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TITLE INTRACUTANEOUS TESTS USING FILTRATES PREPARED FROM PATHOLOGIC PULPS OF HUMAN TEETH WITH SPECIAL REFERENCE TO RHEUMATOID ARTHRITIS

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INTRACUTANEOUS TESTS USING FILTRATES PREPARED FROM PATHOLOGIC PULPS OF HUMAN TEETH WITH SPECIAL REFERENCE TO RHEUMATOID ARTHRITIS

by

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the University of Michigan

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INTRODUCTION

Diseases of the teeth and their adnexa have long been incriminated as possible causes of certain diseases occurring elsewhere in the human body. Such a statement is no better than a frail hypothesis unless the fundamental facts involved in the phenomenon present a coherent picture.

Among the earliest recorded American observations of dental disease, as related to systemic disease, were those of Benjamin Rush. In his works (1) he refers to "...the acrid and putrid matters which are sometimes discharged from carious teeth, or from ulcers in the gums created by them..." He further points out that such dental diseases may lead to serious systemic involvements. From this early concept it will be noted that the emphasis was placed specifically upon diseased teeth and the degradation products associated therewith.

Through the years, the original concept of diseased teeth affecting other parts of the body has become notoriously distorted. With the advent of bacteriology as a scientific discipline, it was only natural that many of its techniques should have been greatly exploited in an attempt to solve this problem. Frank Billings (2), in 1912, popularized the concept of a focus of infection affecting other parts of the body when he introduced the term "focal infection". This euphonious appellation seems to have worked a certain magic. It conveyed such a definite concept that it appeared, as early as 1915, in the "Index Medicus" as an entity in itself. In justice to Billings it should be said that he was not as dogmatic as the terms which he introduced apparently implied. He simply concluded that all foci of

infection should be removed, but that some other element must be present or lacking in certain patients because many patients with chronic local foci of infection may completely escape systemic infection.

The word "infection" specifically denotes that bacteria are present and multiplying in a tissue which inevitably leads to a structural and functional change on the part of the tissue. A "focus of infection" is just such a process taking place. However, when we couple the words and refer to "focal infection", there is also implied the dissemination of bacteria from the primary focus with the establishment of an infection elsewhere in the body. In 1917, Rosenow (3) seized upon this concept and gave the theory of focal infection an unprecedented impetus. His untempered enthusiasm through the years caused him to ascribe a streptococcal etiology to numerous systemic diseases. Hence, a theory was evolved and championed wholly different from the admittedly vague but original concept of Benjamin Rush. Focal infection, per se, has, as of today, inexorably waned due to considerable contradictory clinical and experimental evidence. Many fundamental aspects of the total focal infection complex must first be brought to light before this or any contingent theory could be regarded as merely plausible.

It is not impossible that the theory of focal infection is a feasible explanation in a certain few isolated instances. However, the theory seems wholly untenable in the vast majority of diseases; the cause of which has been ascribed to such a phenomenon.

In the investigation of the possibility that systemic infection may arise from a local source, a revaluation of at least one aspect of the mechanism by which such a phenomenon may occur has been undertaken.

Foci of infection may occur in virtually any organ or tissue of the body. However, the teeth, tonsils, and sinuses have been most frequently incriminated. In this study, only diseased teeth have been investigated as possible primary foci. More specifically, this investigation has been limited to internal tooth disease or to pulp-involved teeth. From the standpoint of focal infection, a number of organs and tissues have been reported by numerous investigators as having been affected by this phenomenon. The arthritides, ocular diseases, and cardiopathies stand foremost in the list of diseases most often believed to have resulted when the theory of focal infection has been implicated. In this study the only systemic disease investigated has been arthritis. The specific type of arthritis under investigation in this study is the rheumatoid variety. This type of arthritic manifestation was especially chosen because certain symptoms associated therewith are somewhat similar to an inflammatory process. It is now evident that the possible affecting tissue and the affected part, in this investigation, have been reduced to diseased pulps and diseased joints respectively; the latter, even more specifically, to rheumatoid arthritis.

It would, indeed, have been remiss to have ignored the concept of focal infection in evolving the rationale of this investigation. Although this study does not invoke the theory of focal infec-

tion, per se, it is immediately contingent upon a similar mechanism. Rather than assuming the necessity for active infection, and the dissemination of viable bacteria from the diseased pulps of teeth, it was believed more in conformity with known facts to determine first whether or not bacterial products, tissue degradation products, or both, are formed in pulp-involved teeth. To further test this hypothesis, preliminary animal experimentation indicated that the reactivity of a bacteria-free filtrate, prepared from diseased pulps, could possibly be demonstrated by the use of the intracutaneous test.

As a corollary to the demonstration of reactive pulp filtrates, it is apparent that the material should be tested upon individuals manifesting a specific disease. In the latter instance, a number of rheumatoid arthritic patients constitute this group. In order to establish an adequate control procedure, it is also essential to introduce a normal group of individuals. This normal group must be constituted by individuals having no known systemic disease and from whom normal pulp specimens are available.

The purpose of the present investigation will be to determine whether or not skin reactivity occurs to filtrates prepared from diseased pulps and whether or not this reactivity is greater in certain groups of patients than in others.

HISTORICAL

Paleopathologists have discovered that many Egyptian mummies of the predynastic period (3400-1700 B.C.) were the victims of exposed dental pulps as a result of excessive tooth abrasion and that, incident to such exposure, alveolar abscesses not infrequently resulted (4). In 1943, it was the author's good fortune to have had the remarkable collection of skulls in the possession of the late Ales Hrdlicka made available to him for study (5). Among the skulls of the Egyptians of the 13th dynasty, in this collection, it was quite evident that extreme abrasion leading to exposed pulps, with their attendant fistulae, was also common during this later period. Hence, that pulp disease has long been recognized, and has probably occurred from time to time through the ages among the various peoples, is quite evident. Among modern man, the etiology of pulp disease is rarely due to abrasion. This etiology was peculiar to the Egyptians as a result of their coarse foods and their gritty environment. It is assumed that the sands of the Sahara played no little part in effecting excessive abrasion. Today, as is well known, the major etiology of pulp disease is dental caries and trauma.

The Chinese, about 2000 B.C., were apparently among the first to have had some crude concept of an interrelationship between the teeth and the rest of the body. The Chinese employed acupuncture (the insertion of needles in a part for the production of counter-irritation) quite extensively for the relief of toothache. However, at least half of the points of acupuncture were not near the teeth but in such structures as the hands, the elbows, and in the feet. Appar-

ently, contemporary Egyptian minds did not countenance a possible systemic relationship. In the encyclopedic Hebraic works, the Talmud, there is also maintained a relationship between the teeth and other organs of the body. That diseases of the teeth may have exerted their effects on other organs of the body did not escape the astute Hippocrates as recorded in the year 460 B.C. However, it was apparently Celsus, in 30 B.C., who was the first to understand the cause of alveolar fistulae and suppurations therefrom. Fabricius (1560-1634) maintained the cure of a "general malady" by the extraction of diseased teeth. In 1728, Pierre Fauchard published his monumental "Le Chirurgien Dentiste", in which he intimated that many of his extractions apparently cured various constitutional ailments (6).

In 1818, Rush reported an apparent cure of a woman suffering from "chronic rheumatism" by having extracted a diseased tooth. Thus was born a concept which Billings, almost a century later, referred to as focal infection. Then Ludvig Hektoen's irrepressible pupil, E. C. Rosenow, began to extoll the important virtues of his bacteriologic procedures as proof of focal infection (7). He particularly invoked dental foci and maintained that focal infection is invariably due to a streptococcus. He believed that the streptococci obtained from diseased pulps did not have to be of the same species that affected some other region of the body. Nevertheless, he did maintain that the latter originated from the former. He explained this "transmutation" on the basis that by appropriate means he had succeeded in converting "the various organisms of the streptococcus group, one into the other"

(8). Rosenow conveniently developed explanations to exonerate certain apparent fallacies in the focal infection complex. Even at the beginning of this decade a staunch Rosenow adherent (9) believed that, because of the transmutation theory of Rosenow, "a streptococcus living an avirulent existence in some neglected point of anatomy, might any day become the progenitor of a highly virulent strain with mutilating and death-dealing properties." This proponent of Rosenow theories believed that where the organism "had previously lived as a mere saprophyte, it changes to a pathogen". He carries the battle cry to a feverish pitch in suggesting that chronic streptococcal disease be called the "Billings-Rosenow syndrome". Such veneration for the theory of focal infection is quite the exception today. However, it serves to remind us that, only two or three decades ago, this was the consensus among many practitioners of the healing arts. During this era an inconceivable number of teeth were sacrificed at the altar of focal infection. Rosenow (10) believed that he had demonstrated an apparent relationship between oral and systemic disease. He insisted that bacteria within a focus at one site in the body can be carried via the blood stream and elect to infect another site. His implications of elective localization ascribe an avidity on the part of the streptococci for certain tissues. This imputes to streptococci certain attributes which, following the law of parsimony, we more reasonably believe to reside in the nature of the tissues infected. Yet we cannot deny that both features are mutually operative in the causation of infection. Obviously, the tissues must be suitable for the organism and the organism must possess a biochemical economy enabling it to

utilize the tissues. The present-day opinion discredits his experiments as having been inadequately controlled. Rosenow isolated bacteria, especially streptococci, from various lesions in the human body and injected them by different routes into animals. He demonstrated his ability to isolate these bacteria from the analogous organs of animals, i.e., the same organs from which they were originally recovered from the human source. The use of overwhelmingly large numbers of organisms with which he injected his animals would indeed cause them to disseminate throughout every organ and tissue of the body. Having used as much as 10 ml of inoculum, this would be equivalent to approximately a half liter of inoculum in man. His best results were accomplished by employing the intravenous route which most naturally would cause a complete diffusion of the inoculum throughout the animal body. Numerous attempts to confirm his observations and contentions have been wholly unsuccessful.

Meyer (11) was among the first to completely dispute Rosenow's conclusion on the ground that he had observed that streptococci were very prone to infect areas which had been injured either by disease, or by experimental or accidental trauma. Meyer believed that the streptococcus was a bacterium of opportunity and yet Rosenow maintained "elective localization".

From the dental aspect, it is important to remember that not all pulp-involved teeth are infected. All may have been infected at some time but they may not remain infected. Dental pulps are demonstrably infected in only approximately 50 percent of pulp-involved

teeth as reported by Ostrander and Crowley (12). Not all of the bacteria found in pulp canals necessarily find their way to the pulp through some discontinuity of the coronal structure. Traumatized teeth which are structurally sound may yield a positive culture when an inoculum is aseptically procured from the pulp canals. Csernyei (13), and Robinson and Boling (14) demonstrated that trauma may produce an anachoretic pulpitis. In this situation it is conceivable that the hematogenous route may initiate infection at the immediate site of an apex or the apex of an affected tooth. However, the majority of periapical lesions are not the result of such retrograde pathogenesis. Perforating carious lesions more frequently and obviously cause an infection of the dental pulp by direct extension and by ultimate contamination with the bacteria from the saliva. Another condition which may conceivably occur is an anachoretic pulpitis as a result of an inflamed pulp due to the irritation caused by an imperforate carious lesion, a silicate restoration or, in fact, any traumatically deep restoration. Clinically, the intervening bridge of dentin between the most advanced part of the cavity and the pulp chamber may appear structurally sound and may be from one to two millimeters in thickness. That bacteria may be present in the dentinal tubules far in advance of actual cavitation has long been recognized. However, this is not invariably the case and should the irritation prove sufficient without actual microbic invasion from the cavity, a transient bacteremia of any origin may possibly infect the pulp. Hence, a deep cavity may irritate the pulp into a state of passive hyperemia.

This inflamed pulp may then become an ideal substrate in which bacteria, transiently passing through the vicinity of the apex of the tooth, can become established. Whereas the bacteria in the cavity may have simply irritated the pulp without invading it, those in the blood stream may actually infect it.

Streptococci from the saliva have a greater mathematical probability for invading the dental pulp and have also been demonstrated to appear in the blood following the extraction of a tooth (15), (16). Even the mere manual manipulation of a tooth, without extraction, is capable of producing a bacteremia of short duration. There is little doubt that during normal mastication, especially in individuals manifesting paradontal disease, a transient bacteremia may occur more frequently than is actually demonstrable. Nevertheless, the inflamed dental pulp is a potential substrate in which a bacterial focus may occur. It is essential to remember that a focus of infection is actually a "locus of infection" and that it does not necessarily imply any focal aspect. Grossman (17) points out that "the terms focal infection and focus of infection have been used indiscriminately and interchangeably at times. They do not mean the same. A focus of infection may be present without causing focal infection. The former denotes an infection of tissue or of an organ while the latter presupposes dissemination of microorganisms from the focus with the establishment of an infection elsewhere in the body."

It is evident that the decomposition of the pulp has been incriminated by a number of dental practitioners as the primary source of periapical disease (not necessarily infection), leading to tissue

changes observable indirectly by radiography and directly by surgical exposure. The histopathological picture of involved pulps has been abundantly demonstrated (18), (19), (20), (21), (22). Clinically, there is considerable evidence that pulps degenerate in a number of ways and undoubtedly pass through various stages of hyperemia, necrosis, putrefaction, and liquefaction. If only the pulp is involved, and because of the unique structure of a tooth, the only cardinal symptom which can be manifested is pain. An extension of this pathologic process inevitably results in periapical disease. Whether or not this stage has been reached, the fact remains that an investigation of the substances produced in the acute or chronic disintegration of the pulp are of utmost importance. It is quite possible that the degradation products formed in a tooth are quite distinct from those formed within diseased gingival tissues. Should this be true, then one form of dental disease may exert more serious systemic influence than another.

Bullied (23) regards the dental cyst as being more important than any other form of dental infection. Here again is an expression which may lead to misinterpretation. A radicular cyst is invariably the result of pulp disease, and the noxious products of the degenerating pulp, over some period of time, are responsible for its occurrence. Most investigators agree with Crowe (24) who, in reference to oral disease, states that "the commonest foci of infection are undoubtedly the teeth...experience teaches that pyorrhea is not as dangerous as root infection."

The orgy of the extraction of teeth, in the hope of alle-

viating various maladies, has met, as is known, with far greater disappointment than with any appreciable measure of success. The question is often asked: "What has been cured or definitely improved?" To answer this, one can simply say that almost every non-specific disease has been claimed at some time or other to have been improved by the extraction of "suspicious" teeth. The frequent allusion to "suspicious" or "involved" teeth having been removed with resulting amelioration of constitutional symptoms, makes a critical analysis quite difficult. When we add to this the rather non-descript and vague designation of the systemic symptoms, a critical analysis is well-nigh impossible. One can never be quite sure whether or not the improvement is the result of the extraction or in spite of it. Unfortunately, irrespective of the measures that may have been taken to eliminate dental foci of infection, the improvement may only have been temporary, and the literature abounds with the immediate so-called spectacular results without adequate follow-up in the case history.

The day of the "post hoc ergo propter hoc" in focal infection is long past. Nevertheless, the newest textbooks in internal medicine, arthritis, allergy, dermatology, otolaryngology, ophthalmology, as well as dental textbooks and many others, without exception, admonish the practitioner not to overlook dental foci before relegating the disease to idiopathy. Various diseases of the eyes, ears, sinuses and other adnexa have, at times, dramatically responded to the elimination of dental foci of infection. These situations do not cogently support the theory of focal infection because of the proximity of the

part to the offending tooth. In these conditions the infection may not be "metastatic" but may express itself by extension along blood or lymph vessels, nerves, fascial planes, or simply by tissue continuity. Of considerable importance is the fact that the involved organs or structures themselves may not demonstrate infection but are physiologically altered. The incriminated tooth, on the other hand, may be bacterially involved. This lends credence to the fact that some product of bacterial infection of the pulp may strike at some susceptible site somewhat removed from its origin. This is definitely not focal infection; yet it is "focal", but it is not infection. If these physiologically active substances, produced within the dental pulp, may be considered toxins then we are dealing with a different concept - "focal toxicosis".

When attempting to evaluate just a fraction of the tremendous amount of material on focal infection, the thoroughly nebulous state of the entire subject is as perplexing as it is distressing. In years past, the number of men believing some or all arthritides to have their origin in various foci of infection was legion. To the arthropathies we may add such vague clinical entities as fibrositis, neuritis, myositis, and neuralgia, which even today are believed, on occasions, to stem from foci of infection. Grossman (25) maintains that "despite the inherent weakness of such case reports, it cannot be denied that periapical infection can, and sometimes does, cause infection elsewhere in the body, or that it may contribute to ill health. The frequency with which such infection exists and causes systemic disease, is open

to question, however, and has without a doubt been greatly over-emphasized in the past."

The irrefutable proof offered by endodontic therapy further inveighs against the all-important diseased pulp and exonerates the maligned periapical lesions. A tooth showing obvious radiographic evidence of periapical involvement will demonstrate only ephemeral improvement if periapical curettage should be instituted without effecting adequate obturation of the root canal with some such inert substance as gutta percha or silver.

Statistics offer considerable evidence that dental disturbances are certainly not the sole determining factors in the causation of any systemic disease. The case analyses by Frankel (26), of about 17,000 people, and that of Appleton (27), of more than two and a half million men from the World War I draft, conclusively substantiate this fact. The clinical studies of Cecil and Angevine (28), in determining the effects of the elimination of apparent foci in 200 cases of rheumatoid arthritis, showed that no benefit accrued to 47 of the 52 patients from whom dental foci of infection were removed. To their astonishment the three remaining patients in this group actually experienced an exacerbation. Their results by the elimination of foci of infection from the tonsils and sinuses follow a remarkably similar course.

The clinical results of many other observers in relation to gastric, cardiac, nephritic, and ocular diseases have indicated only occasional improvement when dental foci were eradicated. Radio-

graphic studies of patients with and without disease, undertaken by a number of dental institutions, have completely failed to demonstrate any relationship between periapical involvements and the clinical manifestation of disease. There is, however, abundant histologic and radiographic evidence affording complete proof that the common periapical lesions are the result of pulp infection or degeneration. Periapical disturbances may frequently be observed to return to a completely normal structure as demonstrated, radiographically, some months or years after instituting root canal therapy only. There are in such instances no objective or subjective symptoms and the tooth functions quite normally; often as serviceably and as long as other normal vital teeth in the same individual.

From the data of Haden (29) and Burket (30) it would appear that nonvital teeth show bacterial contamination about 80 percent of the time and that vital teeth do so approximately in 50 percent of all cases. However, the likelihood of contamination by their methods is exceedingly great.

Fish and MacLean (31) found that streptococci are present only in necrotic areas of bone and also that they do not diffuse through the granulation tissue about the root apex. Similarly, Grossman (32) mentions that "in grossly infected pulpless teeth, the infection is limited to the root canals or can be found in the pus of the associated abscess. Even in such cases, the neighboring alveolar bone and soft tissues are sterile, although they may be irritated by the diffusion of toxic products."

Using a tracer organism, Kanner (33) found that bacteria within the gingival crevice find their way to the pulps of vital teeth while in the process of being extracted. Contamination occurred even after the surface "sterilization" of extracted, intact, vital teeth. Using a local anaesthetic containing a vasoconstrictor, Burket and Burn (34) obtained a transient bacteremia in as few as 17 percent of all vital teeth. In the superior method used by Gunter and Appleton (35), whereby an inoculum was obtained from the pulps of vital teeth in situ, no growth was obtained.

Despite a number of attempts to develop a test for determining whether or not a particular tooth is actually a focus of infection, none has proved valid. Most tests have been confined to blood studies, e.g., Schilling index, differential count, blood uric acid content, blood sedimentation, also the combination of blood sedimentation as induced by such provocative radiation tests as short wave diathermy.

Of interest are the skin tests that were proposed for diagnosing the presence of foci. Kauffer (36) used, in principle, a modified Dick test. Although testing as many as four-hundred patients less than 20 percent gave a positive reaction. Prader (37) employed a skin test using an autogenous vaccine from bacteria recovered from the pulps of teeth. He injected from 0.05 to 0.1 ml intracutaneously, and referred to a hyperemic response as an "allergic" reaction and an exudative, edematous response as a "toxic" reaction. He reported only nine cases and the results are, therefore, difficult to evaluate.

Although clinically, there appears to be evidence that the environment within certain pulp-involved teeth is microaerophilic, there is no proof that it is actually anaerobic. However, the moribund dental pulp undoubtedly affords an unusual substrate. It is conceivable that bacteria, per se, need not leave this site to produce systemic disturbances elsewhere. Toxins, of a particular type, may be formed in such an environment and upon such a tissue. These alone may leave the site of origin to cause damage or disease elsewhere. Bacteria removed from this unique environment and substrate, to artificial media, may no longer produce toxic products absolutely identical to those formed in the tooth. It is not impossible that streptococci, within the diseased pulp, like the diphtheria bacillus which is found only at the site of the lesion, cause systemic disturbances due to the dissemination of soluble products. The criterion for optimal conditions for streptococci would then be the production of a highly "toxicogenic" strain and not only abundant growth. Sometimes the by-products of proteolysis of the pulp seem more important than the bacteria. In this respect it may be well to consider the strong implication of Miller (38) when he referred to "...alkaloids which doubtless form in putrid pulps..."

Cecil and Angevine (39) are now more than ordinarily skeptical in their opinion. They state that, "Many of us who originally accepted the theory of focal infection with enthusiasm have watched with interest and some trepidation its rapid development in the various fields of medicine, but are now wondering if the time has not arrived for a re-

valuation of the whole theory. Many thoughtful students today question seriously its validity, and some are quite willing to throw it completely overboard...Scientific men are becoming a little wearied of the universal acceptance of a theory as though it were an established fact."

