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Staff Meeting Report

Desoxyribonuclease and the Versatility of Group A Streptococci*†

Lewis W. Wannamaker, M.D.‡

Clinical investigators and epidemiologists have long been impressed by the versatility of Group A streptococci. This ingenious group of organisms has been implicated in a wide variety of clinical pictures; a partial list of these, given in Table 1, includes such diverse clinical diagnoses as scarlet fever, tonsillitis, meningitis, septicemia of the newborn, osteomyelitis of infancy, puerperal fever, impetigo, erysipelas, endocarditis, acute rheumatic fever, and acute glomerulonephritis.

TABLE 1

A PARTIAL LIST OF CLINICAL PICTURES ASSOCIATED WITH GROUP A STREPTOCOCCI

Scarlet fever	Septicemia of the newborn
Tonsillitis and pharyngitis	Osteomyelitis of infancy
Otitis media	Streptococcosis of infancy
Sinusitis	Puerperal fever
Mastoiditis	Impetigo
Meningitis	Erysipelas
Endocarditis	
Pneumonia	Acute rheumatic fever
Empyema	Acute glomerulonephritis

Approaching the problem from a somewhat different point of view, bacteriologists, also, have been struck by the complexity and versatility of Group A streptococci. As is shown in Table 2, Group A streptococci are made up of a number of recognized cellular components and give rise to a variety of extracellular products.[1] This impressively long list includes many substances that are both antigenic and biologically active. The C polysaccharide is the group

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on October 18, 1957.

†The investigations reported in this paper were completed while the author was on leave of absence at the Rockefeller Institute for Medical Research, New York City.

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specific substance of the streptococcus and is a major component of the cell wall. The division of Group A streptococci into some forty-odd types is based on a similar number of serologically distinguishable variations in their M protein. The type specific immunity to streptococcal infections that has been demonstrated in both animals and man appears to be related to the development of antibody to M protein.

TABLE 2

COMPONENTS OF GROUP A STREPTOCOCCI

Cellular	*Extracellular*
C carbohydrate	Erythrogenic toxin
M protein	Streptolysin O
T protein	Streptolysin S
R protein	Proteinase and precursor
	Hyaluronidase
Beta glucuronidase	Streptokinase
Lipoproteinase	Desoxyribonuclease
	Ribonuclease
Nucleoprotein fraction	Amylase
Ribonucleic acid	Diphosphopyridine nucleotidase
Desoxyribonucleic acid	
Hyaluronic acid	

Among the extracellular products, streptolysin S is unique in that it is apparently non-antigenic. On the other hand, many of the other streptococcal products are decidedly antigenic. Thus, good precipitating antisera have been produced for proteinase and its precursor; and neutralizing antibodies for streptolysin O, streptokinase, hyaluronidase, and desoxyribonuclease have been demonstrated in patients' sera following natural infection. In studies of the immune response as it occurs following natural infection, two interesting paradoxes have been observed, one in the case of hyaluronidase and another with desoxyribonuclease. Only strains of types 4 and 22 produce appreciable amounts of hyaluronidase. Nevertheless, in most patients with streptococcal infection, regardless of the infecting type, antibodies to this enzyme are developed. Dr. Dennis Watson has been interested in this problem and has suggested that *in vitro* conditions may be less favorable than *in vivo* conditions for production of the enzyme.[2] With desoxyribonuclease, a paradox of a reverse sort has been reported.[3] Virtually all strains of Group A streptococci produce this enzyme in high titers and under a wide variety of con-

ditions, yet antibody responses to desoxyribonuclease have been notably irregular. Neutralizing antibody for this enzyme has been demonstrated in less than half of the patients observed following streptococcal infections. The reason for this has not been clear, although it seemed possible that under conditions of natural infection this enzyme might be less antigenic than some of the other extracellular products.

In spite of this imposing accumulation of knowledge concerning the biology of the Group A streptococci, there is very little available information on the possible role that any of these intracellular or extracellular products might play in the development of the numerous clinical manifestations of streptococcal disease. Some of the products, such as desoxyribonuclease, do not appear to attack living cells. Streptolysin O, on the other hand, is thought to have a cytotoxic effect, but the significance of this in terms of the pathogenesis of human disease is not clear. The development of acute nephritis has been related to infection with strains of certain types,[4] but the biological property of these strains which is responsible for the nephritogenic potentialities has not been identified.

The most widely accepted association of a streptococcal product with a clinical manifestation is that of erythrogenic toxin and the rash of scarlet fever – and even this is uncertain. Although most strains and types of Group A streptococci can be shown to produce erythrogenic toxin, infected individuals who lack antitoxin may fail to show a skin rash. Two immunologically distinct erythrogenic toxins have been described, but even these do not appear to account for many of the discrepancies in clinical behavior. One difficulty in further studies of erythrogenic toxin is that its activity can at present only be defined and measured in the rather unsatisfactory terms of its effect on the skin of a living organism. Precise methods of determining biological activity are available, however, for many of the other extracellular products, particularly those whose activity is enzymatic. But further investigation of the relationship of individual streptococcal products to pathologic processes is limited by the fact that these products have been available only as crude mixtures containing a number of known streptococcal products, considerable streptococcal material of undefined nature, and a generous contribution of *gemisch* from the supporting media. Only in the cases of the C carbohydrate and of the streptococcal proteinase and its precursor have streptococcal products been obtained in anything approaching purity.

