogenic type streptococci (18 per cent of the total streptococci in the swab) in a swab from the pharynx, but there were 5,000,000 (36 per cent of those in the gingival swab) in a swab from the gum margins, many of which showed evidence of inflammation. (See also Ruggier and Chapman.[3])

b. *Use of improper cultural methods.*—Laboratories that are infrequently called upon to make cultural studies of focal infection have difficulty in maintaining a supply of suitable culture media. Hence some of them use the Loeffler medium slants, supplied by departments of health, which are frequently dry and unsatisfactory for this type of work. Others use "blood agar" plates of varying composition, poured so thin, or which are so old, that they are dry and unsuitable for adequate growth of pathogenic bacteria, many of which require abundant moisture. Usually the material is spread on only one plate, resulting in a crowded growth that makes the study of individual colonies difficult.

Unless appropriate cultural methods are used, streptococci, particularly anaerobic types, pneumococci, some members of the Hemophilus group, and other fastidious organisms may not be recovered. Much tedious work can be eliminated by using the newer selective isolation methods. Penicillin, recently discussed by Chain et al.,[4] and Abraham et al.,[5] is useful in the selective isolation of the Hemophilus group. Bromthymol blue lactose agar and phenol red mannitol agar[6, 7] give superior results in the selective isolation of probable pathogenic staphylococci. Crystal violet, sodium azide, tryptose blood agar[8, 23] is of considerable assistance in isolating pathogenic streptococci from mixed cultures.

c. *Taxonomic errors and unsuitable taxonomic considerations.*—Often an organism diagnosed as "hemolytic streptococcus" may be a hemolytic member of the Hemophilus group, a hemolytic staphylococcus, or even an α- or γ-hemolytic streptococcus. The presence of a small hemolytic colony on the *surface* of "blood agar" does not warrant a diagnosis of "hemolytic streptococcus." Similarly, a mucoid colony of staphylococci or of streptococci is sometimes mistaken for *Klebsiella pneumoniae*. Organisms received as "staphylococci" were found

to be "enterococci" and *Neisseria catarrhalis,* and a "Type IV pneumococcus" proved to be *Streptococcus salivarius.*

Sometimes an organism is designated by an insignificant property. For example, numerous publications refer to "hemolytic" staphylococci but it has been shown[9] that the power of staphylococci to produce hemolytic zones on blood agar has only secondary significance.

d. *Error of random selection of colonies.*—When colonies are selected at random from a mixed culture they may not be representative of apparently similar colonies elsewhere on the plate. Only rarely in this type of work do we deal with cultures in which all the cells have similar properties. Therefore, in selecting a colony at random, even from an apparently pure culture, it is possible to select one that does not represent the entire culture.

e. *Influence of the age of the culture.*—Most bacteriologists harvest a culture after "overnight" incubation, an interval which may include extremes of sixteen and thirty hours. Such differences in age are often accompanied by differences in properties of the cultures.[10, 11] (We have found that late logarithmic cultures make more efficient antigens.) Metabolites, particularly hydrogen ions, may have an unfavorable effect on bacteria and their antigens. Polysaccharides, which may be related to the specificity of a bacterial antigen, are not produced uniformly throughout the period of growth of the culture. Autolytic products, the result of prolonged incubation, may alter the antigenic complex. For these reasons, it is important to know the influence of age on the antigenic structure of different cultures.

f. *Fallacy of considering the predominant organism as the focal invader.*—Many bacteriologists in medical fields consider only the gross features of a culture and make no more than a casual inspection of the plates. The relative number of the different types of organisms is estimated on the assumption that the organisms present in largest numbers are most likely to be the focal invaders. While this is often true, the rich growth of saprophytes in many parts of the body precludes the possibility of recognizing focal bacteria on the basis of predominance.

g. *Inadequacy of methods used to differentiate etiologic bacteria from contaminants.*—Because cultures from suspected foci often contain bacteria which have no relationship to the infection, it is important that attempts should be made to differentiate these contaminants from the organisms causing the infection.

Intradermal tests, complement fixation reactions, agglutination reactions, and qualitative bacteriostatic tests have been shown to be inadequate for such differentiation.[12, 13] There is no evidence that organisms showing specific localization produce better antigens than do those not localizing in particular tissues.