Gay and his associates (40) summarize the problem quite succintly: "The sounder concept of focal infection has, as Billings has pointed out, unfortunately become confused with the controversial question of selective tropism as developed by Rosenow...Specifically it has been assumed that the rheumatic state and numerous less well defined conditions are due to streptococci which are distributed from an original focus particularly in the teeth or tonsils to various parts of the body. As an alternative explanation in view of the fact that bacteria are frequently not found in the secondary lesions it has been further assumed that toxins may be spread from the original focus. This restricted conception of focal infections might well serve as a working hypothesis worthy of investigation."

It will be noticed that an alternative suggestion that any substance which may leave the tooth is in the nature of a "toxin" or toxic substance. This is far from any new concept as Pasteur, in 1860, stated that "various diseases are caused by ferments". The broader connotation of "ferments" in that day also embraced the concept of protein hydrolysis which today is ordinarily referred to as proteolysis.

It is quite obvious that there is no indisputable proof that diseased teeth can cause diseases of a systemic nature. Should we not predicate a bacterial incursion, it is then first necessary to study the local-systemic complex and to determine what substance, if any,

issuing from a diseased pulp, could possibly wreak havoc in some remote site. The role of bacteria, and under what conditions they exist, are important factors to consider, should we assume that they play some part in the elaboration of "toxic" substances which escape from diseased teeth. Rickert, et al., (41), proposed the hypothesis that "...the streptococci chiefly responsible for root canal and periapical infections of the teeth all belong to the same type; that this type is highly unstable and appears as S. fecalis, mitis, or salivarius, or another form, depending on the pressure of the environmental conditions, coupled with intrinsic mutational capabilities." This observation, of some twenty-five years ago, does not hold particularly well today when using more refined bacteriological techniques.

Investigations upon animals in an attempt to determine the possible relationship between streptococci and arthritis, particularly the rheumatoid variety, have been numerous. The results on the other hand, have been exceedingly disappointing to the critical investigator. Crowe's works (42) are well known and afford a typical example of what may be expected by such an approach. He has apparently demonstrated that certain strains of streptococci seem to have the power to develop best in a particular tissue and to retain this power upon animal passage. His so-called "osteotrophic strains", originally isolated from the roots of infected teeth, when injected in small doses into young rabbits, immediately after isolation, produced joint infection in many of the animals. Ninety percent of the rabbits were affected with a granulomatous bony infection with bone absorption and fibrous tissue formation. Holman (43) points out an anatomical reason for what is

frequently believed to be an organotropism. He states: "If, for example we give an intravenous injection of bacteria into healthy animals there is a marked tendency for these to localize or be stopped in the finer capillaries of certain tissues...Now it happens that the tissues about and in the joints are the most liable to show evidence of such localization. This fact I demonstrated long ago by the use of Rosenow's results, our own, and the experience of many others. If we use bacteria with any tendency to localize that is what occurs - they localize chiefly in and about the joints. This has been defined as the arthrotropic character of such bacteria." If rheumatoid arthritis required the presence of viable streptococci in the body it is inconceivable why numerous investigators usually have obtained no growth from their aspirates taken from the joints and other regions of their arthritic patients. Animal investigations of this nature are fraught with many variables. The joint symptoms, in fact, are of the nature of an infectious arthritis and are not of the rheumatoid variety. In Crowe's investigation it is apparent that he invokes another possible mechanism by stating that allergy undoubtedly plays an important but secondary role. Williams (44) points out an apparent fallacy among many bacteriologists who have worked with streptococci in relation to rheumatic fever. She states: "Lastly, and of greatest significance, they [the bacteriologists] did not appreciate the extremely important role played by the host with his varying susceptibility and power to react in peculiar allergic ways." Here we have a statement referring to rheumatic fever and the "allergic capacity" of those individuals so afflicted. Cecil (45), and many other investi-

gators of the rheumatic diseases, emphasize the similarity between rheumatoid arthritis and rheumatic fever. Both tend to occur in young people, although rheumatic fever is commoner in children and rheumatoid arthritis in young adults. Both diseases are characterized by swollen painful joints. Both may present all the appearances of an acute infection in the early stages, so much so that it is often impossible to differentiate them clinically; both may progress to a chronic form, and about 5 percent of patients with rheumatic fever progress into a form of chronic arthritis indistinguishable from rheumatoid arthritis. Both diseases may at times involve the muscles and tendons as well as the joints and in both diseases subcutaneous nodules develop. Hence, as the total picture of possible meaningful relationships between the manifold aspects of the local-systemic complex are considered, there can be little wonder as to why certain pitfalls in these investigations are virtually inescapable.

Boland, Headley, and Hench (46) reported an important observation on their experience with the use of penicillin on ten patients with a confirmed diagnosis of early, but progressive, rheumatoid arthritis. They administered large doses of penicillin but laboratory and clinical results were negative. As a result they concluded that neither Streptococcus hemolyticus nor any other penicillin-sensitive organism was directly responsible in the causation of rheumatoid arthritis. From the dental standpoint, it is of interest that the Str. hemolyticus is an infrequent invader of the diseased pulp. In cultures from the pulp canals of pulp-involved teeth, Sommer and Crowley (47) have encountered only about a two percent incidence of the

hemolytic streptococci. In connection with hemolytic streptococci Crowe (48) cites an instance of a severe tonsillitis due to this organism occurring in a rheumatoid arthritic patient. He states: "This case rather weakens than otherwise the hypothesis." One may wonder at this juncture, why the arthritides especially, have become so inextricably woven into focal infection lore. It should be explained that "rheumatism" is a broad term embracing conditions characterized by pain and dysfunction occurring chiefly in the articulations, muscles, and ligaments. Encompassing this huge variety of many vague and little understood maladies, it is not surprising that rheumatism is the commonest cause of chronic illness in this country and that it ranks second in causing temporary and permanent disability. In fact, Comroe (49) expresses the magnitude of this chronic disease problem of rheumatism as being commoner"...than the total number of cases of tuberculosis, diabetes, cancer and heart disease combined", and that, "because of its ability to cripple without killing, it should be put at the head of all other chronic diseases as of preëminent medical, economic and social importance." There is little wonder then why Osler described rheumatoid arthritis as "one of the greatest of the human afflictions."

An exceedingly interesting and equally inexplicable phenomenon is the increase in the agglutinin titers of rheumatoid arthritic patients for certain strains of hemolytic streptococci. Comroe (50) states that these patients show agglutinins for hemolytic streptococci (group A) in only about half of all cases although the test is seldom

positive in individuals whose rheumatoid arthritis is of less than a year's duration. The usual titers in these patients is not ordinarily greater than 1:160 or 1:320; it is negative in most children and adolescents and with a spontaneous remission of the affliction, a fall toward normal occurs. Comroe avers that, "agglutination in a titer of 1:160 or higher rarely occurs in any other disease except frank hemolytic streptococcus infection. Such agglutinins tend to disappear as the individual recovers. This reaction suggests an etiologic relationship between arthritis and hemolytic streptococci but certainly does not prove this. Agglutination reactions with other organisms are essentially negative; sera from individuals with other diseases do not produce such agglutination."

Wallis (51) found that many sera from rheumatoid arthritic patients differ from normal sera in their ability to agglutinate suspensions of fine collodion particles and that the former will non-specifically agglutinate acapsular pneumococci and also that such sera will agglutinate selected strains of hemolytic streptococcus of Group A due to a non-specific enhancement of normally present agglutinins.

Crowe (52) is loath to completely deny that bacteria, may in some way, play a part in the causation of rheumatoid arthritis. It is his opinion that "as time goes on and experience widens, I feel more and more convinced that a microbic factor is present in 99 per cent of all cases of so-called chronic rheumatic disease. The fact is that arthritis can be produced in very many ways, not only with living cultures, but also by the injection of sterile filtrates into the joint." Kling (53), however, could not produce any changes in the joints of rabbits by repeated intra-articular injections of even large amounts of strains of

viable streptococci which were supplied by a laboratory which claimed to have produced chronic arthritis, resembling the rheumatoid type, by the use of the organisms. He does believe, however, that the serological reactions performed with joint fluid does indicate a ready entry of antigen into the joint effusion from the blood.

Among a group of ten children afflicted with a variable degree of periarticular swelling of the soft tissues, pain in the affected joint, and in some a palpable joint effusion, Freiberg (54) found the X-ray findings negative. Having prescribed only dietary regulation, catharsis, and abdominal massage, the joint symptoms were alleviated in all cases with an average duration of only 26 days. From this Freiberg believes that, "this strengthens the hypothesis that some substance is either formed in the intestinal tract, or at least, absorbed from the intestinal tract in a certain type of case, and is a causative element in the production of joint symptoms." Thus it is apparent that the intestinal tract as well as other sites in the body could conceivably play some part in the occurrence of joint symptoms. Such an observation may not have been unanticipated as the literature abounds with reports of arthritis associated with bacillary dysentery. Along this line of reasoning, Freiberg states: "In the reports on bacillary dysentery epidemics, already mentioned, from 1 to 3 per cent of the patients developed arthritis, usually monarticular, but often polyarticular, in which no organisms were found either by smears or by cultures of the joint fluids. Of interest was the time when these arthritides appeared, from twelve to twenty days after the onset of the intestinal symptoms, or

later, a stage of the intestinal infection when intestinal signs and symptoms had usually disappeared. If a soluble toxin formed by the bacteria or a substance released on the destruction of the bacteria in vivo was the etiologic factor, would not this delayed appearance of the joint lesions be difficult to explain on any grounds other than a sensitization reaction?"

The concept of intestinal intoxication also had its harbingers in antiquity. Aurelianus, in the 4th century A.D., considered arthritis as of gastro-intestinal origin somewhat admixed with a hereditary predisposition and Paul of Aegina regarded the intestinal tract with its disorders as contributing to gout and arthritis. Bassler (55) found that with a dietary correction "in 181 cases of primary biotoxic intestinal conditions, practically 68.5% showed marked improvements in reduction of swelling, usefulness of joints and control remissions, and about 26.5% more were distinctly improved, there being 9.2% of failures, these latter being old cases with much deformity." He believes that "a reasonable conclusion from these results is, that, while other causes than intestinal have importance in chronic arthritis, significant etiologic and therapeutic factors are contained in the digestive tract, and of these, infections of intestinal origin comprise an important group." Traut (56) has found "the streptococci of the mouth and teeth very similar, if not indistinguishable from the streptococci of the bowel." He found that "the green streptococci isolated from the bowel have usually been of low virulence for animals. One cubic centimeter of a 24-hour broth culture injected intraperitoneally

does not kill mice; and even doses as large as 5 c.c., has failed to produce arthritis in rabbits." He maintains, however, that arthritis could occasionally be produced by inoculating the streptococci into the colon. The same strains were without effect on intravenous injection." By inference one may conclude that there may be an absorbable intestinal putrefactive by-product which may be responsible for the symptoms observed in the foregoing investigations. Grave concern over the putrefactive products found in diseased teeth was given great impetus by Hunter (57). In referring to periapical disease, he stated: "This form of sepsis is particularly severe and injurious, because it is dammed up in the periosteum and alveolus and cannot be eliminated by any ordinary medical antisepsis the doctor can administer, moreover it being locally painless and insidious in action, it goes on accumulating in severity without giving any symptoms or warning." Grieves (58) believes that he demonstrated a slight rise in temperature among rheumatoid arthritic patients having chronic alveolar abscesses. He maintains that this occurs "whenever the superficial tissues heal over closing the fistula and causes temporary retention." He also maintains that "...in blind periapical infections, where we have the blood stream gradually sensitized for months by hourly dosage of toxins without the safeguarding formation of the active anti-bodies characteristic of acute infection...this condition would appear almost to be a phase of anaphylaxis induced by continuous injection, extending over months, of toxins or ptomaines propelled by the gases arising from the decomposition of pulp tissues..." This latter statement, promulgated over 35

years ago, contains many interesting elements of thought and study resulting principally from clinical observations. Miller (59), however, was among the first to express the opinion that systemic infection is not likely to occur from a gangrenous pulp without the intervention of abscess formation. Similarly, Hatton (60), believes that "small encapsulated infections are more likely to be associated with systemic involvement," and that, "large diffuse infections are associated with bone absorption and extend locally rather than by the blood stream and lymph channels to remote parts of the body." Rickert (61) envisaged a slightly different mechanism in his statement that "...the normal pulp-canal circulation is destroyed, the diffusion rate is unfortunately diminished to a point where the circulatory elements, by stasis and probably catalysis, become toxic to living protoplasm. This may take place even in the absence of infection as a result of lytic principles present in the general circulation." Rhein (62) is of the opinion that "...we have very greatly underestimated the number of teeth with so-called blind abscesses, in which the septic absorption is much greater than where an open sinus exists." Although Buchanan (63) maintains that "an abscess is always infected, [and that] a gramuloma may or may not be infected", he believes that "a granuloma may more readily cause systemic trouble than an abscess." Steinbügler (64) believes that "the most dangerous forms of dental infections are pulpless teeth and apical abscesses. They are free of symptoms and hence unsuspected. Being situated in the osseous tissues, no expansion can take place, and their only drainage is into the circulation." Meade (65), in his experience, found that "a dying or decomposed

pulp may be a focus of infection without definite periapical symptoms." Haden (66) assumes chronic periapical infection to be "by far the most common infection responsible for systemic disease," and that, "once a tooth is infected it is always infected. It is most probable that such structural changes as were evident in the radiographs usually indicate the earlier presence and activity of bacteria. The finding of a negative culture is the best evidence that the areas were bacteria-free at the time the cultures were made...Arthritis is the most common systemic manifestation of focal infection in man." As early as 1916, Thoma (67) recorded some interesting observations which were quite unfettered by focal infection hypothesis. He believes that "this process of bacterial action is a decomposition, resulting in various combinations of the by-products of the metabolism. These by-products can almost always be recognized by the sense of smell and it is small wonder that such substances if absorbed into the system cause diseases of all kinds...The deeper the infection is seated in the tissue, and the greater the pressure of the accumulated bacterial products, the larger is the amount of absorption...An abscess enclosed by bone gives no chance for infiltration or extension; therefore the pressure is great and the bacterial products are absorbed readily... The part of the body affected and the disease produced by absorbed toxins ...depends upon several factors. The different toxins have special affinities for a certain tissue...The natural outlet from the pulp chamber is the apical foramen, or the apical foramina, and therefore we find these openings the most common mouths of the infection, since they are the natural passages through which infected matter may pass from the dental pulp chamber into the surrounding tissues of the apex of the tooth." Thoma

continues, stating, that "granulomata usually are due to direct entrance of the disease through the root canal but may also be caused as a secondary manifestation due to bacteremia, that is, the presence of micro-organisms in the blood...if we look at the unfilled root canal containing remnants of diseased pulp, or the acute and chronic dental abscess and the granuloma as a focus from which not only bacteria may become absorbed and distributed to other parts of the body, but where protein poisons may be generated and taken up by the circulation, thus causing general toxemia or local disease of certain delicate tissues. We have already seen that the protein poisons which are formed during infection differ, among other things, according to the species of the invading bacteria and it is therefore desirable to know which of the bacteria encountered in these lesions produce secondary disease, either by direct infection or by the formation of pathogenic poisons which become absorbed and may cause autointoxication similar to that of intestinal origin." Referring to the granuloma and its encapsulating membrane, Thoma states that "...this capsule does not prevent absorption as it contains a meshwork of capillaries and is penetrated abundantly by larger vessels and therefore its contents are in direct communication with the circulation. Bacteria and toxins may be absorbed by the capillaries in the granuloma and be transported to other parts of the body." It will be noticed that, among the various investigators, Thoma particularly emphasizes the liberation of protein toxins from pulp-involved teeth.

If toxins should be formed in diseased teeth, it is only natural that we should be interested in the way in which they are formed and

in their chemical nature. As early as 1883, Brieger (68) began his experiments with the basic products of putrefaction. From putrefied meat, fish, glue and yeast, from old cheese and digesting fibrin, and from pure cultures of pathogenic bacteria, he isolated a large number of ptomaines, some non-poisonous and others exceedingly poisonous. Of particular interest is the fact that his pure cultures of streptococci yielded especially large quantities of trimethylamine. Vaughan and Novy (69) credit the Italian toxicologist, Selmi, with the introduction of the word "ptomain" which, derived from the Greek, means "cadaver". They define a ptomaine as an organic chemical compound, basic in character, and formed by bacteria on nitrogenous matter. Since all putrefaction is due to bacteria, it follows that all ptomaines result from the growth of these microorganisms. The kind of ptomaine formed, they maintain, is due to, the species of the organism, the nature of the substrate, the amount of oxygen present, the duration of the process, the temperature, and the pH of the process. As the kind of ptomaine found in a given substance will depend upon the stage of putrefaction the substances may be looked upon as transition products in the presence of putrefaction. Vaughan and Novy state that Panum, 1856, "published a most important contribution to the knowledge of the nature of the poison present in putrid flesh." Panum's aqueous extract, unlike that of pre-bacteriologic investigators, certainly contained a chemical as it retained its toxic properties after treatment insuring the destruction of organisms. The chemistry of putrefaction is rather complex and only a brief account will be given of one of Panum's simple procedures.

He extracted the putrid flesh with distilled water,

> and upon distillation the chemical remained in the retort. He found that the poison was not destroyed by boiling or evaporation or by completely dessicating at 100°C. It was found to be insoluble in alcohol and could, therefore, be washed with this agent. It was, however, quite soluble in water. The putrid poison was found comparable to snake venom, curare, and certain vegetable alkaloids. Twelve milligrams of the poison, obtained by his method of extraction, dried at 100°C and washed with absolute alcohol, proved almost lethal when injected intravenously into a small dog. The nervous symptoms were especially marked and upon autopsy, the blood was found to have infiltrated the tissues, to have been dark and imperfectly coagulated. Panum also obtained a narcotic substance, the two agents having been separated by the solubility of the narcotic in alcohol. This narcotic, upon injection into the jugular vein of a dog, caused it to remain in a deep, unbroken sleep for 24 hours.

The foregoing is patent testimony to the early realization that substances of profound pharmacologic activity may result from proteolytic degradation. There exists a variety of specific actions by certain ptomaines, e.g., convulsant, paralytic (cardiac and respiratory), and mydriatic. Brieger (70) demonstrated that the cholera bacillus, growing upon a blood serum medium, produces especially large quantities of putrescin, and that although neither putrescin or cadaverin are toxic upon intramuscular injection, either will cause necrosis of the tissue. Brieger also showed the importance of only a small amount of oxygen to assure the greatest yield of the most toxic ptomaines, and that, in culture, the toxic bases begin to appear about the seventh day of putrefaction, and if the process is permitted to continue for a very long time, that they will disappear.

Although streptococci are not ordinarily regarded as very

significantly proteolytic, they are not uncommon in diseased pulps that have undergone obvious putrefactive decomposition. In fact, Prevot (71) described 28 strains of anaerobic streptococci which he isolated from various systemic gangrenous conditions. It is maintained (72) that "...hemolytic streptococci can break down peptones and casein, but do not attack serum or serum albumin. Apparently anhemolytic streptococci and viridans streptococci are incapable of splitting proteins." Holman (73), in referring to the important factors in infective foci, states: "These products may be the soluble fractions of the bacterial bodies, the toxic or other irritating substances produced in the bacterial metabolism, or a combination of these with the substance derived from the local reacting tissue." In 1903 Godlee (74) expressed the opinion that "many of the chronic diseases of middle life are due to the absorption of small doses of poison from chronic localized infections." Haden (75) also maintains that "in a certain group of cases the reaction in the joints is due to the absorption of toxins." "No one will question", says Gay (76), "that the disintegration of protein liberates chemical substances that are primarily toxic, nor that such disintegration takes place in varying degree in the course of infections. The modern viewpoint, however, would regard such protein poisoning as secondary and incidental to a biological process initiated by micro-organisms, as was maintained by Schwann and Pasteur."

Recent experimental work by Angevine, Rothbard, and Cecil (77) suggests that bacterial allergy may play a definite role in the etiology of rheumatoid arthritis. They found that "a smaller number

of intravenously injected bacteria were required to produce arthritis in rabbits which had received previous intravenous injections with heat-killed streptococci than in normal or intradermally immunized animals." In other experiments, these workers "injected repeated small amounts of heat-killed streptococci or nucleoprotein fraction into the right knee joints of rabbits. When these animals were later injected intravenously with live cultures of the same bacteria, the previously injected joints were more susceptible to infection than other joints injected with heat-killed staphylococci or horse serum." Despite such findings it is important to remember that most investigators at present find that blood cultures are negative in patients with rheumatoid arthritis (78). On the other hand Pollia (79) states: "Where no growths are obtained it is assumed that the bacteria in the focus elaborate a foreign substance which sensitizes a tissue, producing inflammation without the presence of microorganisms. This is called anaphylaxis or protein sensitization and has been studied very extensively with disappointing results."

At this juncture it is only natural to reflect on the possibility of some particular sensitizing substance leaving a diseased pulp, to be carried by the circulation to some susceptible site in the body, particularly the joints. Primarily, could the amount of sensitizing substance be large enough and sustained enough to produce such an effect? Beck (80) has "...succeeded in sensitizing guinea pigs with a single dose of crystallized egg albumin as small as one twenty millionths of a gram. Large doses are less effective in sensitizing

guinea pigs than small, e.g., one milligram of most proteins will usually be much more effective than 100 milligrams." Should we dare assume that a similar or comparable mechanism is operative in man, then such a phenomenon may be possible. There is, of course, no evidence that the same is true for man. Now that some investigators have invoked an "allergic hypothesis", it behooves us to further investigate this ramification which was alternatively proposed as a result of the ignominious fate of the focal infection concept. In 1929, Birkhaug (81) assumed that "...a non-specific common allergenic factor is present excessively in the bacterial products of indifferent and viridans streptococci, to which cases with either acute rheumatic infections or chronic infectious arthritides react hyperergically." Unfortunately, the results are not as attractive as one would be led to believe unless Birkhaug had been working with an inordinately thermoduric substance. To what extent these positive skin reactions may have been non-specific may be illustrated by the fact that he found "hypersensitiveness" to non-hemolytic streptococcal products marked among active cases of rheumatic infection (72 percent); these same patients reacted almost equally well (67 percent) with filtrates boiled for four hours. Crowe (82) implies that the allergen must, perforce, be a whole viable bacterium in his statement that "...if rheumatic joints are allergic in character, the tissues of those joints have got to be sensitized to start with, which seems to be impossible unless they have been in contact with the living active germ." In contradistinction, Appleton (83), in referring

to a "form of infection", believes that "some of the products of this dissolution, diffusing into the blood or lymph, may sensitize, in an allergic sense, various tissues of the body. A later liberation of dissolution products, diffusing from the focus, and reaching the already sensitized part, would call forth an allergic reaction."