The studies reported in this paper describe another instance of the versatility of Group A streptococci. The original purpose of these studies was to explore strain variation as revealed in the extracellular products of Group A streptococci. This was done by preparing concentrates of the extracellular products from various strains and comparing their patterns by starch zone electrophoresis. The distribution of protein peaks was noted and the location of known enzymes was identified. It was thought that this method might also result in separation of some of the recognized streptococcal products for further study,

The initial problem was to devise a suitable medium. In this regard certain environmental conditions are known to favor the elaboration of particular extracellular products. For example, Bernheimer[5] has shown that the presence of a ribonucleic acid fraction and of maltose greatly favors streptolysin S production, and Slade[6] has reported that the addition of reducing agents to certain media may increase the production of streptolysin O. Increased yields of streptococcal hyaluronidase, whose production is known to be adaptive, can be obtained by incorporating substrate in the medium.[7] That pH may also be important has been demonstrated by Elliott,[8] who showed that the precursor of streptococcal proteinase is either not elaborated or not released by the cells until the pH drops to 6.5 and below. In the present problem, however, the goal was not to favor the production of any single extracellular component, but rather to assure satisfactory elaboration of most or at least several of them. It was also important that the media used should contribute no non-streptococcal constituents to the final concentrate. The advantages of a well-defined or at least protein-free medium for electrophoretic studies of this sort are obvious. Unfortunately, Group A streptococci are a fastidious lot. Although several defined or semi-defined media have been described, these often support cell multiplication and enzyme production to a limited extent and are suitable for the growth of only a few rather undiscriminating strains. After considerable experimentation, a fairly satisfactory medium was devised, which is a modification of the Dole dialysate medium.[9] It consists of a combination of dialysates of Pfanstiehl peptone, casamino acids, and meat extract, plus dextrose, salts, and sodium bicarbonate. This medium supported excellent growth of a number of strains of Group A streptococci and good production of at least several of the known extracellular products. Moreover, when uninoculated portions of this medium were brought to 0.8 saturation with ammonium sulfate, no precipitation occurred, while media prepared with other peptones

gave a gummy precipitate when brought to this salt concentration.

To prepare the concentrates, streptococci were grown in this dialysate medium for 18 hours, and cells were removed by centrifugation. Chloroform was added to the supernatant fluid, which was then brought to 0.8 saturation with ammonium sulfate, and the resulting precipitate was removed by filtration through potato starch. The precipitate was redissolved in buffer, and further concentration was accomplished in a collodion bag under negative pressure. The resulting concentrate was dialyzed free of ammonium salt and either placed directly on a starch block or frozen and dried for storage.

As these studies progressed, it soon became obvious that relatively large amounts of culture supernate would be required; for, although enzymatic activities were high, the amount of total material obtained in terms of weight was only about ten mg. per liter of original culture supernate. Fig. 1 shows an electrophoretic pattern

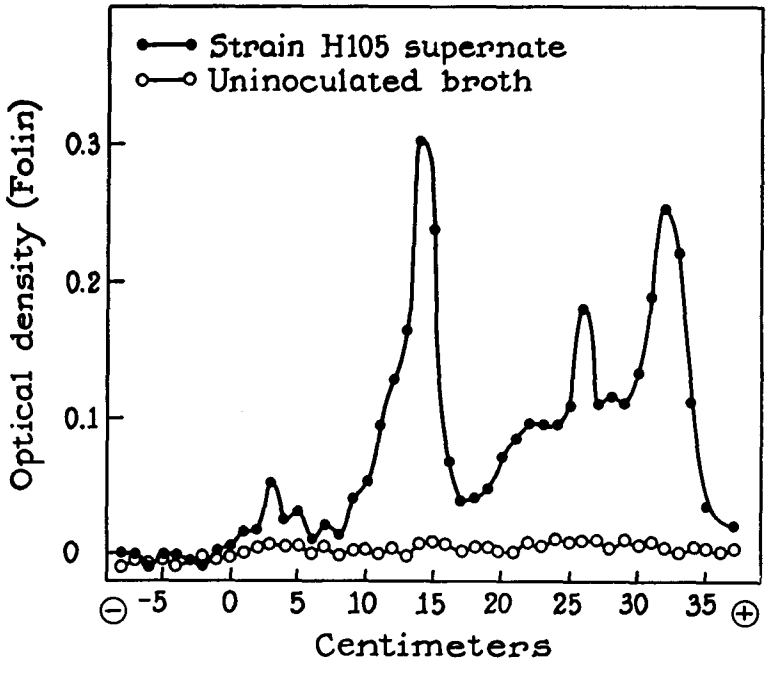

Fig. 1. Comparison of the electrophoretic pattern of a concentrate of extracellular products (culture supernate) with the pattern of a concentrate of uninoculated broth.

made with a concentrate of culture supernate compared with the pattern obtained with a concentrate of a broth blank. In this figure, as in those that follow, the origin is at zero. The anode is on the right, the cathode on the left. Relative concentrations of protein were determined by the modified Folin-Ciocalteu reaction. The curve obtained with the blank preparation is essentially flat and does not rise above the base line, indicating that the culture medium did not contribute appreciably to the electrophoretic patterns obtained. In contrast to this low flat curve, the pattern obtained with the concentrate of culure supernate shows considerable protein with distribution into several peaks.

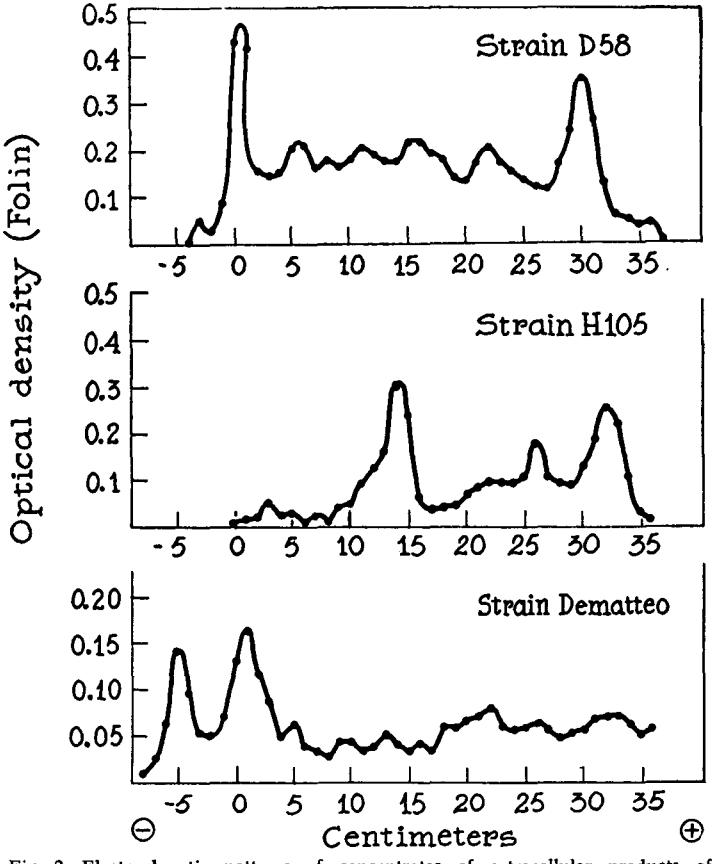

Fig. 2. Electrophoretic patterns of concentrates of extracellular products of three different strains of Group A streptococci.