Our researches[13] indicate that differentiation between causative organisms and contaminants can be made by methods based on the probability that bacteria causing infection are more likely than are contaminants to possess properties associated with pathogenicity. (It is possible that strains of low pathogenicity may be responsible for infections in tissues having low resistance; but such instances are uncommon and only a mild infection usually results.) Because of

the difficulty of applying animal inoculation tests, studies were made of in vitro properties. It was found that certain of these properties were parallel with pathogenicity for animals,[9, 14, 15] were parallel with other findings[2, 9, 16, 17, 23] in the patients from whom they had been isolated, and, because they were easily reproducible, were suitable for studying large numbers of cultures.

h. *Selection of a nonpathogenic variant.*—Even though accurate methods are used to differentiate pathogenic from nonpathogenic strains, the culture selected may still be nonpathogenic because pathogenic cultures often contain nonpathogenic variants. If different colonies are tested separately, an apparently pure culture will be found in many cases to consist of a mixture of dissociants (or variants) in which different properties may be distributed in different patterns among the different cells of the culture.[3] Because this phenomenon occurs so frequently in α- and γ-hemolytic streptococci, cultures of these organisms should be considered, not as stable homogeneous masses, but as dynamic mixtures of cells with a strong tendency to instability of the biochemical, physiologic, immunologic, and pathogenic properties of the different cells of the cultures. To be fully representative, a culture must not only be genetically related to the etiologic (pathogenic) culture but must also possess its pathogenic properties.

Not much attention appears to have been paid to the possibility that the antigenic structure of a culture may change along with the dissociation of other characters. If the colony or colonies selected for preparing the growth for the antigen should lack any of the antigenic properties of the parent culture, then the antibodies produced from injection of this antigen may be qualitatively and quantitatively different from those necessary to neutralize the antigenic complex produced by the invading microorganisms.

LACK OF SPECIFICITY OF THE ANTIGEN

Even though considerable care is exercised in selecting the culture, there is still a possibility that the antigen prepared from it may not be efficient for the following reasons:

a. *There may be loss or degeneration of the antigenic properties during cultivation of the microorganism.*—Any degeneration of the culture during growth is likely to be associated with degeneration of the antigenic complex. Therefore, steps should be taken to minimize degeneration by preventing excessive variations in hydrogen-ion concentration and excessive oxygenation, and by harvesting the culture as quickly as possible.

b. *Physical or chemical changes may be produced in the antigen after the culture has been harvested.*—Heat so injures many bacterial antigens that it is necessary to inject several million bacteria to produce a physiologic effect. In our experience 0.5 per cent phenol does not impair the immunogenic properties of staphylococci, streptococci, and Klebsiella, and does not appear to be injurious to the patients in the dosages used. Only rarely do we find sensitivity to phenol. With properly prepared antigens a physiologic effect may be produced with amounts representing less than a single bacterial cell. The antigens should be stored in resistant glass bottles to minimize the accumulation of alkali.

The essential property of a bacterial antigen is that it should be an efficient antibody-stimulating preparation. If the concentration of specific antigen

should be too small (as in heated or nonspecific vaccines), the dose required to provoke a high titer of antibodies will contain so much protein that the protein itself may produce local or even general reactions.

UNSUITABLE METHODS OF VACCINE ADMINISTRATION

a. *Fallacy of attempts to force the patient to produce a high titer of antibodies.*—The usual aim in vaccine therapy is to force the patient to produce large amounts of antibodies. For this reason most schedules call for the administration of progressively larger doses to the limit of tolerance, even though the immediate results should be unfavorable. Not only is an unfavorable effect ignored, but quite often it is considered a favorable sign, an indication that the vaccine is having an effect on the patient.

On the contrary, such an unfavorable effect, if more than transitory, usually indicates that the patient is unable to produce a sufficient quantity of antibodies to neutralize the antigen. Consequently the excess antigen acts as a toxin. Recovery might be possible if sufficient opportunity were given for the tissues to produce more antibodies, but often the patient is made worse by another injection before he has had a chance to recover. When an injection is given too soon after the previous one it augments the residual antigen, resulting in even greater toxicity.

b. *Use of empirical methods for determining dosage.*—Because the laws governing response to the introduction of an antigen into the body are poorly understood and because of the difficulty in determining the precise effects, it is customary in administering antigens to follow a fixed schedule such as is suggested by manufacturers of biologic products. While such a procedure works satisfactorily in the prophylactic immunization of healthy persons (e.g., against typhoid), it is not reasonable to expect that it would work equally well in immunizing body tissues against an organism with which they are already infected. The varying amounts of antigen produced by the pathogenic organisms present, the varying damage done to tissues by these organisms, as well as the varying effects of nutritional and endocrine deficiencies, combine to alter the ability of the body to respond to the introduction of additional antigen. In our experience best results have been obtained by adjusting the dosage (i.e., both the dose and the interval) according to the response to each injection of antigen. This subject, including our methods of estimating response to antigen injections, will be discussed in a later paper.

c. *Improper spacing of injections.*—The response to an injection of antigen is a function of the properties of the antigen, which are constant, and of the tissue reactability and antibody concentration, which vary with the interval. Therefore, it is important to space the injections to take advantage of these variations. If an antigen injection is given too soon after a previous injection, enough unneutralized antigen may still remain in the tissues to make the amount being injected too large. If given too late, the antibody level may have dropped to such a low level that several injections may be required to overcome this slump and obtain optimum response.

d. *Errors in the size of the dose of antigen.*—An inadequate dose of antigen will have only weak power of stimulating the production of antibodies. The

closer a dose is to the optimum, the greater will be the degree of stimulation. However, maximum stimulation comes only from a quantity of antigen which is close to an overdose. Consequently, considerable care must be exercised when a particular dose produces marked stimulation, because a slightly higher dose, or the same dose repeated too soon, may cause unfavorable symptoms.