Cooke (84), is of the opinion that "the absorption of bacterial substance or the products of their growth from areas of acute infection or from a chronic focus may produce any type of allergic disease...The organisms usually recognized in these cases are the streptococcus, staphylococcus, and the pneumococcus of all types. Other bacteria and viruses may be causes but are less frequent... Chronic foci of infection that are a source of continuous absorption of bacteria or their products are as great, if not greater, in importance than the chemicals mentioned [drugs and non-protein allergens], for they act not only as sensitizer but as the underlying cause of many of the inflammatory and degenerative diseases of man...Practically every site in the rabbit has been tested and these investigations have shown that the same type of reaction can be elicited in any part of the animal body by direct injection of the antigen. Inasmuch as inflammation is a process in which primarily the connective tissue - vascular system is involved, one may conclude that this system, the mesenchyme of the body, is sensitized, or in other words is a shock organ." Cooke throws further light on the question: " How much allergen is required to produce an allergic manifestation?" Apparently infinitesimal quantities may suffice in hyperallergic individuals, for

he states that eggs are the most common provocative food substance of allergic manifestations. "Nausea, vomiting and asthma from the odor of an unbroken egg [may occur] or sudden urticaria from handling one." In an instance where the skin was manifestly sensitized, Cooke relates, "any focal infection, however, may be a cause, especially a suppurative one." He cites the following: "I have known a persistent urticaria to disappear after the removal of a single tooth with a small granuloma at the apex." Yet he also has observed that intercurrent infection by the Streptococcus hemolyticus does not cause an exacerbation of rheumatoid arthritis, but states that "regarding solely the sensitization aspects, good evidence has been produced experimentally and clinically which indicates allergy is an important factor in the pathogenesis of diseases due to streptococci." In his immediately previous statement he was, of course, referring to the rash of scarlet fever and the unique dermatologic lesion - erysipelas.

Clawson (85), using streptococci, believed that he was able to distinguish allergy from resistance (immunity) and he concluded from his experiments that allergy bears no relation to general resistance, and that it seems to be a useless and at times harmful concomitant phenomenon. Swift and Derick (86) found that focal lesions were necessary to incite the allergic state, which could then be maintained by small doses of non-hemolytic streptococci that had no effect on non-allergic rabbits. One of the significant conclusions from their study of rheumatic fever is the fact that there is no specific agent or even

a particular strain of streptococci that plays an etiologic role. This, according to Swift and Derick, supports the theory of allergy for it explains how many different types of streptococci may all induce the same clinical and pathologic disease which is a feature quite common to all allergy.

What a certain group of investigators has called "toxins" another group has called "allergens". If such a substance does exist in certain diseased pulps of teeth there should exist a precursory material from which these substances may originate. The pulps of teeth are rich in protein elements as found in the collagenous connective tissue, vascular tissue, nervous tissue, and the special cells - the odontoblasts. Assuming for the time being, only bacterial intervention in the elaboration of a unique substance, we must admit that the substrate, i.e., the pulp, is an adequate precursor. Hedström (87) points out a common clinical observation, namely, that "in the tooth-pulp the connective tissue cells probably die before the nerve cells, a phenomenon very likely observed by every practitioner who has noticed conductivity of the nerves to remain long since disintegration of other pulpar tissues has taken place...On microscopic examination of a gangrenous pulp, not yet completely disintegrated, we can recognize tissue elements, disintegrating hemoglobin, crystals of amino acids, triple phosphates and bacteria...The bacterial disintegration of proteins has in recent years been subject to elaborate investigations and a great many of our most poisonous substances from the animal and vegetable kingdom arise therefrom...Bacteria are able to split amino acids into

severe poisons...Carboxylytic bacteria which first split the carboxyl radical and then deaminize give rise to highly toxic ptomaines...These toxamines are of different kinds...Their most prominent toxic effects, when introduced into the blood in non-lethal doses, are an increased blood pressure and a thickening of the arterial wall...where we have to deal with these severe poisons in gangrene of the pulp, these must be held responsible for the severe toxic symptoms sometimes occurring in this condition...It is not the bacteria...but rather the enzymes formed by them during their vital processes that give rise to disintegration of the tissue cells and to the production of substances of alkaloid nature which cause the toxicity in infections...The action of putrefactive bacteria on tryptophan gives rise to the formation of indol and skatole...Cadaverine [is derived] from lysine by decarboxylation...and putrescine from argenine...In the course of putrefaction amino-acids are often reduced to fatty acids...The highly toxic neurin, vinyltrimethylammoniumhydrate, is a dehydrate of the innocuous choline, and is obtained from the decomposition of blood, flesh, nerve, and brain substance...Ptomaines are obtained in putrefaction of fibrin by action of streptococci, also the putrefactive base, mydin, is produced in the putrefaction of bone in man...H_2S has long been known as a product of putrefaction...In certain concentrations it is even poisonous to micro-organisms which die off, when during the process of putrefaction the sulphuretted hydrogen has reached a sufficient quantity... Methyl mercaptan and other mercaptans are products of protein decomposition and are exceedingly offensive...These sulfur-containing products

of putrefaction originate from the sulfur-containing amino-acids, cystine and cystiene. Pharmacodynamically, they are considered the most severe kinds of poison...Bacteria thrive in traumatized tissues and chemically convert these substances (tissues and juices), chiefly albumins, to poisons which further cause the death of cells."

We cannot blithely dismiss the fact that there are some investigators who have maintained that periodontal disease may be more important in the causation of systemic disease than the various periapical involvements. Pollia (88) states: "The teeth must be considered from the standpoint of periodontoclasia or pyorrhea and periapical disease. I consider the former more important as a focus of infection because of the richness of the bacterial flora and the wide surface exposure, in spite of the fact that it also has free drainage." Antithetically, Beck (89) points out that "in certain parts of Russia, arthritis is very uncommon and yet pyorrhea is universal." In Comroe's experience, he believes that periapical disease may definitely be the origin of certain cases of arthritis, and that the frequency with which many diseases present infective foci "is greater than would be accounted for by a chance coincidence of the two diseases" (90). In fact, Comroe lists infected teeth as the most probable focus in the order of importance in rheumatoid arthritis. He does believe, however, that "periodontal disease (pyorrhea alveolaris) may be as important as periapical infection: there is no decisive evidence as to which is more important." As a rejoinder to his own doubt, Comroe reiterates: "The acute dental abscess is probably of little significance in chronic rheumatoid arthritis."

However, he maintains that the "dental granuloma may be an exciting or aggravating factor in rheumatoid arthritis." Haden (91) definitely asserts: "In my experience pyorrhea and such pockets are much less important than periapical infection as sources of systemic disease of focal origin." Price (92) maintains that "...individuals with a very marked susceptibility to rheumatic group lesions tend, in general, to be free from extensive periodontoclasia...When infected teeth produce disturbances in other parts of the body, it is not necessary that the quantity of infection be large, nor is it demonstrated that it is necessary that organisms pass throughout the body or to the special tissues involved, but the evidence at hand strongly suggests that soluble poisons may pass from the infected teeth to the blood circulation, or both, and produce systemic disturbance entirely out of proportion to the quantity of poison involved. The evidence indicates that this toxic substance may under certain circumstances sensitize the body or special tissues so that very small quantities of the organisms, which produce that toxin, may produce very marked reactions and disturbances." Price and co-workers maintained that they were "dealing with products in freshly extracted, infected teeth, which differ essentially from those developed by culturing the organisms from the same." He states: "We, accordingly, made determinations of the difference in the reactions when the organisms were removed from the washings, and found that in many instances that after passing the washings of the crushed tooth through a fine Berkefeld filter very similar disturbances were produced in animals even though the cultured filtrate developed no organisms. It was found that, whereas the organisms, when injected, tended definitely

and regularly to produce lesions in various organs and tissues, the filtrate produced disturbances of metabolism and nutrition, with its principal effects upon the digestive tract and nervous system. It is evident that we are dealing, in infected teeth, with a substance which has very profound effect in very small quantities. The substance is a very complicated one which does not lend itself readily to concentration." Using the filtrate of crushed extracted teeth he states: "In many of these cases we have found patients sensitized to the toxic substance as extracted from infected teeth of other individuals, and in other instances, evidences of a marked specificity to the infection of their own teeth. In cases where the toxin was extracted from the patient's own tooth, the test, when positive, developed more rapidly and usually more severely...The boiling or even autoclaving, of teeth does not always destroy all the toxic substance...I have shown the results of studies comparing the toxin of dental infections with histamine, and it was shown that there were some factors in common, one being the marked engorgement of the mesenteries, dilatation of the blood vessels of the small intestine, and one sensitized rabbit died within two minutes after receiving its infection [sic] . Further studies of this toxic substance indicated that it had some characteristics differing very distinctly from those of histamine." Hence, Price concludes that "infected teeth contain substances other than bacteria to which the individual may become sensitized and which substances may, in addition, have strong toxic properties." Those students, of the subject of diseased teeth in relation to systemic disease, who have

labored through the late Dr. Price's tremendous volumes, would immediately recognize his characteristic quasi-philosophic style of presenting his material, even in the absence of his name. Price, unfortunately, injected considerable personal bias into his experimental observations and colored his statements with sweeping assertions. As an allegedly wealthy man, he had his own private research laboratory and hired persons trained in the various basic sciences to work with him. Hence, from his writings, it is difficult to determine whether or not he actually made certain observations or whether or not they were those of his associates. His procedures were never very well defined, and the number of times a particular experiment may have been performed is in many instances unknown. Of particular concern to the critical investigator are his extremely dubious control procedures and, especially, the complete lack of them in the majority of his experiments. The author wishes to go on record as not intending to discredit, defame, or to speak irreverantly of Dr. Price or the results of his experimental observations. On the contrary, the author is delighted to have known this man, and to have attended a number of his lectures some fifteen or twenty years ago. Dr. Price was a distinguished gentleman, who believed in what he thought to be true, and it is not within the province of one mortal to judge another.

Nevertheless, it is conceded that the procedure of crushing a diseased tooth, making a washing of it, filtering this through a Berkefeld filter, and demonstrating skin reactivity therewith, is indeed a pioneer attempt to demonstrate a "toxic" or "allergic" factor. In his

method, Price did not define "diseased tooth", whether possessed of periodontal remnants, how long after extraction before the filtrate was made, how greatly diluted, and the method of filtration other than the fact that a Berkefeld filter was used. His skin reactions were apparently not controlled with either physiologic saline solution, or crushed normal tooth filtrate. The method is fraught with considerable error to the extent that it may be said that this experiment does not unequivocally support the contention that "toxins" or "allergens", if actually present, are formed in or around the tooth while in situ.

This review of the literature constitutes only a small fraction of all that has been written on the subject. Essentially, the articles most germane to the development of the present investigation have been incorporated. Some works have been included to point out various discrepancies and contentious aspects in this field. Others, and even a few virtually ludicrous statements, have been presented in an attempt to develop a tolerably clear and unbiased picture of how, through the years, various workers thought, how they experimented, what they accomplished, and what deductions they made. The author has interjected comments only where necessary in order to bridge certain gaps in the sequence of presentation and to elaborate on certain facts, pertinent and incidental, which were not otherwise overtly expressed.

In reflecting on the panorama of the various and numerous investigations undertaken in this field, it appears particularly important to demonstrate first, by precise and controlled methods, whether or not some demonstrable "toxin" or "allergen" is actually formed in pulp-involved

teeth *in situ*. Secondly, if such a substance is demonstrable, whether or not particularly rheumatoid arthritic patients are more sensitive to it than are normal controls. To determine how this may best be accomplished a revaluation of certain aspects of the problem must be initially undertaken. There is no doubt that radiographically negative teeth may be infected (93). Contingent upon this, no tooth can be assumed to be infected unless a bacteriologic culture is made, under aseptic conditions, from the tooth *in situ*. Apparently, the possibility of demonstrating a transient bacteremia occurring within a normal tooth is rather uncommon (94). It is well known that the improvement that may be expected, particularly in rheumatoid arthritis, upon the extraction of a pulp-involved tooth, is quite the exception. Comroe (95) believes that this could be due to "additional foci present in the body" or that "the disease may be of such long standing that the foci are no longer actively concerned in the further course of arthritis." "Bacterial toxins", he maintains, could be carried from foci to areas of lowered resistance; or the protein fraction of the bacteria could act as an antigen and sensitize tissues such as synovial membrane, so the further additional bacteria or protein coming into contact with the synovial tissue could produce an allergic reaction."

We may, at this point, ask ourselves: "Is there any link of evidence, relative to rheumatoid arthritis, that would indicate its being of an allergic nature?" Like certain allergies, it may be slow or abrupt in its onset, and there are periods of remission and those of exacerbation. Both exhibit heightened physiologic activity at the sensitized site, viz.,

some degree of the cardinal symptoms. However, such analogies may be wholly fortuitous. Nevertheless, the recent findings of Campbell (96) may explain why certain rheumatoid arthritic patients are substantially benefitted when placed on sustained salicylate therapy. Campbell found that "the animals which were premedicated with acetylsalicylic acid showed striking protection against anaphylactic shock...There is nothing to indicate any protective effect against histamine shock...It is interesting to note that the effects of the drug were temporary and that the treated animals showed considerable hypersensitivity 3 weeks later... Benadryl gave good protection, in similar experiments, against histamine shock but was without effect on anaphylactic shock. The present drug acetylsalicylic acid, however, effectively protects against anaphylactic shock but not against histamine. It is, with the other salicylates, apparently a true antianaphylactic drug in that it interferes with the antigen-antibody reactions to prevent or decrease the untoward results of the challenging dose of antigen." If this should in any way support an allergic hypothesis, it is equally necessary to investigate any findings which may disprove it.

In 1925, Hench (97) spoke of two types of chronic non-specific infectious arthritis. One he stated was metabolic and one he maintained was infective. He quoted Haden to the effect that many infected teeth may be negative radiographically and advocated the removal, therefore, of all devital teeth as a prophylactic measure in the presence of arthritis. Recently, Hench, et al. (98), found that the administration of a hormone of the adrenal cortex (more recently called "Cortisone") to rheumatoid

arthritic patients caused considerable improvement in the clinical features of the disease and in the blood sedimentation rates in a period of only a few days. However, when the administration of the hormone was withheld, a complete relapse promptly ensued. These investigators state that "even though the pathologic anatomy of rheumatoid arthritis is more or less irreversible, the pathologic physiology of the disease is potentially reversible, sometimes dramatically so. Within every rheumatoid patient corrective forces lie dormant, awaiting proper stimulation. The inherent reversibility of rheumatoid arthritis is activated more effectively by the intercurrence of jaundice or pregnancy than by any other condition or agent thus far known." As remissions also followed the use of general anesthetics, which stimulate the adrenal cortices, he believed that some substance therein was possibly involved. They maintained that the substance or agent effecting relief "could be neither hyperbilirubinemia nor a unisexual (female) hormone since neither of these is common to both pregnancy and jaundice...It became increasingly difficult to harmonize the microbic theory of the origin of rheumatoid arthritis with the phenomenon of relief of the disease by jaundice or pregnancy." Despite the fact that the lack of some endocrinologic factor may be the immediate cause of rheumatoid arthritis, the etiology may rest in what they believe to be "some basic biochemical disturbance".

Obviously, the question ultimately to be answered is: "What brings about this basic biochemical disturbance?" There can now be little doubt that, from a dental point of view, the most propitious course to

follow is first to demonstrate that a toxic or allergenic substance is actually formed in pulp-involved teeth. If these substances can be obtained from diseased teeth it seems that the best way to demonstrate their presence is by the use of skin tests. Williams (99) and her co-workers have shown that "the skin test properly controlled has a wide application in demonstrating the presence of exotoxins of hemolytic streptococci...There is no question but that a hypersensitiveness may be developed by the activities of several kinds of streptococci and together with the many important contributing causes may explain largely phases of the condition known under the general term 'rheumatic state' which includes rheumatic fever and chronic infectious [rheumatoid?] arthritis." Holman (100) cites the results of skin tests to nucleoprotein fractions of streptococci which were positive in 70 percent of rheumatoid arthritic patients tested, as compared with a 44 percent positivity among normal controls. This, it was believed, constituted an expression of allergy to the nucleoproteins of hemolytic streptococci but there was no correlation between the positive skin test and the presence of agglutinin in the blood serum. In 1934, Griffth (101) reported on a series of 137 rheumatoid arthritic patients in which intracutaneous tests with ground-up hemolytic streptococci were negative in acute cases, positive in "stable" cases, and either positive or negative in the remitting types. In 1935, Wainwright (102) mentions that he found streptococci in the blood of only one rheumatoid arthritic patient in "many attempts". He also states that "this lack of uniformity in the results obtained by different observers leaves us in doubt about the frequency with which

streptococci occur in the blood stream of patients with chronic rheumatoid arthritis, and makes it impossible conclusively to maintain that the joint reaction in this disease is a focal or metastatic lesion. This does not mean that the streptococcus plays no part in the pathogenesis of the disease or even that it may not be the chief cause, but merely puts in question the manner in which the joint reaction is produced." He was forced to draw these conclusions by virtue of the fact that, using a saline solution of heat-killed hemolytic streptococci, he found positive skin reactions, in rheumatoid arthritic patients, in as high a proportion as 90 percent, whereas only 10 percent reacted positively to a saline solution of Streptococcus viridans. Antithetically, McKenzie and Hangar (103) "failed to find any relationship between skin reactions obtained with filtrates and extracts of hemolytic and non-hemolytic streptococci and any disease process." Comroe (104) seriously questions the validity of the high percentages of positive skin tests reported by various investigators as occurring in rheumatoid arthritic patients, and states: "The pathologic changes are so different from those of specific infectious arthritis that one wonders how any known bacterial agent could produce it. The chronicity of rheumatoid arthritis is very unlike any specific infectious process."

From the foregoing it is apparent that any test made with pathologic pulp substance must be made with an extract prepared from all of it. In this way bacterial protein, bacterial products, tissue degradation products - one, two, or all three, should appear in the extract. It seems as if the intracutaneous test should readily lend itself to such a proce-

dure. The mere fact that it has, up to the present, not been found particularly helpful may simply indicate that the specific reactive substance has not been found. Quite recently an abbreviated report (105) has come from Czechoslovakia to the effect that "it was proved that all cases of actual dental focal infection have a positive skin test with the streptococci alpha and gamma from the dental foci. From the result of the desensitization and immunization, used therapeutically, it became evident that allergy plays a part in focal infection and that the streptococci alpha and gamma are responsible for dental focal infection in most cases." No details on this procedure are available and it cannot be evaluated.

If a skin test is employed in any investigation there are many rather abstruse factors that must be considered. Allergy has been defined (106) as "a phenomenon of altered reaction initiated by first contact with a substance and manifested after an interval (period of incubation) upon further contact with the original or an identical substance." It is also maintained that the skin-sensitizing antibodies are those antibodies bound to the cells of the skin making possible the skin test as an indicator of allergy. Even secondary protein derivatives may be reactive, as Cooke (107) demonstrated a positive skin test to the proteoses of the whey of cows' milk but not to milk, per se. Furcolow (108) has recently advanced an interesting theory that cannot be ignored when performing skin tests upon any group of individuals who are ill. Using tuberculin and histoplasmin, he finds that critical illness may exert a depressing effect on skin sensitivity, and that the depressing effect on skin sensitivity,

in critical illness, becomes more marked with advancing age. He found that about 40 percent of those insensitive when critically ill, reacted positively to skin tests again after they began to improve. He maintains that the evidence suggests that the depression is nonspecific with respect to the antigens used and to the cause of illness and that these results call into question the value of any type of skin test on persons critically ill from any cause. Such a phenomenon may indeed be a serious deterrent to any skin tests performed, especially on rheumatoid arthritic patients. Kahn and his co-workers (109), on the other hand, believe that "the capability for positive skin reactions is one of the most permanent responses in immunity and is, in our opinion, not dependent upon circulating antibodies, but is based upon inherent immunologic changes within tissue cells."

Walzer (110) takes a rather optimistic view of the general applicability of the skin test. He states: "However, the fact that skin testing has not turned out to be a simple and completely reliable technic does not detract from the fact that when it is intelligently and skillfully performed, it remains the most effective diagnostic procedure in reaginic allergic disorders." The skin is not ordinarily thought of as being a very complex structure, however, as Cooke (111) points out, it has been "estimated to constitute nearly 15 per cent of the total body weight, and is as vital to life and well-being as any other principle organs of the body...Evidence of the action and reactions of the skin is seen in the response that may be obtained to tuberculin, brucellergin, typhoidin, trichophytin and other fungus preparations, and to the polysac-

charide and proteins of the pneumococcus. Such specific skin reactions occurring in persons previously infected indicate the biocellular activity of the skin even though no skin lesion is apparent as part of the disease."

Because of the welter of fact and fancy with which the literature is amazingly replete, the author reiterates the importance of first demohstrating whether or not a toxic or allergic substance is formed within pulp-involved teeth and secondly, should such a substance exist, that it be tested in an adequate group of rheumatoid arthritic patients and compared with substantial control groups.