Fig. 2 (p. 43) shows the differences in protein patterns encountered when several strains were compared under identical electrophoretic conditions. The prominent peak in the first strain, which is also apparent in the third, seems to be completely absent from the second strain. In the center pattern there is a peak that is certainly less obvious in the other two. The third strain, a nephritogenic one, shows an outstanding peak that is completely absent in the first two. The location of certain known extracellular products can be shown to correspond with certain protein peaks, but in many instances imposing peaks have been observed that do not correspond with any of the known extracellular products. The striking differences in the electrophoretic patterns of these strains suggests that this may be an important aspect of strain variation among Group A streptococci. To clarify this point further additional studies of strains with known biological characteristics and epidemiologic associations are needed.

In addition to studies of the electrophoretic distribution of total extracellular protein, the mobility patterns of some of the known enzymes have been identified, and several have been partially separated. In an attempt to find the optimal conditions of separation, several different buffer systems and hydrogen ion concentrations have been employed, and the time of electrophoresis has been varied. Fig. 3 illustrates the kind of patterns obtained. In this experiment the locations of four enzymes (streptolysin O, streptokinase, desoxyribonuclease, and ribonuclease) were identified and some were found to correspond with certain deviations or peaks in the total protein pattern, as indicated in the uppermost curve. But a large bulk of extracellular protein and many peaks are unaccounted for, suggesting that a number of extracellular products, probably additional unknown enzymes, have not been identified. The separation of streptokinase and streptolysin O, which was only partial in this experiment, became more complete after longer periods of electrophoresis. In contrast, desoxyribonuclease and ribonuclease show an identical distribution here, and none of the conditions of electrophoresis studied resulted in a separation of these two enzymatic activities. The interpretation of this finding is difficult. Either the activities may represent two distinct enzymes with similar electrophoretic mobilities, or a single protein may be capable of depolymerizing both substrates.

The distribution of desoxyribonuclease activity in Fig. 3 shows a small secondary peak which has migrated out beyond the area of streptokinase activity and is clearly separated from the major peak of desoxyribonuclease activity. Studies of other strains indicated that

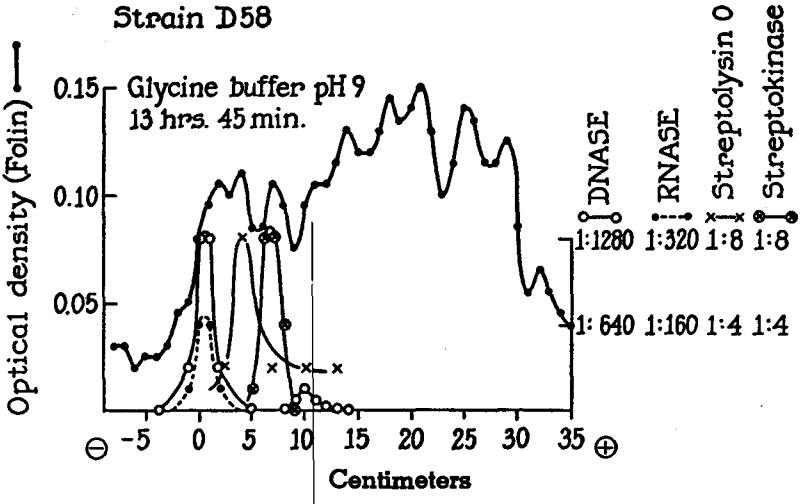

Fig. 3. Identification of the location of specific enzymes in the electrophoretic pattern.

the desoxyribonuclease activity separates into three distinct components recurring at approximately ten-centimeter intervals (Fig. 4.) There is considerable strain variation as to which of the three desoxyribonuclease components — identified as A, B, and C — predominates in the electrophoretic pattern.

The possibility that the three desoxyribonuclease components represented three distinguishable enzymes was strengthened by exploratory studies of the sera of patients with acute rheumatic fever. The ability of these sera to neutralize desoxyribonuclease activity was found often to be quite different, depending on which of the three components was used in the test system (Table 3).

Neutralization of desoxyribonuclease activity by human sera was measured by the alcohol precipitation test,[3] which depends on the fact that unchanged sodium desoxyribonucleate forms a floating fibrous mass upon precipitation with alcohol, whereas after nuclease action only a fine flocculent precipitate is formed. In performing the test, a constant amount of enzyme is added to various dilutions of antibody. This enzyme-antibody mixture is incubated at 37° C. for 30 minutes; then a constant amount of substrate is added to each tube, and incubation is continued for an additional 30 minutes. After the second incubation, absolute alcohol is added. The end-point is de-