At times, following the administration of a dose which is considerably too large, the signs of overdosage may not be dramatic and may be mistaken for failure to respond to the antigen injection. Even if such a dose is repeated several times, the evidence of overdosage may be difficult to detect. We have seen a number of patients, usually of low vitality, who showed no evident change, either beneficial or otherwise, during a series of antigen injections, but who improved after an interval of several weeks without injections. Later these patients responded satisfactorily to smaller doses of antigen.

During an acute illness, or shortly after surgical removal or treatment of foci of infection, there is often so much circulating toxin that even minute amounts of antigen act as overdoses. Consequently, great care must be taken in giving injections in such cases. In general, it is advisable to postpone antigen therapy until a week after any acute illness has subsided, or until the immediate unfavorable effects of a surgical procedure have disappeared and improvement is evident.

In the early stages of treatment favorable response to the injection of antigen follows a curve: increasing improvement, followed by gradual return to the basic level. An injection of antigen will produce better results if it is given at the end of the period of improvement rather than after complete return to the basic level. When the immunity has been raised to a satisfactory level, the antigen may be given in rapidly increasing doses at frequent intervals.

INADEQUATE SUPPORTIVE MEASURES

a. *Failure to reduce the toxic load.*—Response to the injection of an antigen depends not only on considerations of the antigen but also on tissue reactability, which in turn is affected by the toxic load on the system. An undrained (i.e., confined) focus of infection is a source of toxic material. When the focus is drained or removed much of this is eliminated, lightening the toxic load on the body. This is one of the most important phases of bacterial antigen therapy. In many instances in which the patient is sensitive to extremely small doses of antigen it will be found that there is an untreated focus. Numerous patients who reacted badly to small doses of antigen had a beneficial response to much larger doses of the same antigen after foci of infection had been drained or removed.

The toxic load may also be reduced by appropriate chemotherapy and physiotherapy.

b. *Failure to correct endocrine dysfunction.*—A lowered basal metabolic rate has been reported as a common finding in chronic arthritis[18] and in chronic low grade infections.[19] In patients who become drowsy after a dose of antigen that is higher than the optimum amount, the drowsiness may often be reduced by giving adequate amounts of thyroid preparations simultaneously with the

vaccine injection. In patients with more definite evidence of thyroid hypofunction the continued use of thyroid preparations may prove markedly beneficial. Often, in these cases, thyroid function improves as the general health improves, and thyroid therapy may eventually be discontinued.[20]

Sex gland and adrenal deficiencies may also require attention.

c. *Failure to correct liver dysfunction.*—Liver dysfunction is common in arthritis[21] and other forms of low grade chronic illness.[22] Dietary adjustment (low fat and cholesterol, high carbohydrate and high protein) and the administration of bile salts are useful in correcting liver disturbances and in improving the response to vaccine therapy.

d. *Failure to supply adequate nutrition.*—It is generally recognized that resistance to infection is dependent to a certain extent upon nutritional factors. Dramatic response is sometimes noted when an adequate diet is supplied, or even when a single specific dietary factor is administered. The beneficial effects of the appropriate use of vitamin B complex have been especially noteworthy.

With careful attention to the details enumerated and by application of the principles to be discussed in the following papers, we believe it possible to obtain better results in the bacteriologic investigation and treatment of low grade chronic infections than are possible by other methods that have been proposed for this purpose.

SUMMARY

An analysis of some of the factors that might prevent optimum response to the bacterial antigen ("vaccine") therapy of low grade chronic ("focal") infections reveals the following possibilities:

The cultures may be taken from unsuitable foci. The recovery of organisms of suitable antigenicity may not always coincide with clinical expectations.

The cultural procedure may be inadequate from the standpoint of the technique of isolation, the method of selecting the colonies, the incubation period, taxonomic considerations, and the methods used to differentiate the etiologic bacteria from contaminants.

The culture selected may not possess suitable antigenic properties. The antigen may be injured during the cultivation process or in the later manipulation. The injured antigen may contain an excessive ratio of protein to specific antigen, causing it to be toxic and nonspecific.

The dosage may be unsuitable. The injections may be given too often or the amounts may be too large. In other instances the doses may be too small for adequate stimulation of antibodies. The use of a fixed schedule is condemned.

The condition of the patient may not be favorable for optimum antibody response because of endocrine or nutritional deficiencies, liver dysfunction, an excessive toxic load (e.g., an untreated focus), or other factors.

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