METHODS

Evolution of method: It was incumbent upon the author to undertake certain preliminary investigations which were of an exploratory nature. The findings of these preliminary procedures had to be understood before it could be determined which methods could best be used in this investigation. Every attempt was made to reduce certain complex aspects of the problem to simpler situations rather than to become immediately involved in the total complexity. This, the author believes, is a wholesome approach, and is the very spirit and essence of the experimental method. The present method was not spontaneously conceived; it was assiduously evolved and fairly well perfected, by considerable preliminary animal experimentation, before it was carried out upon human beings. Although many of the early investigations and observations yielded important information, such findings will only be briefly incorporated into the discussion where it appears to serve a purpose. In this way, it is believed, the present procedure may be clearly and precisely stated without being encumbered by various technical minutiae.

Source of pulp material: Pulp material, from both normal and pulp-involved teeth, was obtained from patients presenting themselves for treatment at the School of Dentistry at the University of Michigan. The normal pulps were ordinarily obtained from those patients who were to have had normal teeth extracted antecedent to orthodontic, prosthetic, or operative procedures. Only those pulp-involved teeth were selected which, reasonably, did not show evidence of having been contaminated by the bacteria in the saliva. This restricted the choice of pulp-involved

teeth to those which had succumbed to disease, primarily, as the result of the irritation of moderately deep caries or certain restorations. Also in this group are those pulps which have undergone pathologic change incident to rampant erosion, traumatogenic occlusion, exogenous trauma, and excessively rapid orthodontic movement. Fig. 1 illustrates the type of pulp-involved tooth ordinarily chosen. However, the periapical lesion in this case is quite extensive but the considerable improvement as seen in Fig. 2 attests to the importance of investigating the pathologic contents of a pulp canal that play an extremely important part in causing such a degree of destruction.

Procedure for obtaining specimen from pulp canal: Anterior teeth are preferably employed in this investigation as they permit the use of long-handle (type D) files and reamers which constitute a greater assurance of aseptic technique when removing the specimen from these instruments. After having made a radiographic and clinical diagnosis, the pulp-involved tooth is isolated by the use of a rubber dam (Fig. 3). The tooth and the entire operative field is liberally swabbed with "Tincture of Mercresin". This is allowed to dry before the conventional operative approach, by the use of sterile burs, is made. Unlike many investigations in the past, this procedure enables one to carry out a strictly aseptic technique, and to obtain the specimen from a tooth remaining in situ. A size one to three reamer is ordinarily the first instrument employed irrespective of the nature of canal contents. The radiograph will afford sufficient information as to the size that may be most efficiently and should be initially employed. In the event that the pulp should be normal

- Note -

All succeeding figures and tables follow, in immediate sequence, the first reference thereto in the context.

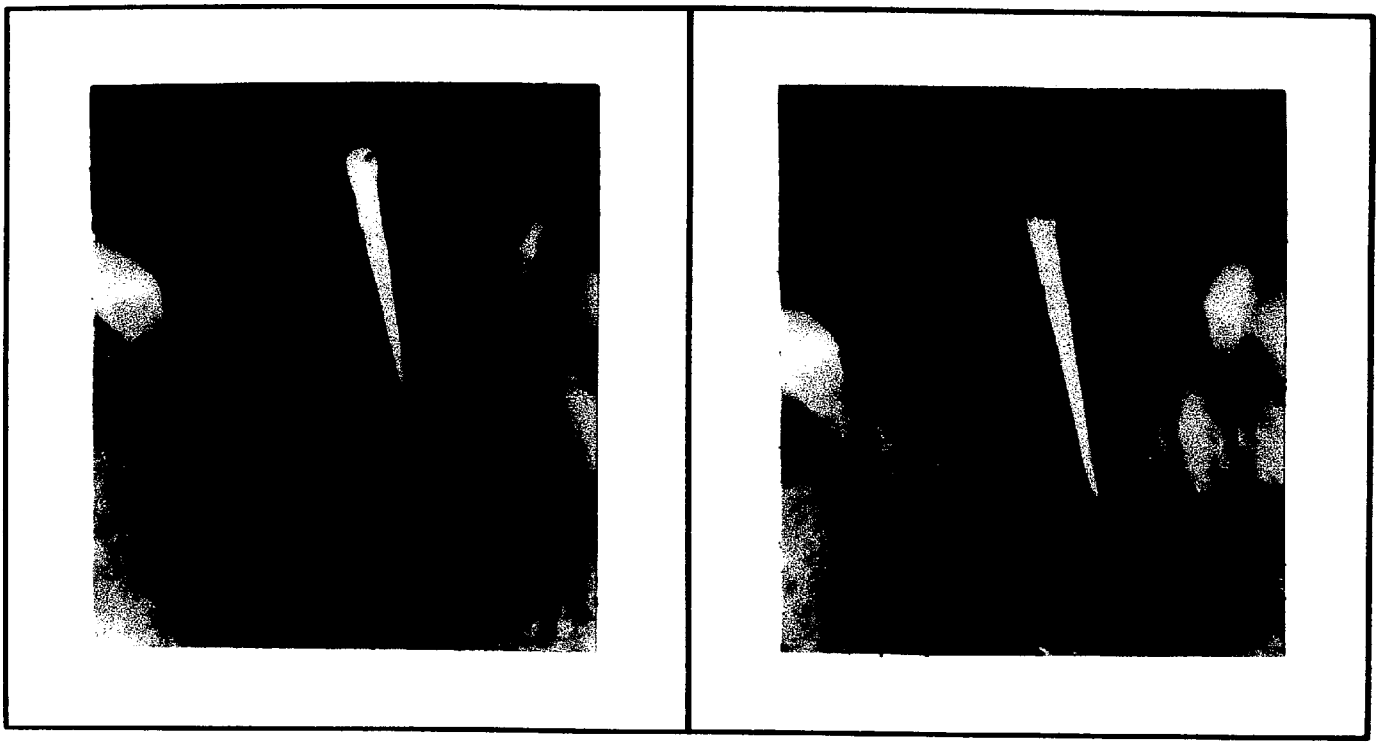

Fig. 1. (left) Radiograph showing the mandibular right central incisor of a patient (R.K.) with extensive periapical destruction originally due to exogenous trauma. A conservative method of treatment was employed because both adjacent teeth were vital. The left central incisor showed a slightly reduced vitalometric response and in this tooth partial calcific obliteration of the pulp canal is evident. The right lateral incisor shows evidence of apical pachymenia although giving a normal vitalometric response. All three teeth within the area of pathological involvement are hypermobile.

Fig. 2. (right) With only complete obturation of the root canal effected, and without any other surgical intervention, the degree of regeneration of the alveolar bone is quite marked in this radiograph taken after a period of 18 months. The lamina dura is partially reconstituted and there is evidence of a faint trabecular pattern throughout the residual demineralized area.

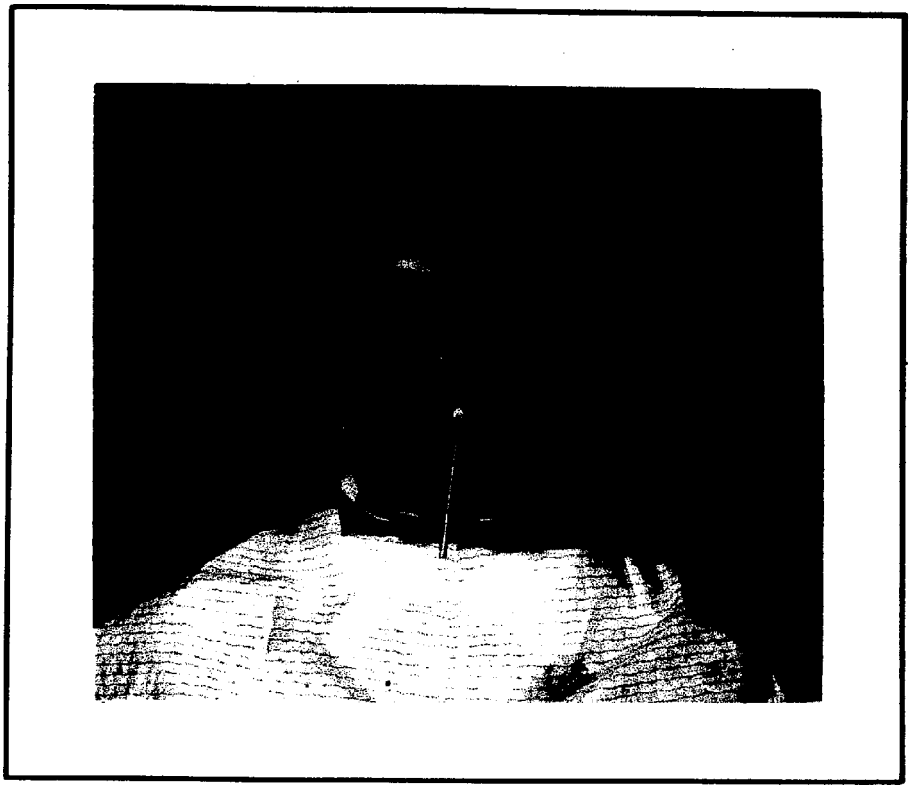

Fig. 3. Photograph showing the maxillary left central incisor isolated by means of the rubber dam. Only the smallest holes practicable are used. The two adjacent teeth are included only if their inclusion facilitates the placement of the dam. In this instance the anterior saddle clamp was sufficiently retentive and the retracting bicuspid clamps were not employed. The rubber dam is brought out slightly over the nasal septum to divert the currents of air, due to nasal respiration, from the field of operation. A brisk jet of air is directed upon the teeth to effect complete surface dehydration before liberally flooding the teeth and the clamp with "Tincture of Mercresin". Then in a centrifugal direction from the isolated teeth, the remainder of the field of operation (as delineated by the rubber dam frame) is also swabbed with the disinfectant. The operative approach is not begun for at least three minutes or until the disinfectant has dried upon the teeth and rubber dam.

or possess fairly good structural integrity, a reamer may be "spiraled" through it. The reamer should be just large enough to bind at the apical constriction to assure cutting off the pulp at this point and to avoid the propulsion of the instrument beyond the confines of the root canal (Fig. 4). This is especially important to observe with pulps of this character as the use of a local anesthetic is frequently required for their extirpation. It is desirable therefore to remove as much of the pulp as possible in a single deft insertion and "twist" of the instrument. Should hemorrhage be provoked from the apical foramen, and this blood obtained, there is some evidence that perhaps the vasoconstrictor (epinephrine) within the procaine may appear therein. Such a specimen may be unsuitable to use in the subsequent skin tests as a trace of epinephrine may produce a mildly blanched reaction and slightly mask the true reaction of the specimen. This was demonstrable in a few instances by "split sample" analysis, e.g., post-extirpation blood, in an adequate quantity, was used as a specimen. Merely using ordinary precaution this effect should never occur.

In the nonvascular degenerated pulps, or those pulp canals filled with liquefied, purulent, or seropurulent matter, as much material as possible is ordinarily obtained. However, when extirpating as much material from these avascular pulps as possible, the first instrument showing the slightest tinge of fresh blood is not included in the specimen. If suppuration ensues from a canal, a large file or reamer is quickly inserted into the purulent material in the canal about two or three times and the amount adhering to the instrument is used as the specimen. This

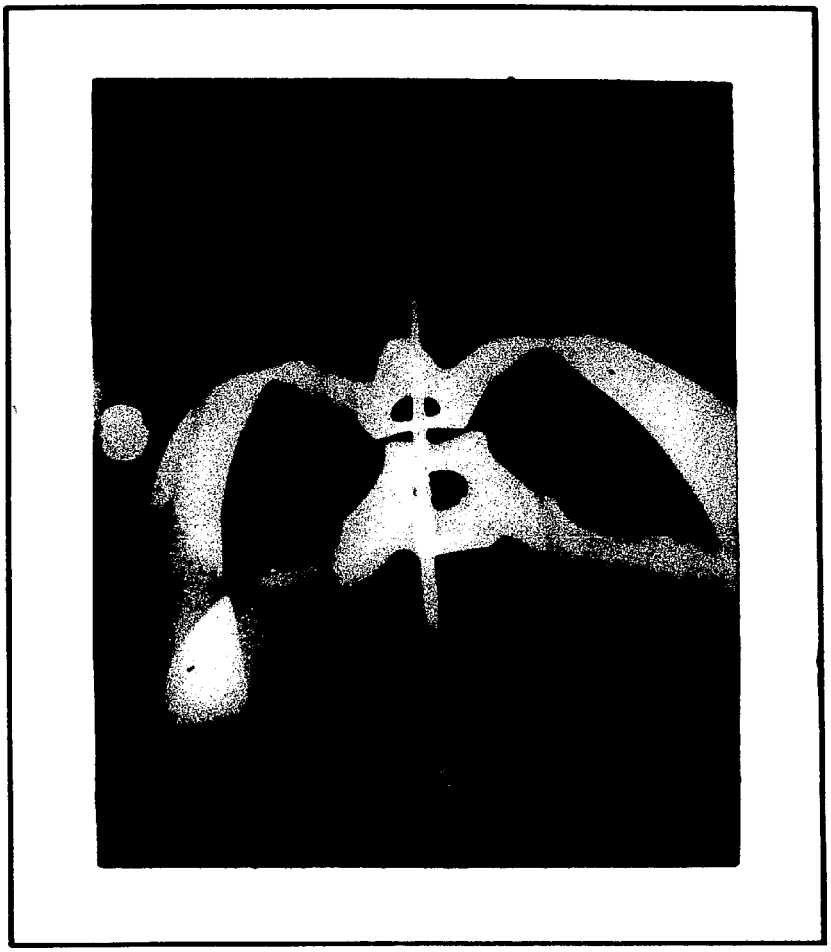

Fig. 4. Radiograph of maxillary left central incisor of patient shown in Fig. 3. The patient, aged 24, sustained exogenous trauma at 17 years of age. Two weeks prior to opening into the tooth, it was painful to heat only. At the time of operation it was found to be completely non-vital, and to be slightly discolored; otherwise it was asymptomatic. Upon extirpation, the pulp appeared grossly normal although somewhat ischemic. No detectable putrescent odor was evolved from the pulp. The apical resorption of the anterior teeth is due to concurrent orthodontic intervention.

amount of specimen is usually quite adequate because of high viscosity of the purulent matter. Taking more than this amount will not be representative of that substance which was present in the root canal but rather that purulent matter which was within the periapical region. In the various conditions encountered, the foregoing criteria are most essential. In observing, primarily, these criteria, whatever additional material may be obtained by the use of files from the walls of the pulp canals is especially important to include (Fig. 4). In any event, as much material must be obtained from the apical third of the root as possible. The removal of an excessive amount of dentin should be avoided for reasons later to be explained.

Essential data obtained before and during pulp extirpation:

A limited general history of the patient is obtained. This includes all pertinent information relative to the patient's past and present state of health that he is able to reliably state. The dental history includes a complete radiographic and clinical examination of all teeth. In the event that a pulp-involved tooth is disclosed, an inquiry into any past or present symptoms is made. The apparent etiology of the condition is recorded and it is tested vitalometrically or by thermal tests as required. Percussion of the tooth and deep palpation of the overlying gingival tissues is performed. The mucosa is minutely examined for the evidence of a healed fistulous bleb, one about to rupture, or a frankly draining sinus. The radiographic diagnosis is reduced to four simple categories to facilitate the analysis of the data, i.e., (1) normal periodontal membrane, (2) rarefying osteitis, (3) apical pachymenia (thickening of the periodontal

membrane at the apex), and (4) cysts. Upon extirpation of the pulp, the following data are recorded: (1) the gross appearance of the pulp or the pulp canal contents, (2) whether or not the amount of specimen procured was scant or adequate, and (3) whether or not a putrescent odor was evolved.

Preparation of specimen suspension: The aseptic technique, as used by Sommer and Crowley (110), originally intended for obtaining cultures through the pulp canal, with the tooth in situ, is precisely followed up to the point of operatively exposing the coronal portion of the pulp. The pulp or pulp canal contents are then removed as previously indicated. After each insertion of a reamer or file into the root canal (or canals) the instrument is energetically shaken into a special micro-tube (Fig. 5), containing 0.5 ml of sterile physiologic saline solution. The vaccine cap, which served as the base supporting the micro-tube, is then aseptically placed over the mouth of the tube. Four micro-tubes and caps may be autoclaved in a sterilizing tray, then set in upright position in the tray, and each filled with the saline solution using a hypodermic syringe. In this way four specimens may be expeditiously obtained from a single patient or one or two each from different patients. Irrespective of the character of the pulp material, it is removed from a reamer more readily than a file when shaken within the saline solution in the micro-tube. Normal pulps, or those exhibiting fair structural integrity, should be crushed against the wall of the micro-tube thereby facilitating the extraction of as much soluble substance within the pulp material as possible.

Fig. 5. Showing the preparation of the suspension. The procedure, as shown, is carried out in a sterile assembly (vaccine cap, which serves as the base, and the micro-tube) within the folds of a sterile towel. Only 0.5 ml of sterile physiologic saline solution is placed in the micro-tube which has a volume of approximately 0.7 ml. Immediately, upon completion of this procedure, the vaccine cap, which served as the base, is securely placed over the mouth of the micro-tube. This capping procedure may be readily performed with the aid of sterile forceps.

The extraction of substances soluble in physiologic saline solution: This is performed by placing the capped micro-tube within the capsule arms of a dental amalgamator (Fig. 6). If the physical character of the pulp canal specimen is readily lyophilic and adequate in amount, this apparatus need be operated for only a minute. If the nature of the pulp canal specimen is readily lyophilic but inadequate in amount, it should be operated for three minutes. Normal pulps and those showing fair structural integrity should also be mechanically agitated for a period of three minutes. Vascular pulp material will show a complete solution of hemoglobin with only white fibrous material remaining undissolved. The greater the color intensity of hemoglobin in the agitated suspension, the greater will be the amount of supernatant froth observed when holding the micro-tube vertically with the vaccine cap down. All agitated suspensions appear quite uniformly homogeneous with the exception of the insoluble fibrous material and unsuspendible dentinal debris.

Sequence of laboratory procedure for each specimen; 1. Bacteriologic methods: The first procedure undertaken in the laboratory is the making of a bacteriologic culture from the suspension (Fig. 7). A loopful of the suspension is removed from the micro-tube and is deeply inoculated into a single tube of culture medium.* In addition, a loopful of the suspension is routinely inoculated upon one-half of a blood agar

* Approximate composition:
0.1% agar
0.2% glucose
5.0% ascitic fluid
94.7% fresh beef infusion broth
Final pH approximately 7.40

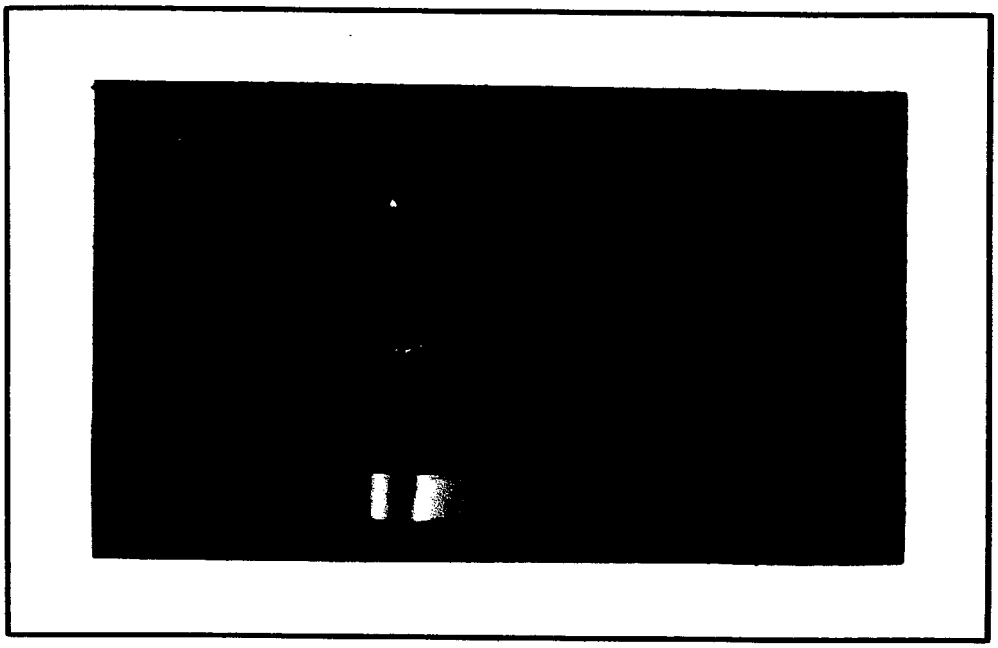

Fig. 6. The efficient extraction, from the pulp canal specimens, of the substances which are soluble in physiologic saline solution, is performed by vigorous mechanical agitation by the use of a dental amalgamator as shown. The arms of this standard device were slightly expanded and modified to accommodate the necessary length of the cap-tube assembly. The approximate 0.2 cc of air space in the micro-tube assures a very thorough mixture and extraction to be effected. The timer facilitates operating the device for periods of one or three minutes depending upon the nature of the pulp canal specimen.

Fig. 7. A standard loop may be used to obtain an adequate inoculum from the suspension in the micro-tube. The cap which sealed the tube may again be used to serve as a base for supporting the tube. Capping and uncapping requires the use of sterile forceps for its aseptic performance. At no time has it been found advisable or necessary throughout the total procedure to flame the micro-tube if all other precautions against contamination are strictly observed.

plate. These are incubated for not less than a week during which time they are observed for growth. With the advent of a positive culture in only the liquid medium, these bacteria are then subcultured upon blood agar plates as required. One culture is then incubated anaerobically, especially for the determination of anaerobic streptococci and actinomyces. Other standard procedures are incorporated into the routine determinative sequence as required. Ordinarily, the streptococci, and certain infrequently encountered bacteria, are carried out to a restricted point of classification. All streptococci tentatively classified as to the alpha and gamma varieties were subcultured on blood agar slants. After adequate growth, these organisms were suspended in about 1 ml of physiological saline solution and preserved by deep freezing. In this way they would be available for further identification if deemed advisable.