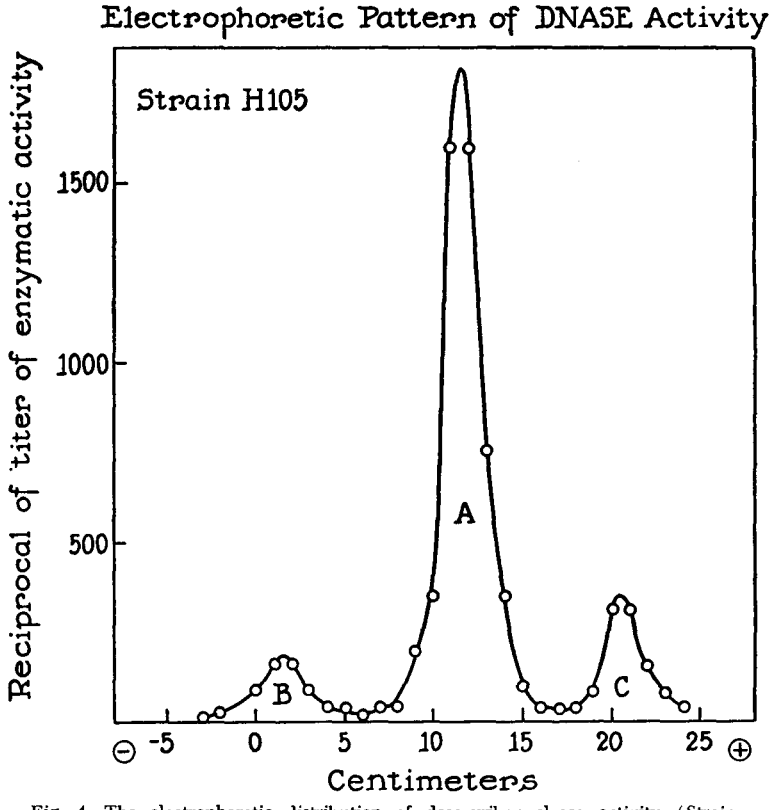

Fig. 4. The electrophoretic distribution of desoxyribonuclease activity (Strain H105).

fined as the highest serum dilution that prevents enzymatic degradation of the desoxyribonucleate (DNA) so that a definite fibrous precipitate forms upon the addition of alcohol. As recorded in Table 3, the pluses indicate the degree of enzymatic activity, and the zeros indicate no enzyme activity or complete inhibition by antibody. It can be seen that there was considerable variation in the ability of this patient's serum to neutralize the enzymatic activity of the three electrophoretic components. This suggested that the three components might be immunologically dissimilar.

The problem was investigated further by preparing antisera in animals. Rabbits were immunized with electrophoretically separated fractions. Each component (A, B, or C) was mixed with mineral oil

TABLE 3

INHIBITION OF THE THREE DESOXYRIBONUCLEASE COMPONENTS BY SERUM FROM PATIENT, RP66

Enzyme component	*Dilutions of Serum* 1:10	1:20	1:40	1:80	1:160	1:300	1:640	1:1280	1:2560	1:10240
A	0	++++	++++	++++	++++	++++	++++	++++	++++	++++
B	0	0	0	0	0	0	0	0	++	++++
C	+	0	0	0	0	++	++++	++++	++++	++++

0 = Intact fibrous precipitate (complete inhibition of enzymatic activity).
++++ = No fibrous precipitate (no inhibition of enzymatic activity).

TABLE 4

SPECIFIC INHIBITION OF THE THREE ENZYME COMPONENTS BY HOMOLOGOUS RABBIT ANTISERA

Antiserum	*Enzyme*	*Dilutions of Antiserum* 1:10	1:20	1:40	1:80	1:160	1:320	1:640
Anti-A	A	+	0	0	0	0	+++	++++
	B	++++	++++	++++	++++	++++	++++	++++
	C	++++	++++	++++	++++	++++	++++	++++
Anti-B	A	++++	++++	++++	++++	++++	++++	++++
	B	0	0	0	0	0	0	++++
	C	++++	++++	++++	++++	++++	++++	++++
Anti-C	A	++++	++++	++++	++++	++++	++++	++++
	B	++++	++++	++++	++++	++++	++++	++++
	C	++++	0	0	0	++++	++++	++++

and aquaphor to form an adjuvant emulsion. The results of neutralization tests with these rabbit antisera, illustrated in Table 4, show that each antiserum neutralized its homologous enzyme but failed to neutralize the heterologous enzyme components. The enzyme activity seen in the low dilutions of homologous antiserum does not represent streptococcal desoxyribonuclease which has not been neutralized, but is the result of the serum nuclease often present in rabbit sera.

These observations indicate that the three components represent three different desoxyribonucleases, all attacking the same substrate but immunologically distinct. The three desoxyribonucleases have been designated DNASE A, DNASE B, and DNASE C. The serological differences were also demonstrated by the more sensitive viscosimetric test (Fig. 5), in which enzyme activity is measured by its effect on the relative viscosity of the substrate. Fig. 5 reveals that enzymatic activity is inhibited by the homologous antibody but not by normal rabbit serum or by antibody to the other two enzymes.

The three enzymes also show certain differences in optimal pH of activity and in inhibition by citrate. DNASE A is most active at about pH 8.5; DNASE C is most active at pH 6; and DNASE B shows a broad range of optimal activity in the neutral range. Al-

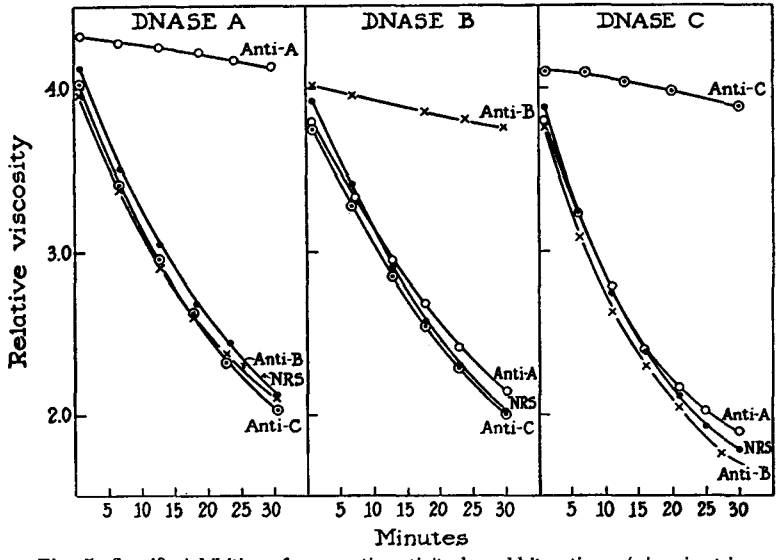

Fig. 5. Specific inhibition of enzymatic activity by rabbit antisera (viscosimetric test). NRS = normal rabbit serum.

though all three enzymes appear to require magnesium for activation, DNASE B is more completely inhibited by citrate than are the other two.

No information is yet available concerning possible differences in end-products obtained after digestion of desoxyribonucleate with the individual streptococcal desoxyribonucleases. It is conceivable and perhaps likely that in depolymerizing DNA, the three enzymes attack the substrate in somewhat different ways, but this possibility needs further study.