2. The suspension smear: A routine smear is made from a loopful of the suspension (Fig. 8). This is allowed to dry naturally and is then fixed in the flame of the Bunsen burner. The suspension smear serves as an index as to whether or not non-viable bacteria are present in the event that the cultures are negative. The smear is Gram-stained and only a morphologic clue is given as to the possible presence of bacteria.

3. The determination of the pH of the suspension: This is carried out by the use of a special micro-beaker in conjunction with a Beckman pH meter (113). This method enables a quite reliable pH determination to be made by the use of only 0.1 to 0.2 ml of specimen (Fig. 9).

Fig. 8. Showing the routine method of making a suspension smear. The patient's name or initials and the tooth number, from which the specimen was obtained, is marked on the slide. This scheme of designation is carried through on all the equipment used in conjunction with a single specimen. A smear made after thoroughly shaking the micro-tube assembly by hand and another after mechanical agitation are much different. The latter is smooth and homogeneous; long chains of streptococci, if present, are noticeably fragmented.

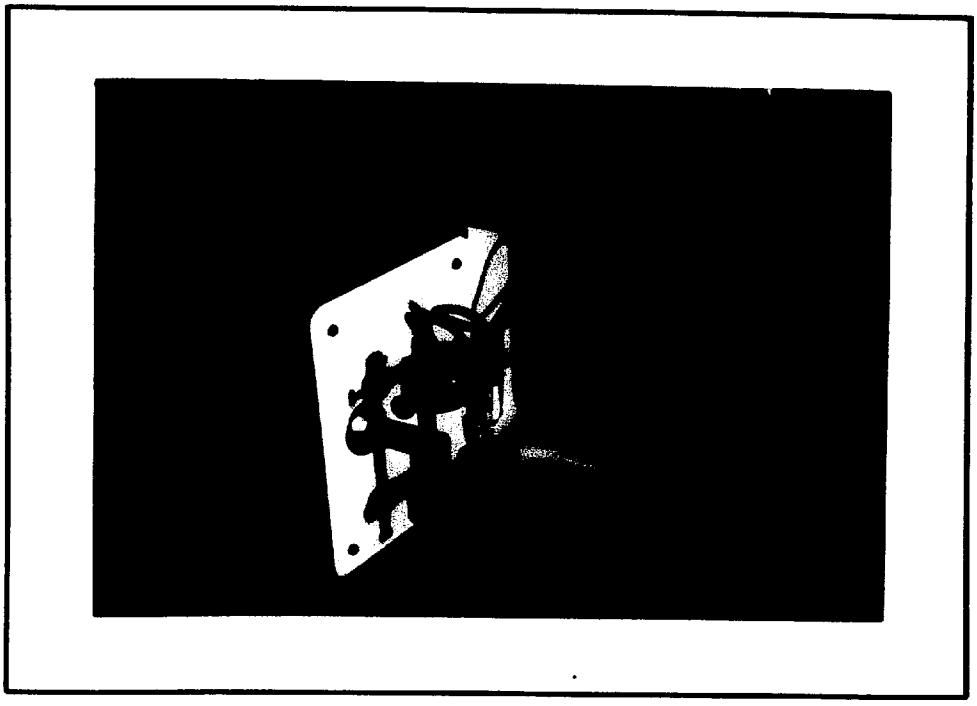

Fig. 9. The use of the micro-beaker in conjunction with the Beckman pH meter (model G) for the determination of the pH of a small quantity of the specimen suspension. The asymmetry potential is now believed to be more accurately corrected by the conventional use of the standard 5 ml beaker first. A pH 7.0 buffer solution is preferably used. In addition, the use of certain chemicals upon the micro-beaker, or permitting it to become dehydrated between use, will lead to inaccuracies in reading. When not in use, it is suggested that the micro-beaker be maintained in a fresh pH 7.0 buffer solution; also, that it merely be rinsed thoroughly with distilled water and dried with a soft cleansing tissue before use. This method assures an accuracy to within 0.05 of a pH unit.

4. The preparation of the specimen filtrate: The amount of suspension remaining is reconstituted to its original volume of 0.5 ml. This suspension is then breech-loaded into a 5 cc Luer-Lok syringe having a micro-Seitz filter adapter attached. This sterile assembly is first placed within the mouth of a sterile 5 cc bottle (Fig. 10). Exactly 0.5 ml of sterile physiologic saline solution is again added to the micro-tube, it is also once again capped, and thoroughly shaken by hand. This volume is also added to the Luer syringe thereby bringing the volume up to 1 ml. Filtration is then slowly effected. When filtration is complete, the syringe is removed, the neck of the filtrate is bottle flamed, and a long (00) sterile rubber stopper is placed therein.

Clinical procedure: the intracutaneous test: Using the filtrates prepared from the specimens obtained from the pulp canals of normal and pulp-involved teeth, at least 6 intracutaneous tests are performed with each specimen. A normal physiologic saline solution, or normal pulp filtrate control, or both, are routinely employed. Because of the very substantial positive skin reactions obtained in some of the earlier cases, using 0.1 ml of filtrate, this quantity was, for reasons of economy, reduced to 0.025 ml. All injections are made into a glabrous area on the volar surface of the forearm (Fig. 11). After a period of 15 to 20 minutes the skin reaction is read. By this time any pseudo-reaction on the part of an equal quantity of a physiologic saline solution control has disappeared (Fig. 12). The criteria, as established in this investigation, for a skin reaction to be considered positive, are

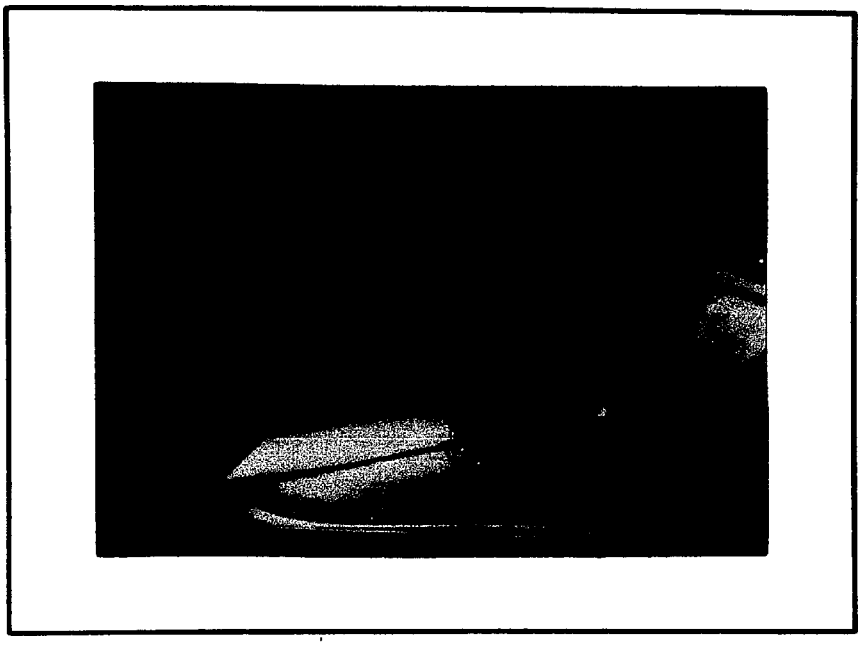

Fig. 10. Filtration of the specimen suspension by the use of the micro-Seitz (Swinney) syringe. A well fitting plunger in the Luer syringe is essential for its efficient function. The micro-Seitz syringes are preferably autoclaved just an hour or two before use. It may be observed that, during filtration, an air space is maintained between the surface of the suspension and the face of the plunger. In this way a maximum yield of filtrate may be expected. Ordinarily, there is about a 20% loss of volume incident to filtration with the resulting volume of filtrate being approximately 0.8 ml. Filtration should not be hastened; two to three minutes usually being required. When the last drop is about to be expelled from the syringe, it is allowed to fall upon the unstreaked half of the blood agar plate. This constitutes the sterility control for the filtrate.

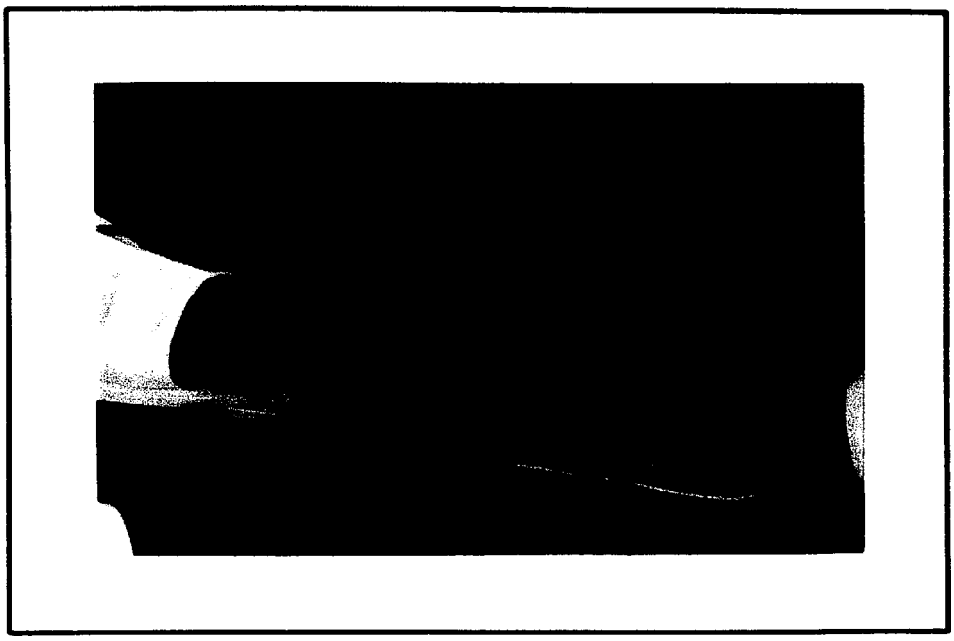

Fig. 11. The technique of performing the intracutaneous injection. The surface to be injected is thoroughly swabbed with propanol-2 and is permitted to dry before making the injection. A standard tuberculin syringe with a 5/8", 27-gauge needle is used. The injection is made slowly and carefully with only a minimum of trauma. Multiple injections should be spaced not less than two inches apart. Note the long (00) rubber stopper used in the small filtrate bottle which greatly facilitates the aseptic withdrawal of the filtrate.

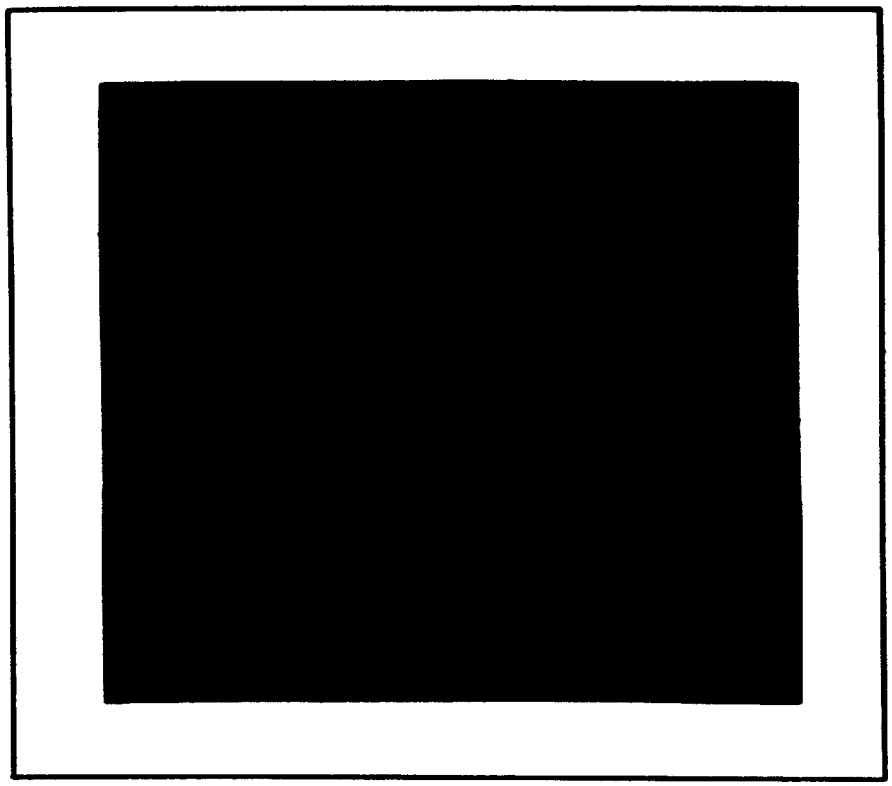

Fig. 12. A positive skin test (a), and a negative control(b), Any erythematous response on the part of the control must be discounted from the reaction of the filtrate. Normally, the reading is made within 15 to 20 minutes after injection or when any pseudo-response on the part of the control has faded. Centripetal fading usually begins within a half hour after injection and there may be peripheral stippling as shown here. A rather prominent wheal is evident around the "pinpoint" site of injection.

(1) the flare must be at least 10 mm greater in diameter than the control and (2) the intensity of erythema must be at least moderate.

EXPERIMENTAL

The primary purpose of this investigation is to determine whether or not skin reactivity occurs to pathologic specimens* obtained from the pulp canals of pulp-involved teeth and whether or not it is greater in certain groups of patients (particularly rheumatoid arthritic) than in other groups.

The Skin Tests.

Of the variety of cutaneous tests such as the patch (Vollmer), prick (punctiform), scratch (von Pirquet), or intracutaneous (Mantoux technique), the intracutaneous (intradermal) technique is the method of choice. The intracutaneous test may be more accurately controlled as to the quantity of material used and as to the exact site at which it is deposited. This test is especially applicable when using test materials that are only mildly physiologically active.

1. The Plan for Performing the Intracutaneous Tests.

The skin reactions among patients falling within the following categories were extensively investigated with filtrates prepared from the following materials:

A. Pathologic specimens from the pulp canals of patients with no known systemic disease.

B. Pathologic specimens from the pulp canals of rheumatoid arthritic patients.

C. Normal pulps from persons with no known dental or systemic disease.

* The term "pathologic specimen" is usually used rather than "pathologic pulp" as in the latter condition the pulp material is not infrequently completely absent as a result of the pulp having been replaced by an exudate or purulent matter. The simple use of the word "specimens" is intended to imply any material obtained from a root canal whether pathologic or normal.

Specimens obtained from the preceding groups were injected according to the following plan:

A. A pathologic specimen obtained from an individual with no known systemic disease, inject into -

1. Patient from whom pathologic specimen obtained.
2. One other patient with a pathologic pulp and with no known systemic disease.
3. Two rheumatoid arthritic patients (with or without pulp-involvements as available).
4. Two individuals with no known dental or systemic disease.

B. If pathologic specimen obtained from a rheumatoid arthritic patient, inject into -

1. Patient from whom pathologic specimen obtained.
2. One other rheumatoid arthritic patient (with or without pulp-involvement as available).
3. Two other patients with pathologic pulps and with no known systemic disease.
4. Two individuals with no known dental or systemic disease.

C. If normal pulp obtained from an individual with no known dental or systemic disease, inject into -

1. Patient from whom normal pulp obtained.
2. One other individual with no known dental or systemic disease.
3. Two patients with pathologic pulps and with no known systemic disease.
4. Two rheumatoid arthritic patients (with or without pulp-involvements as available).

The foregoing plan constitutes the basic pattern in the overall scheme of injections. It also constitutes the minimum number of injections (six) with any single filtrate. Should a sufficient amount of filtrate remain, this pattern may be expanded to include more injectees within any group when necessary. In this investigation the control pattern was occasionally broadened to the virtual limit consistent with the amount of filtrate available from any single specimen. This scheme proved to be especially helpful in ruling out any possible false positive reactions. Occasionally, it was found discreet to employ as many as 7 or 8 injections with a single filtrate rather than the basic six. As the situation demanded, the retest was either performed on the same subject or a third injectee was incorporated into a particular group. In the latter instance, "two out of three" were the deciding factors as to whether or not a few "borderline" reactions were positive or negative.

It is well recognized that the treacherous aspect of analyzing multiple data on skin reactions is even greater when we are dealing with a variety of unquantifiable, unknown, skin reactive substances. Consistent with the previously established criteria for a positive skin reaction, the requirements for two injectees within a group to be considered positive are as follows:

(1) Each injectee may demonstrate a moderate intensity of erythema 10 mm greater in diameter than the respective control.

(2) Should one injectee of a group be positive to the filtrate and the other completely negative, the positive reactor must demonstrate an area of erythema 20 mm greater in diameter than the combined reactions of the controls.

(3) Generally, the average of the reactions of the two filtrates must be at least 10 mm greater in diameter than the average of the diameters of the controls. In addition, a moderate degree of erythema is requisite in view of the fact that in the few instances in which normal pulp filtrates gave positive reactions the erythema varied from a light to moderate intensity and has never been found to be bright.

Minutiae of the patients' dental and systemic histories, as well as certain other details were found, after a thorough attempt to effect any positive or negative correlations, to be of no significance. This enabled the remaining pertinent data to be overtly expressed and reduced to a common denominator wherever possible. The essential data obtained from 65 pathologic specimens may be observed in Tables 1 and 2. Table 2 constitutes an extension of Table 1, the only difference being in the broader control pattern in the intracutaneous tests as indicated in Table 2. In the latter table all designations, as to "positive" or "negative", already constitute an average of the results of at least two injections. These are designated in conformity with the aforementioned criteria as to what constitutes a positive or negative reaction among a group.

In Table 1, column "a", under Intracutaneous Tests, the "homologous" designation refers to the injection of the patient from whom the pathologic specimen, the filtrate of which is used, was removed. Column "b" refers to a rheumatoid arthritic patient in which the same filtrate is used. Column "c" refers to patients with normal pulps (N.P.), and, it is to be understood, with no known systemic disease. In Table 2, column "a", under Intracutaneous Tests, the designation as to "positive" or "negative" refers to the result as determined by the average response upon the homologous patient and also upon another patient with

TABLE 1. PATHOLOGIC SPECIMENS FROM PULP CANALS OF PATIENTS WITH NO KNOWN SYSTEMIC DISEASES.

I	II	III	IV	V	VI	Intracutaneous Tests		
Patient	Radiographic Interpretation	Clinical Condition of Pulp	pH	Smear	Culture	[a] Hemolysins	[b] Rh. Arth.	Pt. with S.P. [c]
1. B.B./#8	Rarefying osteitis	Liquefaction necrosis, non-vital, fistula, no putrescent odor.	7.80	Neg.	Str. anhemolyticus	Pos.	Pos.	Neg.
2. V.Z./#9	" "	Purulent substance, non-vital, fistula, putrescent odor.	7.70	Str.	No growth	Pos.	Neg.	Neg.
3. M.M./#27	" "	Liquefaction necrosis, non-vital, fistula, putrescent odor.	----	Neg.	No growth	Neg.	Neg.	Neg.
4. M.R./#8	Radicular cyst	Purulent substance, non-vital, putrescent odor.	7.40	Variety of bacteria	No growth	Neg.	Neg.	Neg.
5. J.A./#8	Condensing osteitis	Degenerated pulp, non-vital, fistula, putrescent odor.	7.10	Str.	No growth	Neg.	Pos.	Neg.
6. B.B./#8	Rarefying osteitis	Degenerated pulp, non-vital, fistula, putrescent odor.	7.50	Str.	No growth	Neg.	Neg.	Neg.
7. R.B./#8	" "	Degenerated pulp, non-vital, putrescent odor.	7.50	Variety of bacteria	No growth	Pos.	Neg.	Neg.
8. S.B./#8	" "	Liquefaction necrosis, non-vital, putrescent odor.	7.40	Neg.	Lactobacilli Str. viridans	Pos.	Neg.	Neg.
9. C.B./#18	Normal periapical region, deep restoration.	Incipient pulpitis, hyperesthetic, normal appearance and vascularity.	7.30	Neg.	No growth	Neg.	Pos.	Neg.
10. R.B./#24	Rarefying osteitis	Liquefaction necrosis, non-vital, putrescent odor.	7.70	Neg.	No growth	Neg.	Neg.	Neg.
11. P.C./#6	Normal periapical region, imminent exposure.	Pulp appeared normal although quite fibrotic and avascular.	7.50	Str.	No growth	Pos.	Neg.	Neg.
12. M.C./#10	Rarefying osteitis	Liquefaction necrosis, non-vital, putrescent odor.	7.35	Str.	No growth	Pos.	Pos.	Neg.
13. F.D./#8	" "	Purulent pulp, non-vital, putrescent odor.	7.50	Str.	Str. hemolyticus	Pos.	Neg.	Neg.
14. E.K./#8	" "	Liquefaction necrosis, non-vital, putrescent odor.	7.70	Variety of bacteria	No growth	Neg.	Neg.	Neg.
15. E.K./#9	Normal periapical region, jacket crown close to pulp.	Incipient pulpitis, hyperesthetic, normal appearance and vascularity.	7.30	Variety of bacteria	Str. hemolyticus	Pos.	Pos.	Pos.
16. J.L./#24	Apical pachymenia	Decomposed pulp, non-vital, putrescent odor.	7.45	Str.	No growth	Neg.	Neg.	Neg.
17. M.L./#21	Rarefying osteitis	Partial liquefaction necrosis, hyperesthetic.	7.50	Neg.	No growth	Neg.	Neg.	Neg.
18. M.M./#27	" "	Degenerated pulp, non-vital fistula, putrescent odor.	7.50	Neg.	No growth	Neg.	Pos.	Neg.
19. D.M./#8	Normal periapical region, deep carious lesion.	Clinically normal appearing pulp, electively devitalized, hyperesthetic.	7.10	Variety of bacteria	Str. anhemolyticus	Neg.	Neg.	Pos.
20. D.M./#10	" "	" "	7.55	Variety of bacteria	Str. viridans	Neg.	Neg.	Neg.
21. A.M./#7	Minute rarefying osteitis	Non-vital otherwise pulp normal in appearance.	7.50	Neg.	No growth	Pos.	Neg.	Neg.
22. L.R./#6	Rarefying osteitis	Seropurulent pulp, non-vital, fistula, putrescent odor.	7.30	Variety of bacteria	Str. anhemolyticus (anaerobic)	Pos.	Pos.	Neg.
23. B.R./#9	Normal periapical region, imminent exposure.	Pulp passively hyperemic, hyperesthetic otherwise normal.	7.60	Neg.	No growth	Neg.	Neg.	Pos.
24. D.S./#10	Periapical cyst calcifically circumscribed.	Seropurulent material, non-vital, putrescent odor.	7.75	Str.	Str. anhemolyticus (anaerobic)	Pos.	Neg.	Neg.
25. L.W./#9	Normal periapical region, tooth extensively restored.	Pulp hypoesthetic otherwise clinically normal.	7.60	Neg.	Str. viridans	Neg.	Neg.	Neg.
26. M.W./#10	Normal periapical region	Exogenous trauma, crown discolored, papescent pulp, putrescent odor.	7.45	Str.	No growth	Pos.	Neg.	Neg.

TABLE 2. PATHOLOGIC SPECIMENS FROM PULP CANALS OF PATIENTS WITH NO KNOWN SYSTEMIC DISEASE.

I	II	III	IV	V	VI	Intracutaneous Tests (revised controls)		
Patient	Radiographic Interpretation	Clinical Condition of Pulp	pH	Smear	Culture	Ar. Hem.[a] + Pt. + P.P.	Ar. B.A.[b]	Pts. + B.P.[c]
27. P.P. #7	Rarefying osteitis	Liquefaction necrosis, non-vital, putrescent odor.	6.90	Neg.	Lactobacilli & gram pos. organisms	Neg.	Neg.	Neg.
28. M.T. #25	" "	Liquefaction necrosis, non-vital, putrescent odor.	7.00	Str.	No growth	Neg.	Neg.	Neg.
29. W.J. #8	Normal periapical region.	Crown discolored, non-vital, pappescent pulp having brown color.	7.00	Neg.	No growth	Neg.	Neg.	Neg.
30. G.K. #7	Rarefying osteitis	Liquefaction necrosis, non-vital, putrescent odor.	7.25	Neg.	No growth	Neg.	Neg.	Neg.
31. G.S. #7	" "	Liquefaction necrosis, non-vital, putrescent odor.	7.30	Neg.	Actinomyces	Neg.	Neg.	Neg.
32. D.A. #9	Apical pachymenia	Exogenous trauma, hyperesthetic, otherwise pulp appeared normal.	7.00	Neg.	Str. viridans	Pos.	Pos.	Neg.
33. D.A. #8	" "	Exogenous trauma, hyperesthetic, pulp sparse and avascular.	7.90	Neg.	No growth	Pos.	Neg.	Neg.
34. R.C. #8	Rarefying osteitis	Liquefaction necrosis, non-vital, fistula, putrescent odor.	7.30	Str. and variety	Str. anhemolyticus	Neg.	Pos.	Neg.
35. R.M. #7	Radicular cyst	Purulent material, non-vital, completely odorless.	6.90	Str.	No growth	Pos.	Pos.	Neg.
36. R.M. #8	" "	" "	7.40	Neg.	No growth	Neg.	Neg.	Neg.
37. M.P. #20	Normal periapical region, extensive carious lesion.	Pulp brown in color and ischemic, structural integrity good, no odor.	7.25	Neg.	No growth	Neg.	Neg.	Pos.
38. J.D. #25	Radicular cyst	Tooth discolored, non-vital, pulp with some structural integrity but avascular.	7.10	Neg.	Str. anhemolyticus	Pos.	Neg.	Neg.
39. P.K. #9	Normal periapical region	Incipient discoleration, non-vital, pulp avascular, otherwise normal.	7.80	Neg.	No growth	Pos.	Pos.	Neg.
40. M.L. #8	" " "	Periosementitis, degenerate pulp, quite ischemic, non-vital, putrescent.	7.00	Neg.	Neisseria catarrhalis	Neg.	Neg.	Neg.
41. R.B. #11	Normal periapical region, deep silicate restoration.	Tooth gave high vitalometric response (13), pulp appears normal although ischemic.	7.35	Str.	Str. anhemolyt. & Lactobacilli	Pos.	Neg.	Neg.
42. J.C. #23	Normal periapical region, imminent exposure.	Partially vital. Pulp tissue quite normal but ischemic.	7.60	Neg.	No growth	Neg.	Neg.	Neg.
43. D.P. #7	Rarefying osteitis	Liquefaction necrosis, non-vital, fistula, no putrescent odor.	7.70	Neg.	Actinomyces	Neg.	Neg.	Neg.
44. C.S. #13	Apical pachymenia, imminent exposure.	Liquefaction necrosis, possibly microscopic exposure. Putrescent odor.	7.10	Neg.	Variety haet. (contaminants)	Neg.	Neg.	Neg.
45. P.V. #11	Rarefying osteitis	Seropurulent material in abundance although no odor.	6.90	Neg.	Actinomyces	Neg.	Neg.	Neg.
46. L.J. #30	" "	Dry, flaky, avascular pulp, no odor.	6.80	Neg.	No growth	Neg.	Neg.	Neg.
47. W.P. #7	" "	Avascular degenerated pulp, non-vital, no odor.	7.65	Neg.	No growth	Pos.	Neg.	Neg.
48. D.G. #9	Normal periapical region	Liquefaction necrosis of pulp, non-vital, putrescent odor.	7.40	Neg.	No growth	Pos.	Pos.	Pos.
49. G.L. #24	Apical pachymenia	Structural integrity of pulp fair, with normal vascularity. Putrescent odor.	7.45	Str.	No growth	Pos.	Neg.	Neg.
50. G.L. #25	Rarefying osteitis	Liquefaction necrosis, material sparse, putrescent odor.	7.25	Str.	No growth	Pos.	Neg.	Neg.
51. B.I. #23	" "	" " and fistula.	7.10	Neg.	No growth	Neg.	Neg.	Neg.
52. B.I. #24	" "	" " "	7.05	Neg.	No growth	Neg.	Neg.	Neg.
53. B.I. #25	" "	" " "	7.10	Neg.	No growth	Neg.	Neg.	Neg.
54. F.B. #7	Rarefying osteitis	Pulp tissue with good integrity but black. Non-vital, putrescent.	7.30	Neg.	No growth	Neg.	Neg.	Neg.
55. J.S. #10	Large cyst (origin from #9)	Pulp vital, integrity good but completely ischemic. No odor.	6.90	Neg.	No growth	Neg.	Neg.	Neg.

TABLE 2. (cont.) PATHOLOGIC SPECIMENS FROM PULP CANALS OF PATIENTS WITH NO KNOWN SYSTEMIC DISEASE.

I	II	III	IV	V	VI	Intracutaneous Tests (revised controls)		
Patient	Radiographic Interpretation	Clinical Condition of Pulp	pH	Smear	Culture	Av. Pt. [a] Hem. & P.P.	Av. R.A. [b]	Pts. & N.P. [c]
56. D.S. #7	Rarefying osteitis	Fistula, purulent material, no odor.	7.10	Neg.	No growth	Neg.	Neg.	Neg.
57. L.B. #9	Large cyst (embracing #10)	Degenerated pulp, putrescent odor, non-vital.	7.20	Neg.	No growth	Pos.	Pos.	Pos.
58. L.M. #13	Periapical region is normal, extensive carious lesion.	Partially vital pulp, normal in appearance, no odor.	6.90	Neg.	Variety bact. (contaminants)	Neg.	Neg.	Neg.
59. M.K. #10	Rarefying osteitis	Fistula, pulp material sparse and avascular, some loss of integrity.	6.95	Neg.	No growth	Pos.	Neg.	Neg.
60. S.F. #7	(extension from #8) Rarefying osteitis	Pulp quite normal although non-vital, no odor.	7.50	Neg.	No growth	Neg.	Neg.	Neg.
61. J.L. #4	Normal periapical region, deep carious lesion	Pulp normal in appearance. Was quite hyperesthetic.	7.55	Neg.	Str. anhemolyticus	Neg.	Neg.	Neg.
62. A.M. #8	" "	Pulp appeared passively congested. Non-vital, no odor.	7.35	Neg.	No growth	Pos.	Neg.	Neg.
63. A.L. #22	Rarefying osteitis	Fistula, non-vital, material sparse avascular and shreddy.	7.60	Variety of bacteria	No growth	Neg.	Neg.	Neg.
64. M.M. #7	" "	Material sparse and avascular, non-vital, putrescent odor.	7.10	Neg.	No growth	Neg.	Neg.	Neg.
65. M.P. #8	Apical pachymenia Deep carious lesion	Pulp normal in appearance, minimal vitality, no odor.	7.70	Neg.	No growth	Neg.	Neg.	Neg.

a pathologic pulp (P.P.). Column "b" refers to the result as determined by the average response upon at least two rheumatoid arthritic patients. Column "c", similarly, refers to the result as determined by the average response upon at least two patients with normal pulps and having no known systemic disease.

2. Clinical and Laboratory Observations on the Sixty-Five Pathologic Specimens Obtained from the Pulp Canals of Patients with No Known Systemic Disease.

The preceding data (Table 1), relative to the 65 filtrates prepared from pathologic specimens obtained from pulp canals, were obtained from 55 different patients judged to be systemically normal. The broad over-all pattern was derived from over 500 injections on these patients and others, as well as upon a number of rheumatoid arthritic patients and numerous individuals comprising the normal control groups.

Because of the necessity for introducing certain technical modifications as this study progressed it should be pointed out that patients 1-4 (column I) were given injections of 0.1 ml quantities; patients 5-26, 0.05 ml quantities, and patients 27-65, 0.025 ml quantities. Considering the potency of the filtrates, various technical aspects, as well as certain anatomical and physiological features of the skin occasionally encountered, the optimal quantity to use in this procedure is 0.05 ml. This quantity is usually superior for this purpose than either of the other quantities mentioned. However, for reasons of economy, the smaller quantity was chosen for the latter part

of this investigation in order that the control pattern could be vastly broadened. At the same time it was desired that about 0.4 ml of each filtrate remain, so that certain supplemental analytical tests could be performed. The use of the smaller quantity was not particularly objectionable in view of the fact that when 0.05 ml of a filtrate was injected in one site, and 0.025 ml in another site, the latter was usually not half, but was about two-thirds to three-quarters the size of the former. Hence, the reactions were not physiologically proportionate to the dilution.

In column II, radiographic terms of only the simplest designation have been used which are essentially broad enough to categorize the various periapical conditions encountered. Hence, this column embraces only four arbitrary categories, viz., (1) normal periapical region, (2) apical pachymenia, or the incipient thickening of the periodontal membrane within the region of the apex, (3) rarefying osteitis, which embraces the various granulomata and periapical dento-alveolar abscesses, and (4) cysts.

In column III, the condition and gross clinical appearance of the pulp or specimen are briefly described, the degree of vitality of the tooth is indicated, vascularity is noted, as is the presence of a fistula, and whether or not a putrescent odor was detected.

In column IV, the pH of the specimen suspension is indicated. Unlike normal pulps which consistently demonstrate a pH value of 7.35-7.40, the pathologic specimens vary from 6.80-7.90. It is believed that a few of the low values (below 7.35) may not be entirely accurate because

of the nature and sparsity of the pulp material occasionally encountered. In these few instances, despite the fact that the specimen is suspended in only 0.5 ml of physiologic saline solution, the buffer capacity of the material may have been inadequate. The average of the 64 pH determinations (in column IV, it may be noted that the third reading was unavoidably missed) is 7.32. As the low values cancel the high, this information is of no value. However, in the absence of gross structural changes the following criteria may be considered diagnostic of a pathologic pulp:

(1) If the pH value of a specimen is greater than 7.45.
(2) If the material is adequate and lyophilic and less than pH 7.30.

These criteria have been corroborated in a number of patients experiencing only the vague subjective symptom - pulpalgia. In a few of these situations we are dealing with incipient pulp disease in which the above criteria are apparently satisfied before actual histopathologic evidence can definitely incriminate the pulp as being pathologic. It is not unexpected that such cytochemical changes, determinable by an aberrant pH reading, may ordinarily precede structural changes as indicated by Menkin (114). This is especially true due to the fact that, incident to the most delicately performed pulpectomy, some cellular damage is inevitable. In addition, the histologic picture of even a fairly young normal pulp may occasionally appear questionable due to certain normal physiological retrogressive changes occurring in a perfectly sound tooth. However, the primary and original reason for performing the pH analyses was to determine whether or not the skin reactions may be in-

fluenced by this factor. It may now be stated with assurance that, from the lowest value obtained (pH 6.80) to the highest (pH 7.90), it plays no known part.

Column V is concerned with the presence of bacteria as determined in a smear made from the thoroughly mechanically agitated suspension. The smear is Gram-stained and viewed microscopically. The kaleidoscopic picture frequently obtained with normal specimens is due to pulpal shreds, lysed blood, and deranged tissue cells. Pathologic specimens may be even more bizarre. The only microorganisms occasionally absolutely identifiable, on a pure morphologic basis, are the streptococci. These are frequently present in long chains. Actinomyces-like organisms are occasionally encountered but are easily confused with cellular debris. Hence, when these organisms may have been present it was believed safer to consider the smear negative than to be in error. This was decided upon when a few smears which had been considered to be positive for actinomyces gave no growth after maintaining the semi-solid and aerobic blood agar cultures for at least a week in the incubator. In a few other instances, subculture upon blood agar plates, incubated anaerobically, produced typical actinomyces colonies in those specimens where the smear had been considered negative for these organisms. Actinomyces-like organisms were also believed to have been observed in a few instances where a vast heterogeneity of bacterial forms were encountered, yet no actinomyces were subsequently recovered in the cultures made from these specimens.

In the category designated as "variety of bacteria" in column

V, occasionally Gram-positive bacteria of such morphologic forms as lactobacilli, diphtheroids, and a "chaining" thread-like organism were encountered. The latter (marked with asterisk) was not directly observable in the smear but was cultivable aerobically and proved to be hemolytic. Gram-negative rods and diplococci, if either are present, would easily be confused with certain fragmented elements of the connective tissue of the pulp which assume the safranine stain.

Occasionally Gram-positive diplococci, some of which were apparently capsulated, were believed to have been observed. These bacteria, when cultivable, invariably gave a subsequent growth of streptococci only. Hence, original designations as Gram-positive diplococci only, are now changed to streptococci in column V. However, there is no assurance that the bacteria observed in the smear are those which are recovered upon culture. The direct smear alone offers but few significant findings as summarized:

(1) Of the 65 smears observed, 23 or almost one-third showed evidence of bacteria being present.

(2) Of these 23 positive smears, a variety of bacteria was observed 9 times or almost 40 percent of the time.

(3) Of these 23 positive smears, a streptococcus was observed 15 times or about 65 percent of the time.

(4) Only once was a streptococcus definitely observed in conjunction with a variety of organisms.

In column VI is listed, some in their restricted identification, the bacteria recovered upon culture. In summary:

(1) Of the 65 specimens cultured, 21 were found positive, however, only 19 will be considered positive as there is good reason to believe that two specimens were contaminated. Both of these contaminated cultures showed a variety of bacteria including lactobacilli. Radiographically, and clinically, the carious lesions in both of these instances led just barely to the pulp. These two specimens were taken only because the last bit of excavation of the carious debris was performed aseptically. In view of this highly selected group of specimens, and the fact that every operative precaution was observed, it is not surprising that the number of positive cultures is only 29 percent, or roughly expressed, less than a third positive.

(2) Among the 23 smears recorded as positive, bacteria were cultured on only 7 occasions or less than a third of the time. This may indicate that when bacteria are definitely observed in the smear, they may be non-viable about two-thirds of the time.

(3) Streptococci were cultured from all of the 7 specimens in which both the smear and culture were found positive. However, the smear had been reported as showing streptococci in 4 instances and on 3 occasions as showing a variety of organisms.

Of the 19 positive cultures the following species were encountered:

Microorganism	Number of occurrences*
Str. anhemolyticus	6
Str. viridans	4
Lactobacilli	3
Actinomyces	3
Str. anhemolyticus (anaerobic)	2
Str. hemolyticus	2
Neisseria catarrhalis	1
Gram-positive thread-like bacillus	1

* A mixed culture (including only two organisms) was encountered only three times.

As was previously mentioned, all the streptococci, that were restrictively identified (Table 2) as to their hemolytic proclivities, were preserved in physiologic saline solution and maintained in a deep-freeze refrigerator at approximately -20°C. An inadvertant failure on the part of the refrigerator, which escaped notice for two days, may have been a great factor in causing only two strains to have been recovered.

These two strains were initially inoculated into two tubes of semi-solid medium* and were subcultured upon blood agar plates. After colonial and microscopic verification indicated a pure culture for each strain, they were inoculated upon blood agar slants. A few drops of sterile physiologic saline solution were added to the surface of the slant to effect luxuriant growth. After 48 hours' incubation a 5 ml suspension of each strain was made and these suspensions transferred to sterile tubes. Thorough shaking of these tubes ensured a uniform suspension of bacteria. A 0.1 ml quantity of each suspension was inoculated into a tube of beef infusion broth containing 0.1 percent glucose and adjusted to a 6.5 percent salinity (NaCl). After four to five days' incubation, growth was found to be abundant. At the time of the inoculation of the high saline culture media, 0.1 ml of each suspension of streptococci was inoculated into three tubes, i.e., into one each of lactose, salicin, and mannite media with indicator (Difco). After only 24 hours of incubation the colorimetric change was complete and no gas formation was evident in the Durham tubes. Concurrent with these experi-

* Composition previously given in footnote.

ments a 1 ml quantity of each suspension of streptococci was inoculated into a brain-heart infusion broth (Difco) and the culture tubes immediately heated in a water bath maintained at 60°C ± 1°C for one-half hour. The tubes were then removed from the water bath and incubated for 48 hours at which time growth was evident. Recheck smears from all final cultures in the three preceding experiments showed streptococci only. From these tests it was concluded that both of these species are Streptococcus fecalis. It is of interest that one of these strains was originally designated as Str. viridans, (Table 2, patient 32, D.A. #9) and the other Str. anhemolyticus (patient 61, J.L. #4). The only feature in common, as to the origin of these two surviving strains, is the fact that both were derived from quite incipient pulp involvements.

It is interesting to compare the number of positive skin reactions occurring among the various groups of patients when bacteria were observed at least in the smear, at least recovered upon culture, or present in both instances. Eliminating the two occasions where contamination undoubtedly occurred, the data may be summarized as follows:

(1) The group consisting of the "homologous" patients, from whom pulp canal specimens were obtained, and patients with pulp involvements.

a) A positive skin reaction occurred in this group, when filtrates of specimens were used in which bacteria were found in the smear, recovered in culture, or both, 18 times or about 29 percent.

b) A positive skin reaction occurred in this group, when filtrates of specimens were used in which no bacteria were found in the smear or recovered in culture, 8 times or about 12 percent.

c) A negative skin reaction occurred in this group, when filtrates of specimens were used in which bacteria were found in the smear, recovered in culture, or both, 15 times or about 24 percent.

d) A negative skin reaction occurred in this group, when filtrates of specimens were used in which no bacteria were found in the smear or recovered in culture, 22 times or about 35 percent.

(2) The group consisting of two rheumatoid arthritic patients.

a) A positive skin reaction occurred in this group, when filtrates of specimens were used in which bacteria were found in the smear, recovered in culture, or both, 8 times or about 13 percent.

b) A positive skin reaction occurred in this group when filtrates of specimens were used in which no bacteria were found in the smear or recovered in culture, 5 times or about 8 percent.

c) A negative skin reaction occurred in this group, when filtrates of specimens were used in which bacteria were found in the smear, recovered in culture, or both, 26 times or about 41 percent.

d) A negative skin reaction occurred in this group, when filtrates of specimens were used in which no bacteria were found in the smear or recovered in culture, 24 times or about 38 percent.

(3) The group consisting of two individuals having no known dental or systemic disease.

a) A positive skin reaction occurred in this group, when filtrates of specimens were used in which bacteria were found in the smear, recovered in culture, or both, 2 times or about 3 percent.

b) A positive skin reaction occurred in this group, when filtrates of specimens were used in which no bacteria were found in the smear or recovered in culture, 4 times or about 6 percent.

c) A negative skin reaction occurred in this group, when filtrates of specimens were used in which bacteria were found in the smear, recovered in culture, or both, 32 times or 50 percent.

c) A negative skin reaction occurred in this group, when filtrates of specimens were used in which no bacteria were found in the smear or recovered in culture, 25 times or about 40 percent.

From the foregoing data, the most statistically significant feature is the fact that the "pulp-involved" group shows far greater (29 percent) skin reactivity, if bacteria were at least present in the smear, culture, or both, as compared with the normal group showing only 3 percent. The skin reaction is negative for the "pulp-involved" group 24 percent of the time and negative 50 percent of the time for the normal group. Also of statistical significance, but less striking, is the fact that the "pulp-involved" group shows greater (29 percent) skin reactivity, if bacteria were at least present in the smear, culture, or both, as compared with the arthritic group showing only 13 percent. The skin reaction is negative for the "pulp-involved" group 24 percent of the time and negative 41 percent of the time for the arthritic group. This suggests that bacterial products may play an important role in the causation of the positive skin reactions observed among the "pulp-involved" group; but this fact is only evident when compared with the group having no known dental or systemic disease. The mere fact that, within the "pulp-involved" group, almost as many positive skin reactions occur

whether or not bacteria were demonstrably present does not preclude their having been present.