The existence of three immunologically distinct desoxyribonucleases seems to explain the paradox, mentioned earlier, in the antibody response to this enzyme. Previous antibody studies have been done with strains producing primarily DNASE A. Since this is an uncommon antigen, only a few patients show an antigenic response following infection. But antibody for the more common desoxyribonuclease, DNASE B, can be found in high titers in most acute rheumatic fever patients and a rise can be demonstrated in most patients following streptococcal infection.

Fig. 6 shows the distribution of antibody titers for two of the enzymes among a group of patients with acute rheumatic fever and a small group of normal individuals. Among patients with acute rheumatic fever the distribution of titers for enzyme B was quite different from that for enzyme A; in general, titers for enzyme B were much higher, occuring in a dilution of 1:320 or greater, in 34 of 38 patients with acute rheumatic fever. However, none of the twelve normals in the control group showed antibody to DNASE A, whereas all of these normals showed antibody to DNASE B in low titers. (The distribution of antibody to DNASE C, not shown in Fig. 6, is similar to that for DNASE A.)

As yet, too few strains have been examined to establish the prevalence of the three enzymes among various strains or types of Group A streptococci, but two findings – the presence of antibody in low titers among most normal adults, together with the high titers observed in most rheumatic patients – suggests that DNASE B is the most common enzyme.

Fig. 7 illustrates the frequency of antigenic response to DNASE B as observed in a group of patients with acute streptococcal infections. Each point represents a single patient. The abscissa charts the titer of the serum taken during the acute illness, the ordinate that of the serum taken three weeks later during convalescence. It is clear that all

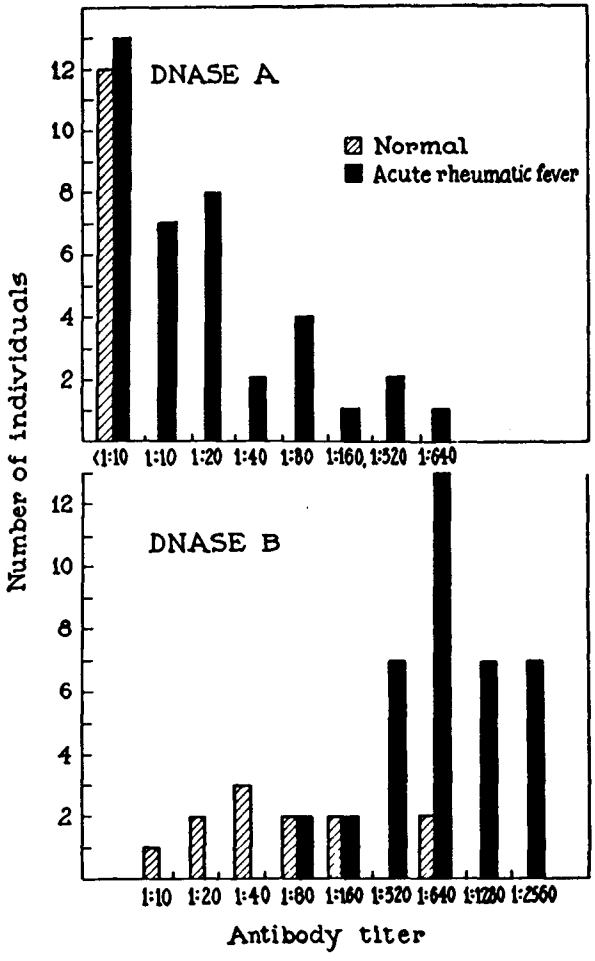

Fig. 6. Distribution of antibody titers among normal individuals and among patients with acute rheumatic fever.

sera showed some increase in titer, although those near the diagonal line showed only a one-tube increase. In about 80 to 85 per cent of patients with untreated streptococcal infections there is a reproducible antibody response to DNASE B; this is comparable to the percentage of patients who show an antibody response to streptolysin O.

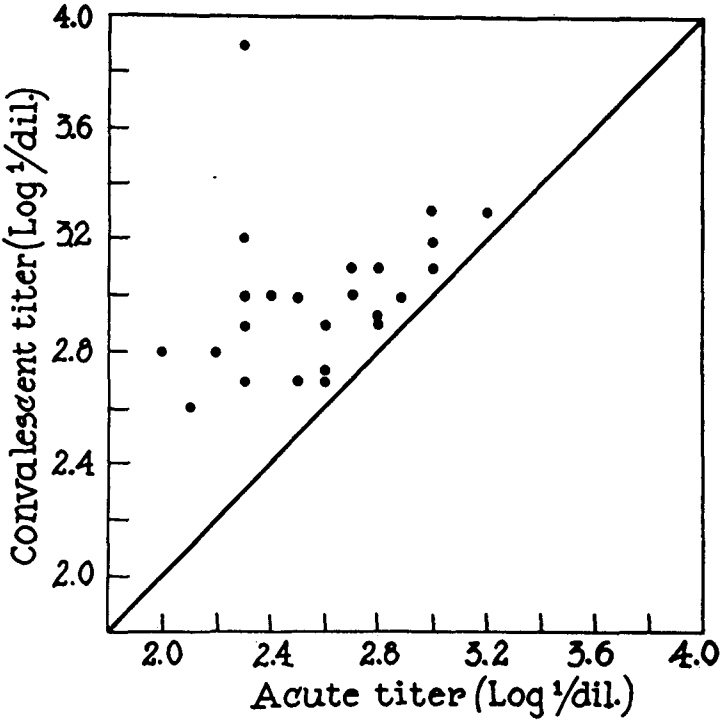

Fig. 7. Antibody response to desoxyribonuclease B following acute streptococcal infection.

The magnitude of the response to streptolysin O and to DNASE B often differs considerably, however, even when measured in the same sera from the same patient, as illustrated in Fig. 8. While the preceding scatter graph compares the acutely ill and the convalescent patients' titers of antibody to a single enzyme, this graph compares the antibody rise to streptolysin O (shown on the abscissa) with the antibody rise to DNASE B (shown on the ordinate). Identical dilution increments were used for the two tests. It is obvious that a patient may show a high antigenic response to one enzyme and a low response to another, a phenomenon that has been observed in comparing antigenic responses to other streptococcal enzymes.