3. Observations on the Fifteen Normal Pulp Specimens Obtained from Patients with No Known Dental or Systemic Disease.

In the data (Table 3), embracing the 15 normal pulp specimens of the control group, no bacteria were ever in any instance cultivable, and the pH values are remarkably consistent from pH 7.35-7.40. The two deviations (both 7.30) are consistent within the limits of accuracy of the method of pH analysis.

4. Observations on Specimens Obtained from Rheumatoid Arthritic Patients.

The pathologic specimens obtained from four rheumatoid arthritic patients also gave no growth, and the pH values did not differ significantly from those pathologic pulps of systemically normal individuals (Table 4). It is exceedingly difficult to find bona fide pathologic pulps among this group as they have, as a rule, invariably been subjected to a not inconsiderable number of extractions. Although filtrate G.C. #7 of this group has been included, there is absolutely no justification for considering it other than normal.

5. A Comparison of the Skin Reactions of the Different Groups to the Various Filtrates.

It seems only logical to begin with certain observations made in the control group so that we may better evaluate the other groups. In Table 3, under intracutaneous tests, column "c", it may be noted that

TABLE 3. NORMAL PULPS FROM PATIENTS WITH NO KNOWN SYSTEMIC DISEASE.

I	II	III	IV	V	VI	Intracutaneous Tests		
Patient	Radiographic Interpretation	Clinical Condition of Pulp	pH	Smear	Culture	Av. Pt. & [a]	Av. R.A. [b]	Av. Pt. & [c]
1. G.Z. # 7	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	---
2. D.F. # 7	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	---
3. W.V. # 6	Normal	Normal	7.40	No bact.	No growth	Neg.	Pos.	---
4. J.W. #11	Normal	Normal	7.40	No bact.	No growth	Neg.	Pos.	---
5. L.W. # 7	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	---
6. P.D. # 7	Normal	Normal	7.35	No bact.	No growth	Neg.	Neg.	Neg.
7. P.D. #10	Normal	Normal	7.30	No bact.	No growth	Neg.	Neg.	Neg.
8. S.K. #29	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	Neg.
9. V.S. # 5	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	Neg.
10. E.J. # 8	Normal	Normal	7.30	No bact.	No growth	Neg.	Neg.	Neg.
11. A.M. #29	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	Neg.
12. Z.K. # 7	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	Neg.
13. S.S. #11	Normal	Normal	7.40	No bact.	No growth	Neg.	Neg.	Neg.
14. C.B. # 7	Normal	Normal	7.40	No bact.	No growth	Pos.	Pos.	Neg.
15. C.B. # 8	Normal	Normal	7.35	No bact.	No growth	Neg.	Neg.	Neg.

TABLE 4. PATHOLOGIC SPECIMENS AND NORMAL PULPS FROM RHEUMATOID ARTHRITIC PATIENTS.

I	II	III	IV	V	VI			
Patient	Radiographic Interpretation	Clinical Condition of Pulp	pH	Smear	Culture	Hem. Test	Other Rh. Arth.	Normal Control
1. C.T.# 7	Rarefying Osteitis	Liquefaction necrosis, material sparse, non-vital, no odor.	7.35	Neg.	No growth	Pos.	Neg.	Neg.
2. C.S.# 8	" "	Pulp material very sparse, no putrescent odor, non-vital.	7.10	Neg.	No growth	Neg.	Neg.	Neg.
3. G.C.# 7	Dentigerous cyst (origin from #6)	Pulp grossly normal, vital, and no odor. NORMAL*	7.35	Neg.	No growth	Neg.	Neg.	Neg.
4. A.H.# 5	Apical pachymenia	Pulp sparse, dry, and necrotic, non-vital, and no odor.	7.50	Neg.	No growth	Neg.	Neg.	Neg.
5. A.H.# 4	" "	Shreddy, rust-colored, partially vital pulp. No odor.	7.30	Neg.	No growth	Neg.	Neg.	Neg.

*Normal stands at this point only because cases are listed in the sequence in which they were undertaken.

the filtrates of the first five patients were not crossed over into systemically normal patients with pathologic pulps. In the total pattern it may also be observed that four positive reactions were elicited. Because of the unaccountable nature of these reactions, these injectees were retested and in all instances a third member was incorporated into the group to rule out any peculiarity in response which may have resided within the patients rather than with the filtrates used. The observations on the use of the normal pulp filtrates may be summarized as follows:

(1) In the total pattern, 4 positive reactions out of 40, or 10 percent, were obtained. This may have been lower had the additional five tests been performed. However, the expanded control pattern had not been instituted at the beginning of these experiments. All reactions were characterized by a light to moderate erythema.

(2) According to groups the reactions were positive:

a) 1 in 15, or 6.6 percent (Average, homologous reaction and one other patient with no known dental or systemic disease)

b) 3 in 15, or 20 percent (Rheumatoid arthritic group)

c) 0 in 10, or 0 percent (Patients with pathologic pulps but no known systemic disease)

The data obtained from the use of filtrates prepared from the pathologic pulp canal specimens of patients with no known systemic disease (Tables 1 and 2) may be summarized as follows:

(1) In the total pattern, 44 positive reactions out of 195, or slightly over 20 percent were obtained. All reactions were characterized by moderate to bright erythema.

(2) According to groups the reactions were positive:

a) 25 in 65, or 38.5 percent (Average, homologous patients and one other patient with pathologic pulp but no known systemic disease)

b) 13 in 65, or 20 percent (Rheumatoid arthritic group)

c) 6 in 65, or 9.2 percent (Patients with normal pulps but no known systemic disease)

6. An Evaluation of the Skin Reactions of the Different Groups to the Various Filtrates.

The small group of five rheumatoid arthritic patients from whom pulp canal specimens have been obtained is inadequate for statistical treatment. However, this group does appear to indicate that there is probably no justification for assuming that the nature or products of normal or diseased pulps in these patients differs from those of systemically normal persons. Further evidence of a lack of significant correlation was the fact that 20 percent of the rheumatoid arthritic patients responded positively by a light to moderate erythema to normal pulp filtrates and also that 20 percent responded positively by moderate to bright erythema to filtrates of pathologic specimens obtained from patients with no known systemic disease.

The difference in a positive skin reaction among persons with no known dental or systemic disease, to normal pulp filtrates, as compared with a positive reaction to filtrates from pathologic specimens, was obviously not significant. The greatest difference appeared in the positive reactions obtained in the homologous test, using the filtrates of

pathologic specimens, and in those reactions obtained when injecting such filtrates into other systemically normal patients having pathologic pulps. The homologous reaction was usually a little more frequent but it was not unusual for the heterologous reaction in this group to be greater. Should we assume that these substances leave the teeth via the apical foramen, it is possible that they may do so to a greater extent in one individual than in another. This concept necessarily presupposes the phenomenon of autosensitization. It would, however, explain the occasional occurrence of greater positive heterologous reactions.

In general, it may be stated that given any particular filtrate prepared from pathologic specimens we may expect:

(1) The homologous patient, and patient with a pathologic pulp, as a group, to respond positively to the filtrate about twice in every five injections or almost 40 percent of the time.

(2) The rheumatoid arthritic group to respond positively once in every five injections or 20 percent of the time.

(3) The normal control group to respond positively almost once in every ten injections or almost 10 percent of the time.

(4) Broadly stated, the first group was twice as reactive as the rheumatoid arthritic patients and the latter group twice as reactive over the normal controls. The statistical validity of these differences has been found not to be particularly significant.

(5) It may also be more significantly expressed to point out that the first group was 30 percent more positively responsive than

the controls. A statistical analysis of this difference has been found to be highly significant.

An Evaluation of Other Clinical and Laboratory Findings.

No correlation of any unusual significance appeared between the radiographic interpretation and the clinical condition of the pulp other than a normal periapical region usually accompanied a pulp of good structural integrity even though it was diseased. Of course, the more extensive and chronic periapical involvements are indicative of completely degenerated pulp tissue.

A pulp may yield a reactive filtrate if it should be only incipiently diseased or frankly purulent. It was also apparent that bacteria may or may not be found viable in any stage of pulp degeneration.

The presence of even a very putrescent odor was wholly unrelated to the reaction of the filtrate. Some pulps evolved an odor indistinguishable from skatole. Patients homologously tested with such a filtrate, and a skatole solution, more frequently gave negative than a positive skin reaction.

The pH value, other than establishing a physical means of corroborating pulp disease, was not significantly diagnostic if the pulp specimen, although diseased, should have been between pH 7.30 and 7.45. The pH values were seemingly unrelated to any of the other data.

Immunologic Investigations.

Early in the course of this investigation, it was believed most desirable to inquire into the possibility of some underlying immunologic mechanism to account for the reason why some individuals re-

acted markedly to certain filtrates whereas others were completely negative. Because of the greater reactivity observed homologously, or upon others with pathologic pulps, it was believed entirely plausible that some substance may have escaped from the pathologic pulp to have possibly sensitized the individual. The ability to demonstrate such a phenomenon would indeed lend considerable credence to an unusually attractive hypothesis. An excellent immunologic procedure for demonstrating the possible existence of specific reaginic antibodies is the passive transfer test or the so-called Prausnitz-Küstner reaction (115). Reagin has been defined (116) as: "One of a group of special antibodies in serum which react with the allergens or atopens responsible for the specific manifestations of hypersensitivity in man, as hay fever, asthma."

Blood serum was obtained from three rheumatoid arthritic patients and from two normal patients, each of whom reacted, substantially, to one of a number of filtrates prepared from pathologic pulps with which they had been tested. A non-reactor was found for each of the several filtrates employed. These non-reactors were injected, intracutaneously, with the Seitz-filtered specific serum required to effect sensitivity. Subsequent injection, three or four days later, of the specific filtrate in the identical site, in each instance, failed to give a single positive test. The test was performed in duplicate on three of the occasions which also terminated uneventfully. Microprecipitation tests, each with its specific filtrate, were performed with all five of these sera and, these too, proved to be negative.

Despite the disappointing results obtained in this phase of the

investigation it is still believed that certain filtrates may be capable of demonstrating the Prausnitz-Küstner phenomenon. However, for the present we must assume that the majority of filtrates from pathologic pulps that give a positive skin test are of a nonspecific nature. Such a hypothesis may seem difficult to reconcile with the variety of skin test responses observed with the majority of reactive filtrates. It may be quite possible that these filtrates are simply not amenable to such tests as passive-transfer and precipitation.

In the eight attempts to demonstrate positive passive transfer tests five different filtrates prepared from pathologic pulps were used. Three of the pulps used in the preparation of these filtrates were in a stage of advanced decomposition and were characterized by a strong putrescent odor. It is only natural, at this point, to inquire into the role of certain products of putrefaction which may be responsible for the original positive skin reactions.

Intracutaneous Tests Using Ptomaines and Other Chemical Agents.

The dental pulp is essentially composed of protein substances. When this protein is hydrolyzed to amino acids by the enzymatic activity of bacteria, and possibly other agents, it is readily further degraded by a series of reactions involving deamination and decarboxylation. Such a degradation on the part of the pulp results in a putrefactive condition, which while chemically demonstrable by the indole test, is easily recognized clinically by the loss of structural integrity on the part of the pulp accompanied by a slight to profound malodorous condition. A variety of ptomaines and other putrefactive products are chiefly responsible for

this odor. Hence, it was believed to be of considerable importance to test all known and available products known to result from putrefaction in order to determine what role, if any, they play in the causation of the positive skin reactions observed. All chemicals used in this undertaking were of either reagent quality or of the highest purity obtainable. There are three major ptomaines which result from the putrefaction of animal protein and a rather complete account of the procedure will be given as performed with these products. The results using other ptomaines and putrefactive products will be, for the sake of brevity, concisely stated, essentially, as the same procedure was used with each chemical. The three major ptomaines are putrescine, indoleacetic acid, and indole. Incident to putrefaction, putrescine is derived from the amino acid arginine which is known to constitute about 5 percent of serum albumin, globulin, and hemoglobin. The other two chief toxic bacterial decomposition products, indoleacetic acid and indole, result from tryptophane degradation.

The putrescine (1,4 diaminobutane dihydrochloride) is quite readily soluble in physiologic saline solution and a one percent solution was prepared. As indole and indol-3-acetic acid are quite insoluble in physiologic saline solution, a supersaturated solution of each was prepared, shaken thoroughly, and allowed to stand at room temperature for 18 hours. The three solutions were then Seitz-filtered into sterile test tubes. "Doubling dilutions" were then carried out aseptically through to ten tubes for each solution. Starting with the highest dilution, the author injected himself, intracutaneously, with 0.025 ml of each. The high-

est dilutions gave no reaction over a 24 hour observation. Successively stronger solutions of each were used until a standard skin test dose (STD) could arbitrarily be established for each solution. The STD for each solution was established as that concentration which, when injected in a 0.025 ml quantity intracutaneously, would give an average 25 mm reaction within 10 minutes. Only 22 intracutaneous tests were required to establish this STD for these solutions with certainty; at least, on the basis of the author's responsive capacity. For the three ptomaines, these concentrations were:

(1) A 50 percent saturated solution of indole.

(2) A 25 percent saturated solution of indol-3-acetic acid.

(3) A 0.125 percent solution of 1,4 diaminobutane dihydrochloride (putrescine).

The intracutaneous tests as performed with these solutions gave the following skin reactions.

Rheumatoid arthritic patient (JMc):

	Saline control	Indole (pH 6.6)	Indol-3-acetic acid (pH 4.2)	1,4 diaminobutane dihydrochloride (pH 5.8)
10 mins.	Neg.	15x25 mm	20x30 mm	20x25 mm
30 mins.	Neg.	10x12 mm	14x22 mm	10x12 mm
6 hrs.	Neg.	5x7 mm	5x5 mm	Neg.
12 hrs.	Neg.	Neg.	Neg.	Neg.
24 hrs.	Neg.	Neg.	Neg.	Neg.

Normal control (VD):

10 mins.	Neg.	25x25 mm	23x27 mm	24x26 mm
30 mins.	Neg.	22x23 mm	22x24 mm	20x23 mm
6 hrs.	Neg.	Neg.	5x6 mm	Neg.
12 hrs.	Neg.	Neg.	Neg.	Neg.
24 hrs.	Neg.	Neg.	Neg.	Neg.

All of these solutions burned quite considerably upon injection and some degree of erythema was sustained for variable periods over an hour. The reactions in these instances were quite definitely not antigenic in nature but were undoubtedly the result of direct chemical irritation. The wheals in all instances, unlike any pulp filtrates, were essentially edematous and were slightly painful when applying pressure to them a half hour after injection. Further experiments with these solutions were advisedly abandoned.

Similarly prepared, were solutions of skatole, histamine, cadaverine, choline, methyl mercaptan, ethyl mercaptan, trimethylamine, phenol, ammonia, carbon disulfide, and hydrogen sulfide. These agents were also dissolved in physiologic saline solution and sterilized by Seitz-filtration. Standardized skin-test doses were then prepared by serial dilution and testing proceeded from high to low dilutions for each solution. Skin tests were performed on seven persons for each solution (with noted exceptions*); these injectees were, two rheumatoid arthritic patients, three dental patients with pathologic pulps, and two normal controls. There were no consistent differences found between any of the groups and none of the reactions resembled those of the pulp filtrates for the following reasons:

Skatole - at a 0.06 percent dilution (STD), produces a very light skin reaction and the characteristic odor is strong.

Methyl mercaptan - odor obviously offensive above a dilution of 0.06 percent and at this the reaction is too weak.

Ethyl mercaptan - concentration needed (0.12 percent) too high for total amount of active substances that could be expected to be present in a filtrate prepared from a pathologic pulp.

Trimethylamine - at a 0.03 percent dilution the erythema is too light and histamine-like.

Carbon disulfide - profound acetone odor at the dilution (0.1 percent) giving only a minimal reaction.

*Ammonia - burns exceedingly at a 0.03 percent dilution with only minimal erythema.

Hydrogen sulfide - a 0.12 percent solution (STD) exceeds the volume of total reactive substance in a pulp filtrate.

Histamine (acid phosphate) - at only 4 parts per million, the wheal is too light and shows pseudopods in certain subjects. The erythema is too bright in too short a period of time.

*Cadaverine - profound burning and chemical irritation far in excess of the erythema.

Choline - quantity required for reaction exceeds the volume of total reactive substance in a pulp filtrate.

*Phenol - quantity required for reaction exceeds the volume of total pulp substance in a pulp filtrate.

The highly volatile liquefied gases (the mercaptans and trimethylamine) were worked with at sub-zero temperatures. Preliminary experiments indicated that if the entire pulp were but one chemical substance, even then the greatest concentration that one could expect in the 1 ml suspension thereof is about 0.5 percent. However, but a minute amount of this exceedingly small quantity passes the Seitz-filter. Hence, for any chemical to correlate with the concentration of the skin-reacting substances of the pulp filtrates, it must not only satisfy clinical, chemical, and physical requirements, but should be positive in a dilution not only far less than 0.1 percent but undoubtedly less than 0.01 percent.

Histamine is the only chemical that satisfies this requirement, yet the characteristic reaction with this agent at even a 4-parts-per-million dilution is quite unlike that elicited by any of the pulp filtrates.

Trimethylamine, which Brieger (117) reported as a metabolic product found in streptococcus cultures, was especially interesting as a skin-testing agent. This chemical, in a 0.03 percent dilution frequently produced a moderate flare with a small blanched wheal similar to histamine but without "pseudopods". However, its use in conjunction with a physiologic saline solution control indicated that it may be a very useful chemical if it should be desired to know the threshold of reactivity of the skin of any injectee. To repeated injections of this chemical, the wheal-flare ratio was seemingly quite consistent for any given patient. The wheal and flare, if both or only the latter were present, were sharply demarcated and facilitated mensuration. It is the author's opinion that it is not always particularly advantageous to have a negative control because it does not answer the question, "How negative is a negative control?" In actuality, it is believed that both physiologic saline solution and some such agent as trimethylamine, or perhaps other tertiary amine, could be used as simultaneous controls and may yield more delicate information. This feature was especially brought to the author's attention when all of 12 intracutaneous tests, with different pulp filtrates, were completely negative in a single individual who was also negative to the trimethylamine. In all tests performed with the chemical solutions the amount injected was 0.025 ml with the standardized concentrations cited. It appears from these experiments that no sim-

ple chemical, among those tested, could, in such a form, be primarily responsible for the positive reactions as observed when filtrates prepared from pathologic pulp material are used.

Experiments on the Dialysis of Various Filtrates.

Aseptic dialysis of approximately 0.5 ml quantities of the standardized chemical solutions used in the preceding experiment was performed as follows:

> A 5 inch strip of Visking standard ($\frac{1}{4}$ inch diameter, collodion) casing was opened and recreased between the existing creases. After the latter creasing it was sharply bent in the center to form a "V-tube". This collodion tube, folded back on itself, was placed in a serological tube with the two open ends toward the mouth of the serological tube. A number of such tubes were prepared simultaneously, cotton plugs were inserted, and they were then autoclaved. A tuberculin syringe was used for the aseptic transfer of the 0.5 ml quantity of each solution into one column of the collodion tube in the serological tube. With the aid of sterile forceps the collodion tube was raised half way out of the serological tube and both columns of the former were tightly tied together with dental floss. The collodion tubes, now as double bags, were clipped just below the rim and inside of a 500 ml beaker. The beaker was then filled with distilled water about a half inch above the level of the solution in the collodion bags. The beaker was placed in a refrigerator at approximately 4°C for 24 hours. After this time the distilled water was siphoned out of the beaker and replaced with fresh distilled water. It was again placed in the refrigerator for 24 hours after which the distilled water was replaced with physiologic saline solution to reconstitute the proper salinity. It was once again placed in the refrigerator for a period of 24 hours or longer. These solutions were exceedingly irritating upon injection unless normal salinity was re-established.

Upon retest, by intracutaneous injection with the dialyzed solutions upon the same subject, all of the skin reactions were either virtually negative or completely negative. Hence, the simple chemicals

in solution were essentially regarded as completely dialyzable.

This technique was repeated with six active filtrates prepared from pathologic pulps which were pre-tested on a single subject. These dialyzed filtrates showed but little or no apparent difference upon being retested. From these experiments it seemed quite evident that the essential skin-reactive substances in the pulp filtrates were not dialyzable and, hence, not of the character of a simple chemical substance but possibly macromolecular in nature. It seemed desirable, at this time, to attempt to characterize, to some extent, the nature of the skin-reactive substances appearing in the filtrates of pathologic pulps.

The Characterization of the Skin-Reactive Substances Found in the Filtrates of the Pulp Canals of Pulp-Involved Teeth.

It was obvious that any attempt to investigate the nature of the skin-reactive substances of filtrates, prepared from pathologic pulps, would be especially difficult due to the minute amount of filtrate ordinarily remaining after having determined its reactivity. Having performed at least six intracutaneous tests with each of the filtrates, usually not over half of the original volume, or about 0.4 ml of the filtrate, remained. Other than the time during which certain tests were performed, the filtrates were always maintained at approximately 4°C in the refrigerator.