In the past, elevated titers of antistreptolysin O, antistreptokinase, and antihyaluronidase have been useful indices of the probability of recent streptococcal infection, particularly among patients suspected

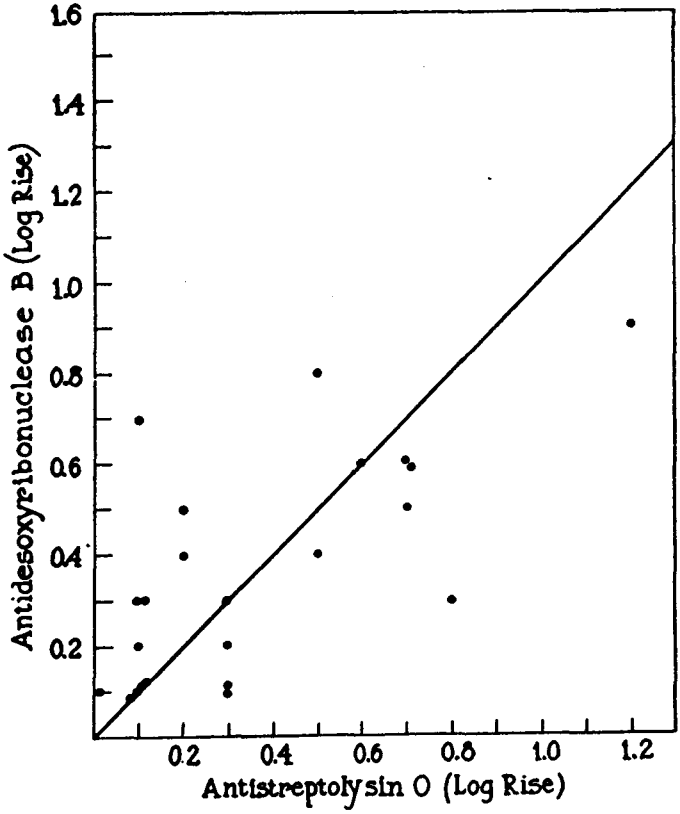

Fig. 8. Comparison of the antibody responses to streptolysin O and to desoxyribonuclease B following acute streptococcal infection.

of having either acute rheumatic fever or acute nephritis. Titers of antidesoxyribonuclease have been less frequently elevated and therefore less useful, probably because measurements were being made for antibody to the less common antigen, DNASE A.

In contrast, the regular antibody response to DNASE B following streptococcal infection and the high titers in patients with acute rheumatic fever suggest that the determination of this antibody might be a logical addition to those tests currently in use. It is not likely that this test for antidesoxyribonuclease B will displace that for antistreptolysin O. However, this new antibody test does have certain advantages over the other commonly used secondary tests: it is easier

to perform and standardize, and it shows a response in a higher percentage of patients.

In summary, this report has presented additional evidence of the versatility of Group A streptococci. Electrophoretic studies of the extracellular products have revealed striking differences in the protein patterns obtained with various strains. Furthermore, it appears that these organisms can elaborate not one but three enzymes which depolymerize desoxyribonucleate. The three enzymes migrate differently on starch zone electrophoresis, are immunologically distinct, and show certain differences in optimal pH of activity and in inhibition by citrate. The three desoxyribonucleases have been designated DNASE A, DNASE B, and DNASE C. Since the prevalence of these individual enzymes is a function of strain variation, these observations provide a logical explanation for the paradox previously observed in the immunological response to desoxyribonuclease. DNASE B is the most common enzyme or antigen, and antibody to this enzyme can be demonstrated in the sera of most patients with acute rheumatic fever or following streptococcal infection. The test for anti-DNASE B would appear to be useful when the diagnosis of streptococcal infection, acute rheumatic fever, or acute nephritis is under consideration.*

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Staff Meeting Report

Cutaneous Biopsy as An Aid to Diagnosis*

Robert W. Goltz, M.D.†

In preparation for this staff meeting, it seemed wise not to limit the discussion to any specific clinical or research aspect of the field of dermatology, but rather to review and evaluate broadly the cutaneous biopsy as an aid to diagnosis, pointing out some of its advantages and limitations, and also to speculate about the probable new developments in this field.

It should be clear at the outset that the skin enjoys a unique position among the organs because of its accessibility for biopsy, as well as for clinical inspection and topical therapy. The dermatologist can, so to speak, do his autopsy before making his diagnosis and beginning treatment. As a result, the histopathology of skin diseases has been investigated and described in minutest detail, and sizable textbooks, even many-volume sets, have been written about it.‡

To review briefly, it will be remembered that the skin is a compound organ arising from two embryonic layers. The ectodermal structures comprise a number of different glandular elements as well as the epidermis, while the mesoderm consists chiefly of connective tissue, and also of muscles, fat, and reticuloendothelial elements. It is not suprising that this complex organ is subject to a wide variety of diseases.

What is perhaps not so generally appreciated is the degree of variation in the skin from one body area to another. Thus the epidermis, which has the same basic structure whatever its area of origin, shows great modification of its components, in accord with its varying functions, on the several parts of the body. Compare, for example, the thick, horny epidermis of the sole with the thin, flexible layer cov-

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on October 25, 1957.

†Clinical Instructor, Division of Dermatology.

‡It may be mentioned in passing that just as the sophomore medical student is introduced to general medicine through his course in pathology, so also may the teaching of dermatology to graduate students be approached in pathologic terms. There is, in fact, much to be gained from introducing the study of the various dermatoses with the extra dimensions added by knowledge of the histologic changes at various stages of these diseases. It may also, incidentally, comfort the neophyte to learn that so formidable a title as "erythrodermie pityriasique en plaque diseminée" refers to what is, after all, only a rather mild inflammation of the skin!

ering the eyelids. The variations in the glandular appendages of the epidermis are equally great: Witness the large hair follicles of the scalp, in contrast to those of the small lanugo hairs of the trunk. By paying proper attention to this sort of detail, one who enjoys playing Sherlock Holmes can arrive at a more or less accurate localization of a given section of skin, and perhaps also some idea of the sex, age, and even occupation of the donor.

This kind of activity is not simply entertaining; it has practical importance to the dermatopathologist, and can help him avoid interpreting certain normal phenomena as pathological. For example, one must be wary of basing a diagnosis of occlusive vascular disease on a biopsy specimen taken from the lower leg, for in that region a certain amount of apparent vessel change is normal. Similarly, it is difficult, if not impossible, to evaluate connective tissue changes in the digits, because of the normal density of the collagen in that area. A knowledge of such facts is important in selecting the optimal site for biopsy, and the pathologist should be fully informed about the site of each specimen on the request slip accompanying it to the laboratory.

Specific Considerations

A. Tumors of the Skin.

It is not the author's intention to review in detail the subject of cutaneous tumors, because this aspect of cutaneous histopathology is already recognized by most physicians, any of whom could diagnose under the microscope such lesions as basal cell and squamous cell epitheliomas. It should be pointed out, however, that the complex structure of the skin gives rise to an extraordinary *variety* of tumors, and, indeed, one of the most interesting avocations of dermatologic histopathologists is speculation about the site of origin of various epitheliomas and adenomas. These tumors may arise from any of the ectodermal glands and their several parts. To complicate the situation further, many cells of the cutaneous ectoderm are pluripotential, i. e., they are capable of producing other kinds of epidermal cells. This quality is an important element of wound healing, and is used to advantage in such surgical procedures as skin grafting and dermabrasion. It may also account for the fact that intergrades between the various tumors, as well as mixed forms, are often encountered.

B. Inflammatory Dermatoses.

Although the diagnosis of inflammatory dermatoses is probably

the most important contribution of dermatopathology, it appears to be the least appreciated by physicians in general.

These inflammatory dermatoses can be classified according to their histology in three groups: The first contains those with a specific and characteristic histologic appearance, making diagnosis unmistakable. Examples are lichen planus, psoriasis, and keratosis follicularis. This group also includes a number of conditions of interest to physicians in fields other than dermatology; among these are lichen sclerosus et atrophicus, necrobiosis lipoidica, hemochromatosis, sarcoidosis, xanthomatosis, amyloidosis, mast cell tumors, reticuloendothelioses of the Letterer-Siwe, Hand-Schueller-Christian group, acanthosis nigricans, and, of course, the many infectious processes (such as the deep mycoses) that can be identified by the presence of organisms in the cutaneous biopsy specimen. General infections, such as tuberculosis, syphilis, and leprosy can also be recognized by the specific histology of their cutaneous lesions.

A second group of conditions lacks an absolutely characteristic histology, but it may arouse strong suspicion; or, the histologic picture may be pathognomonic only at certain stages of the disease, or, in only certain types of lesions, or from certain areas of the body. For example, lupus erythematosus has a characteristic structure when the lesion is a well-developed discoid plaque, is less characteristic in its acute stages, and may be unrecognizable in lesions taken from unusual anatomical locations. In erythema multiforme, only bullous lesions can be certainly diagnosed under the microscope, while other types of lesions show only non-specific inflammatory changes. The same is true of such blistering diseases as dermatitis herpetiformis, cutaneous porphyria, and epidermolysis bullosa, where the pathologist must see a vesicle in order to be able to make a diagnosis. The histology of scleroderma is diagnostic in later stages of the disease, but in early forms and especially in specimens taken from the digits, where the collagen is normally very dense, a definite diagnosis is impossible. This is unfortunate, because it is precisely in such cases as suspected early acrosclerosis that laboratory help would be most valuable.

Finally, there is a large group of dermatoses in which the histology is not specific enough for exact diagnosis but is often helpful in placing the disease into a certain category. An outstanding example is that broad group of conditions known as dermatitis, or eczema. It will be readily appreciated that dermatitis is only dermatitis whether caused by external influences, such as ragweed sensitivity, by food

allergy, psychic factors, or a host of other causes. When the dermatopathologist is asked to diagnose an eruption described as "a widespread scaling rash of four months' duration," it is unsatisfying to all concerned to know the histology is that of a "subacute nonspecific dermatitis". The clinician wants more help than that, but such are the limitations of skin biopsy – limitations that apply to any laboratory procedure. Hence the continuing need for good clinicians. Even in such an instance as this, however, the report may be helpful in ruling out such other conditions as psoriasis or lichen planus, which may have entered into the differential diagnosis.

New Trends in Dermatopathology

Most of the basic histologic changes in the various skin diseases were well described years ago by such masters as Unna, Kyrle, and Gans. In recent years, a few diseases have been re-evaluated and bits of new knowledge added here and there, but the groundwork remains unchanged.

In addition to the routine hematoxylin and eosin stain, various special stains contribute valuable information: mesodermal structures such as collagen, elastic fibers, muscles, and reticulin can now be differentiated, and the various pigments, such as melamin, iron, and silver, identified. Special laboratory techniques can be used to identify such deposits as amyloid, mucin, calcium, and heparin.

Since World War II, dermatopathology has surged forward as a result of the application of various histochemical techniques which have contributed tremendously to our knowledge of the fine structure and function of the skin. Most of this work has necessarily dealt with normal skin and has not yet found its way into general use in histopathology laboratories; but already a few practical applications for these techniques have been found. Perhaps the best established is the use of the Periodic-acid Schiff reaction for the identification of fungi. This technique is also best for demonstration of amyloid and it has some value in the differential diagnosis of certain tumors. By this method basal cell epitheliomas can often be distinguished from similar new growths by their lack of glycogen, while cylindromas are readily recognizable from the great reactivity of the capsule surrounding the tumor masses. Many investigations, including the author's, are being directed along these lines, and it is hoped that the histochemical approach will contribute an increasing number of useful techniques to the field of dermatopathology.

Editorial

Earliest History of Tissue Transplantation in Minnesota

It was Indian Summer and the hour was growing late. The men had been spinning yarns about the campfire, and there was time for just one more story. Paul Bunyan called on Febold Feboldson who hadn't said a word all night. Febold grunted and reluctantly consented to relate an amazing story about Mabel the Mule:

"Mabel was stubborn and had a mind of her own, but she never knew when she had had enough. One day, the lumberjacks found Mabel with her heels in the air in the yard, and they kicked and punched her, but to no avail. Mabel lay motionless. The lumberjacks were chagrined. Now it meant more work for them; they would have to get rid of the carcass. Being thrifty Norwegians, they skinned her and then they went away.

"When they came back the next day to perform the burial rites, they saw a peculiar sight. Mabel was standing bolt upright and shivering violently; she was naked and hairless as a baby. She hadn't been dead at all, but had just been so stubbornly asleep that she hadn't even woken up during the skinning."

"Did they leave her like that?" queried Paul.

Febold went on to tell how the lumberjacks had been butchering sheep and just happened to have a nice fat sheepskin along with them. "So they brushed Mabel off and they threw the sheepskin over her, and since they had no thread to sew it on with, they did the next best thing: they fastened the skin in place with blackberry thorns. What a sight to behold – the erstwhile black mule now clad in a black sheepskin with blackberry thorns. Mabel didn't seem to mind, and went her stubborn way through the winter. Her wool grew thick and in the spring they sheared ninety pounds of it from her back. "And what's more, after the wool was gone, they picked fifty quarts of blackberries from Mabel!"

Postgraduate Education

Physical Medicine for Specialists

The University of Minnesota, with the generous support of the Sister Elizabeth Kenny Foundation, will present a continuation course in Physical Medicine for Specialists at the Center for Continuation Study from November 18 to 20, 1957. The course, which will deal with the evaluation and management of cerebral palsy, is under the direction of DR. FREDERIC J. KOTTKE, Professor and Head, Department of Physical Medicine and Rehabilitation. The faculty will include DR. IRVING S. COOPER, Director, Department of Neurosurgery, St. Barnabas Hospital, and Assistant Professor of Neurosurgery, New York University Postgraduate Medical School, New York City; DR. TEMPLE FAY, neurosurgeon, Philadelphia; DR. CLINTON N. WOOLSEY, Professor of Neurophysiology, University of Wisconsin Medical School, Madison; DR. HARRIET E. GILLETTE, Director of the Physical Medicine and Rehabilitation Clinic, Atlanta, Georgia; and DR. CHESTER SWINYARD, Head, Department of Physical Medicine and Rehabilitation, University of Utah College of Medicine, Salt Lake City, Utah.

Course in Fractures for General Physicians

The University of Minnesota announces a continuation course in Fractures for General Physicians to be held at the Center for Continuation Study from December 5 to 7, 1957. Treatment of fractures most commmonly seen will be stressed. Guest speakers will be DR. GEORGE W. N. EGGERS, Professor, Department of Orthopedic Surgery, University of Texas School of Medicine, Galveston; DR. CLAUDE N. LAMBERT, Orthopedic Surgeon, Chicago, Illinois; and DR. WILLIAM F. NEUMAN, Associate Professor of Pharmacology and Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York. The course will be given under the direction of DR. JOHN H. MOE, Director, Division of Orthopedic Surgery.

Notice

All continuation courses presented by the University of Minnesota are approved for formal postgraduate credit by the American Academy of General Practice. Attendance certificates will be furnished on request.

Further information concerning the above programs or others to be presented may be obtained by writing to Dr. N. L. Gault, Jr., 1342 Mayo Memorial, University of Minnesota, Minneapolis 14.

Coming Events

November 7 . . . . . E. P. LYON LECTURE: *Biophysics and Neurophysiology in the Cochlea*; DR. HALLOWELL DAVIS, Director of Research, Central Institute for the Deaf, St. Louis, Missouri; Room 100, Mayo Memorial; 4:30 p.m.

November 7 . . . . . LEO G. RIGLER LECTURE: *Bullous Emphysema*; DR. DICKINSON W. RICHARDS, Director, First Medical Division, Bellevue Hospital, New York City; Mayo Memorial Auditorium; 8:15 p.m.

November 14 . . . . . Special Lecture: *Recent Advances Relative to Diabetes;* DR. ROBERT H. WILLIAMS, Professor and Executive Officer of Medicine, University of Washington Medical School; Mayo Memorial Auditorium; 9:00 a.m.

November 18-20 . . . Continuation Course in Physical Medicine for Specialists

November 27 . . . . JACK FRIEDMAN LECTURE: DR. ULF G. RUDHE, Acting Chief of the Roentgen Diagnostic Department of Children's Clinic, Karolinska sjukhuset, Stockholm, Sweden; Mayo Memorial Auditorium; 4:00 p.m.

December 5-7 . . . . Continuation Course in Fractures for General Physicians

December 6 . . . . . JOURNAL-LANCET LECTURE: DR. WILLIAM F. NEUMAN, Associate Professor of Pharmacology and Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York; Mayo Memorial Auditorium, 11:30 a.m.

WEEKLY CONFERENCES OF GENERAL INTEREST

Physicians Welcome

Monday,	9:00 to 10:50 A.M.	OBSTETRICS AND GYNECOLOGY Old Nursery, Station 57 University Hospitals
	12:30 to 1:30 P.M.	PHYSIOLOGY- PHYSIOLOGICAL CHEMISTRY 214 Millard Hall
	4:00 to 6:00 P.M.	ANESTHESIOLOGY Classroom 100 Mayo Memorial
Tuesday,	12:30 to 1:20 P.M.	PATHOLOGY 104 Jackson Hall
Friday,	7:45 to 9:00 A.M.	PEDIATRICS McQuarrie Pediatric Library, 1450 Mayo Memorial
	8:00 to 10:00 A.M.	NEUROLOGY Station 50, University Hospitals
	9:00 to 10:00 A.M.	MEDICINE Todd Amphitheater, University Hospitals
	1:30 to 2:30 P.M.	DERMATOLOGY Eustis Amphitheater University Hospitals
Saturday,	7:45 to 9:00 A.M.	ORTHOPEDICS Powell Hall Amphitheater
	9:15 to 11:30 A.M.	SURGERY Todd Amphitheater, University Hospitals

For detailed information concerning all conferences, seminars, and ward rounds at University Hospitals, Ancker Hospital, Minneapolis General Hospitals, and the Minneapolis Veterans Administration Hospital, write to the Editor of the BULLETIN, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14, Minnesota.