1. The Thermostability of the Skin-Reactive Substances in Fifteen Filtrates.

Three individuals with a high reactive capacity, for these fil-

trates, were chosen as the injectees. In this way a number of "borderline" filtrates, which in the original intracutaneous tests may have been considered negative, were included. Only enough filtrate remained to carry this procedure through on one subject for each filtrate. Hence, it was essential that the injectee not only be a good reactor but also a reliable one. Among 25 filtrates originally pre-tested on these subjects, 15 produced reactions which were substantially (10 mm) greater than the saline control. Because of the inordinately small amount of filtrate that remained in each bottle, the heating periods were, of necessity, additive. That is, all filtrates were heated at 56°C for one-half hour, then the intracutaneous tests were performed. They were then boiled for ten minutes and again the intracutaneous tests were performed. Finally, the filtrates were boiled for one hour and the intracutaneous tests were once again performed. The data is summarized in Table 5 in which any response on the part of the control injection of physiologic saline solution has already been discounted.*

Table 5. The Thermostability of Fifteen Pulp Filtrates

Filtrates	Reaction* before heating	Reaction after 56°C for ½ hour	Reaction after 100°C for 10 mins.	Reaction after 100°C for 1 hour	Injectee
1. JA# 8	25x25	20x25	20x20	Neg.	VD
2. SB# 8	20x25	18x20	Neg.	Neg.	"
3. MC#10	20x20	18x20	Neg.	Neg.	"
4. FD# 8	30x40	Neg.	Neg.	Neg.	"
5. EK# 9	30x40	Neg.	Neg.	Neg.	"
6. ML#21	15x20	25x30	Neg.	Neg.	"
7. DM# 8	22x28	Neg.	Neg.	Neg.	"
8. WV# 6	22x30	25x28	20x25	Neg.	"
9. JW#11	27x27	Neg.	Neg.	Neg.	"
10. PC# 6	25x30	25x35	25x30	25x27	CT
11. HW#10	16x20	20x25	Neg.	Neg.	"
12. VZ# 9	15x15	20x20	Neg.	Neg.	"
13. JL#24	18x18	25x25	Neg.	Neg.	CS
14. MM#27	20x25	30x30	Neg.	Neg.	"
15. MR# 8	18x20	20x22	Neg.	Neg.	"

In summarizing the data, as shown in Table 5, the following may be observed:

(1) Eleven filtrates, or slightly over 73 percent, were thermostable as evidenced by giving a positive skin reaction after having heated the filtrates at 56°C for one-half hour.

(2) Three filtrates, or exactly 20 percent, also remained unaltered in reactivity after having heated the filtrate at 100°C for 10 minutes.

(3) Only one of the fifteen filtrates tolerated 100°C for one hour without loss of reactivity.

(4) The four thermolabile filtrates all gave very substantial pre-test skin reactions.

(5) An unanticipated finding was the fact that about a third of the filtrates were seemingly potentiated after having been subjected to 56°C for one-half hour. All of these filtrates, however, were inactivated upon subsequent treatment at 100°C for ten minutes. This was not surprising when we compare this observation with the effect of mild heat denaturation on normal serum, with potentiation demonstrable upon intracutaneous injection.

There is no apparent correlation of this data with the clinical condition of the pulp, radiographic findings, the presence or absence of viable bacteria, the pH of the suspension, or with any other findings obtained.

Filtrate (PC #6), which tolerated boiling for one hour, was exhausted in quantity and could not be further tested. If this filtrate had

not given a virtually identical skin reaction, after each period of heating, it would not have stood out so markedly. In only one other instance was a highly thermoduric filtrate encountered and this was obtained from a rheumatoid arthritic patient and was designated as CT #7 (Table 4). This particular filtrate even withstood standard autoclave treatment with only little appreciable loss of skin-activity. There was no essential correlation whatsoever between other findings and filtrates PC # 6 and CT #7. Unfortunately, in these tests, all filtrates including FD #8 and EK #9 (Table 5) from which cases hemolytic streptococci were recovered, were exhausted and could not be further tested.

From these heat inactivation tests, the suspicion that we may have been dealing with a variety of skin-reactive substances was further confirmed. The ability for certain filtrates to tolerate the third (100°C/1 hour) or even fourth (standard autoclave) heat treatment indicated that any reactive filtrate may contain three or even four different reactive substances. In addition to such qualitative differences, the amount of each substance present in a reactive filtrate may be subject to even greater variation. The author was actually of the opinion that certain observations made on filtrates, highly reactive in one injectee and completely negative in another, were not in reality erratic, but possibly indicative of a heterogeneity of skin-reactive substances being present in the filtrate used. Hence, it is conceivable also that should any given filtrate have been injected into two individuals, with a positive skin reaction resulting in both,

one may have reacted to one substance and the other to a different substance.

2. Tests for Protein in Filtrates Prepared from Normal and Pulp-Involved Teeth.

In a few preliminary tests where three pulp specimens were split, an alcoholic extract was prepared from one half of each specimen and an extract from the other half using physiologic saline solution. The alcoholic filtrates had to be resuspended in physiologic saline solution before injection. The alcohol was slowly eliminated by mere warm evaporation and the filtrate was then made up to volume with physiologic saline solution. It was consistently apparent that the most reactive filtrates were, without a doubt, those prepared with physiological saline solution. Those skin reactions resulting from the alcohol extracted filtrates were only 5 to 10 mm in diameter over the control. They were, however, a rather deep red, slow in onset (20 minutes), and remained, on two occasions, for periods over two hours. Due to possible alcoholic denaturation, these reactions are of little significance.

Hence, it appears, at this point, that the skin-reactive substances in certain pulp filtrates may be protein in nature. This was based on the fact that the substances are soluble in physiologic saline solution; gave an immediate type of skin reaction, not unlike proteins, upon intracutaneous injection; were non-dialyzable; and showed inactivation at the various levels of heat treatment.

Preliminary tests with highly dilute human serum indicated that Robert's modification of Heller's nitric acid test (118) was especially simple to use, extremely delicate, and readily adapted to testing

the very small quantities of filtrate. Four normal pulp filtrates and six reactive filtrates from pathologic pulps were tested. In this experiment, 7x70 mm test tubes were used into which 0.5 ml of Robert's reagent was placed with a capillary pipette. The filtrates, from pathologic pulps, that were used, were those employed in the dialysis experiments, and were carefully stratified over the surface of the reagent using tuberculin syringes for this purpose. Because of the minute amount of protein, the tubes were allowed to stand for a few minutes and were then observed by oblique light against a black background. The four normal pulps, especially two showing a conspicuous tinge of hemoglobin in the filtrate, gave a readily detectable positive test. The filtrates from pathologic pulps were only weakly positive in comparison. Two of these filtrates were so weakly positive as almost to border on the doubtful. The sensitivity of the test is presumed to be not reliably greater than for about 0.01 percent of protein in solution and, as such, bears out a previous point that the amount of reactive substance in a filtrate probably is not much greater than 0.01 percent. There is a good possibility that when the amount of pulp substance obtainable was very scant, it may have been substantially less. Of course, this test definitely does not prove anything other than the fact that a demonstrable amount of protein substance was present and that it possibly is usually present in the filtrate when skin-reactive substances are present. It does not, unequivocally, prove that the protein substance and the skin-reactive substance or substances are one and the same. It was believed particularly desirable to attempt to

inactivate the protein fraction in some physical way in order that it may be injected. In this way it could possibly be determined whether or not the protein fraction and the skin-reactive fractions may be the same substance. To determine this, it was thought that the subjection of filtrates to the action of ultraviolet light may supply the answer.

3. Ultraviolet Inactivation of Pulp Filtrates.

Six filtrates that had been prepared from pulp-involved teeth were divided into equal quantities. Exactly 0.2 ml of each filtrate was aseptically placed in two 5 cc bottles. The small amount of filtrate constituted about a one millimeter stratum in each bottle. One of each set of two bottles, for each filtrate, was unstoppered and placed in virtual contact with a 4-watt, U-tube, 2537 Å, ultraviolet lamp within a special enclosed chamber. The surface of the filtrates was exactly 5 cm from the incident source of ultraviolet light. These filtrates were subjected to a quantum satis dose of ultraviolet light for a period of two hours. Two delicate and reliable reactors were then chosen for this series of injections. Six injections, three irradiated filtrates and their unirradiated controls, were given to each injectee. One reactor demonstrated a slightly increased reaction, over the control, with one filtrate, another filtrate was essentially the same, and the third injection was substantially (10 mm) less than its control injection. The other subject did not respond to one filtrate, i.e., neither the irradiated specimen nor the control; and again one filtrate appeared slightly greater and the other slightly smaller. In rechecking on the source of the filtrates it was discovered that the two filtrates, one in

each injectee, which gave increased responses, were obtained from incipiently diseased pulps of good or fair structural integrity, and that a trace of hemoglobin was present in the specimen demonstrating the greater (10 mm) increase upon irradiation. This is not to be construed as incriminating hemoglobin for the positive reaction. Hemoglobin is non-antigenic and positive skin reactions occur wholly independent of its presence.

Despite such ostensibly erratic results, it was thought that, perhaps, in those filtrates prepared from diseased pulps of good structural integrity, some protein fraction may have been just sufficiently denatured to cause them to give an increased response. Whether or not it could have been a potentiation of the original reactive substance or substances or of another protein fraction was impossible to determine. Many non-reactive proteins are known to become reactive or antigenic upon denaturation. In fact, a coctoprecipitin is defined as "a precipitin produced in an animal by immunization with a boiled antigen, such as serum protein" (119). Denaturation is not a profound change on the part of a protein molecule, in fact "one of the characteristic reactions of most native proteins is their ability to undergo denaturation. Denaturation may be defined as a non-hydrolytic modification of the structure of a protein in such a way that the physical, biological, and in most instances, the chemical properties of the proteins are altered. In the case of most proteins, these changes are irreversible" (120).

At this point in the investigation the potentially reactive filtrates were fast being depleted and a large number would possibly be re-

quired to determine at what ultraviolet dose the original skin-reactive substances could be inactivated without potentiating, perhaps, other protein fractions. It was at least decided to determine whether or not the non-skin-reactive protein fractions could be potentiated. Hence, two normal filtrates were subjected to the same procedure and although one skin reaction with these filtrates was only 6 mm greater in diameter than its control, the other was 10 mm greater in diameter than its control.

These results, although interesting, virtually defy interpretation. Supportively, it does appear to indicate that at least some of the skin-reactive substances seem to be of a protein nature whereas others may be denatured proteins and as such could act as "foreign" proteins. Due to the small neck of the bottles and the fact that, during the two hour period of irradiation, the ambient temperature was 27°C, there was no measurable degree of evaporation. Therefore, the potentiation of the two filtrates could not be attributed to concentration by evaporation. However, no definite conclusions could be drawn from this phase of the investigation and the ultraviolet technique was abandoned in favor of a plan to digest the skin-reactive substances in the filtrates.

4. The Tryptic Digestion of Filtrates Prepared from the Specimens Obtained from Pulp-Involved Teeth.

In this phase of these investigations it was desired to use the minimum amount of trypsin in the filtrate which of itself would not give a positive skin test and yet which would be just sufficiently adequate to digest the skin-reactive substances to non-reactive products. For this purpose twice crystallized, "salt-free" trypsin was used. This crystalline

trypsin*, possessing specific protease activity (121), has been rendered virtually "salt free" by the new Worthington process. If the addition of this crystalline trypsin to a filtrate should cause a filtrate to become completely inactive upon subsequent intracutaneous injection, it should prove that the skin-reactive substances are proteins or of a protein-like nature.

Not to simply presuppose tryptic activity, two serological tubes were filled with 1 ml of skim milk and were autoclaved with a resultant pH of 6.6. To one tube, 0.025 ml of sterile physiologic saline solution was added and to the other, 0.025 ml of a sterile one percent trypsin solution. Within one minute, at room temperature, the opaque light buff color of the autoclaved skim milk, to which the trypsin was added, showed obvious beginning proteolysis. Within two to three minutes, proteolysis appeared complete in this tube with a change from the opaque light buff color to a clear amber solution.

Six of the most highly reactive filtrates were held in reserve for this particular experiment. The injectee chosen for this experiment possessed only a moderate reactive capacity but was an extremely reliable reactor. A one percent trypsin solution was prepared using physiologic saline solution. This solution was Seitz-filtered into a serological tube and "doubling dilutions" were carried out aseptically to a final dilution of approximately 0.0005 percent trypsin in physiologic saline solution.

The reactor was pre-tested with the various dilutions of tryp-

* Obtained from the Worthington Biochemical Laboratory, Freehold, New Jersey.

sin until no skin reaction resulted. An approximate 0.1 percent trypsin solution was initially used and produced slight burning upon injection. A positive skin reaction to this solution was apparent in five minutes. The reaction increased to a "light-bright" erythema of 30x40 mm in diameter in ten minutes at which time a profound edematous wheal was evident. Higher dilutions were consistently used until an approximate 0.001 percent dilution was the last to give a slight (20x25 mm) response. This was considered "slight" in view of the "light-bright" reaction to saline of 15x15 mm in diameter. The next dilution, 0.0005 percent, gave a 15x15 mm response which was equivalent to the control. The physiologic saline solution and the 0.0005 percent trypsin solution were again injected and both gave an equivalent response, i.e., a 15x15 mm "light-bright" skin reaction for each. This reaction was evident in 10 minutes, was sustained in both instances for 20 minutes, then rapidly faded, and was completely lost in one-half hour. There was slight hemorrhage along the line of the needle puncture with this highly dilute trypsin solution. This observation was consistently, and more manifestly, observed with the stronger trypsin solutions.

The six filtrates were then split into equal quantities and to one aliquot of each filtrate an equal amount of the 0.001 percent trypsin solution was added to make these filtrates equivalent to a 0.0005 percent trypsin solution. An equal amount of physiologic saline solution was added to each filtrate control. The twelve bottles were lightly shaken and allowed to remain at room temperature for approximately eighteen hours. The intracutaneous injections, using 0.025 ml of these fil-

trates, were then performed and the reactions were read after 20 minutes.

One filtrate, to which the trypsin had been added, was virtually the same size (about 15x15 mm) as its control. Therefore this filtrate was considered non-reactive for the injectee. It was not unexpected that a negative skin reaction to any filtrate may have occasionally occurred as the remaining quantities of the filtrates were insufficient to have pre-tested this injectee. Two of the filtrates, to which the trypsin had been added, were moderately reduced (8 and 10 mm) over their respective controls and two others were slightly reduced (each about 5 mm) over their respective controls. One filtrate was substantially increased (about 12 mm) over its control. The behavior of this filtrate remains wholly inexplicable. This particular filtrate was prepared from a necrotic pulp which was quite putrescently malodorous. This fact, neither anything else, seemed to account for the peculiar result. Two other filtrates, also obtained from very similar pulps, were those which were moderately reduced.

It is the author's opinion that this experiment only further substantiates the previous evidence that these filtrates contained a variety of skin-reactive substances undoubtedly present in various amounts. The skin reactivity of four of the filtrates was either wholly or partially destroyed. This seemingly attested to the fact that most of the skin-reactive substances were of a protein or protein-like nature. It was also evident that the majority of filtrates contained skin-reactive substances of such a nature.

5. Attempts to Neutralize Various Filtrates of Pathologic Pulps with an Anti-Histaminic Compound.

Incidental observation led to the recognition of the fact that reactive filtrates, prepared from pathologic pulps exhibiting a fair structural integrity, not infrequently gave a larger and somewhat more ischemic wheal as compared with those filtrates prepared from completely degraded pulp material. The author believed it advisable to determine whether or not these added features in the skin reaction may not be due to, possibly, just a trace of histamine. The dialysis experiments were performed with the remarkably stable histamine acid phosphate but the fact cannot be ignored that perhaps a trace of histamine, per se, may at least exert a minor contributory role in certain of the observed skin reactions. When dealing with partially or especially incipiently decomposed pulps, some of the tissue may only be slightly removed from a state of normalcy. However, a filtrate of normal pulp tissue is only infrequently and mildly reactive. Yet the fact remains that it has been observed that "extracts of practically all tissues, but especially of glands and smooth muscle, produce histamine-like or peptone-like effects...These principles, 'vasodilatin',...implies a chemical identity that has not been established. The reactions correspond closely to those of histamine, often with an admixture of choline actions" (122).

Only four potentially skin-reactive filtrates remained for this phase of the investigation. These may be observed in Table 2 as 32. D.A. #9 and 62. A.M. #8 which were obtained, from a clinical stand-

point, from very nearly normal pulps according to their gross appearance. Filtrates 48. D.G. #9 and 57. L.B. #9 were completely necrotic and putrescent. A sensitive reactor was pre-tested with these filtrates and gave a good response over the physiologic saline control to all four filtrates.

One of the newer antihistaminics*, thonzylamine hydrochloride, was prepared in a one percent solution. A 25 mg tablet was simply dissolved in 2.50 ml physiologic saline solution. This solution was then micro-Seitz filtered. This procedure, other than sterilizing the solution, removed the orange coloring matter and also the binding substance ordinarily used in tablet making. This resulted in a perfectly clear one percent solution of the antihistaminic.

A 4-parts-per-million solution of histamine acid phosphate was diluted with an equal quantity of physiologic saline solution. The injection of only 0.025 ml of this solution (now 2-parts-per-million histamine acid phosphate) gave a 30x35 mm reaction in the injectee. Preliminary tests indicated that the injection of 0.025 ml of a 0.5 percent solution of the antihistaminic produced a very slight skin reaction equivalent to the physiologic saline control in this injectee. The reactions to these solutions having been ascertained, the next step was to dilute a 4-parts-per-million histamine acid phosphate solution with an equal quantity of a one percent solution of thonzylamine hydrochloride (the antihistaminic). When a 0.025 ml quantity of this solution was injected the 30x35 mm reaction of the 2-parts-per-million of the histamine acid phosphate was reduced to a 10x12 mm reaction. For

* The brand "Neohetramine".

all practical purposes these quantities of histamine to antihistamine may be considered approximately stoichiometrically correct, from a pharmacologic standpoint, for this injectee. This was assumed on the basis that this sensitive reactor gave about a 10x10 mm reaction to the control injection of physiologic saline solution.

Exactly 0.2 ml of each filtrate was placed in two sterile 5 cc bottles. To one bottle of each of the four filtrates, an equal quantity of a one percent solution of thonzylamine hydrochloride was aseptically added. To the control filtrates an equal quantity of physiologic saline solution was aseptically added. The eight bottles were lightly shaken and the intracutaneous tests were immediately performed. The usual 0.025 ml quantity was injected and the readings were made after an interval of fifteen to twenty minutes.

The two filtrates, prepared from the pathologic pulps showing good structural integrity, were both moderately reduced (5 and 8 mm) in skin reactivity over their respective controls. While this feature was not particularly striking, the secondary features of the reaction were noticeably diminished. The wheals were somewhat smaller and definitely less ischemic in appearance over the controls. All of these features were, however, only questionably diminished when using the two filtrates of the completely degraded pulps as compared with their respective controls.

These observations led the author to believe that histamine, per se, may have played a minor role in some of the skin reactions observed. This was probably occasionally true when using the filtrates prepared from pathologic pulps having good structural integrity. Hence,

histamine, or a histamine-like substance, may play some part in the hyperemic condition of early pulp disease or possibly in incipient apical pachymenia.

The author was immediately reminded of the fact that the few slightly reactive normal pulp filtrates gave skin reactions very similar to a very highly dilute histamine solution. Only one of these slightly reactive normal pulp filtrates (C.B. #7) remained on hand and it was hoped to be able to demonstrate that, perhaps, the slight reaction occasionally encountered with such a filtrate may be due, possibly, to just a trace of histamine alone. Three sensitive and reliable reactors were pre-tested with this filtrate but not a one gave a greater reaction over a physiologic saline control. By this time the remaining volume of filtrate was exhausted. Although one can hardly conclude from a few tests performed with only a single filtrate, it was not unexpected that histamine, which is quite labile in solution, would have decomposed in the filtrate over a period of time. In contradistinction, the essential skin-reactive substances, in the filtrates of pathologic pulps, remained fully active over a period of many months when maintained in the refrigerator.

6. <u>The Cold Flocculation of Pulp Filtrates</u>.

Vascular pulps, whether normal or pathologic, were frequently responsible for considerable hemoglobin appearing in the filtrates prepared from them. In those filtrates in which the hemoglobin was particularly obvious it had been observed that a degree of flocculation may occur despite their having been maintained in the refrigerator. This phenomenon only occurred after a period of several months, and the degree

of flocculation appeared directly proportional to the amount of hemoglobin present. The flocculi were small and were essentially white with only a slight brownish tinge. They were irregular in size and shape and rather rapidly settled after the bottle had been shaken. Concomitant with the appearance of flocculi, there was a progressive loss of the sparkling light and bright clear red color of the hemoglobin in solution. The presence or absence of either these flocculi or hemoglobin, in a filtrate, was apparently quite unrelated to skin reactivity.

Case Presentation.

Because hemolytic streptococci have been variously incriminated in the causation of rheumatoid arthritis, a single complete case presentation, in which instance such a microorganism was recovered, will be made. The filtrate, prepared from this dental patient, stands as 13. F.D. #8 in Table 1, and as 4. F.D. #8 in Table 5. In the latter table, it may be observed that this filtrate was thermolabile. The following case exemplifies how the data was recorded for each patient. The intracutaneous tests in this instance appear quite simple as the broad control group was not introduced until those cases that appear in Table 2 were undertaken. However, all cases included in Table 1 were observed for an "initial reaction", i.e., within the first half hour, then at six, twelve, and twenty-four hours. These "latent" observations were eliminated among those cases included in Table 2. However, they were carried out in all cases appearing in Table 1. Also, in Table 1, all patients from whom the pulps were removed, the rheumatoid arthritic patients, and all other injectees, were subjected to a

modified Dick Test. The latter was modified only by the fact that 0.05 ml of fresh standard Dick toxin was used. This half skin test dose (STD) was preferably employed in order that all injected volumes would be equivalent. The skin-reactive substances in filtrate F.D. #8, as well as in all others, has not yet, in any instance, been found in any way comparable to the erythrogenic toxin of Dick. The Dick toxin is, of course, quite remarkably thermostable and gives a delitescent reaction not appearing at its height for 18 to 24 hours later. In addition, neither Dick-positivity nor Dick-negativity had any apparent influence on the skin reactions observed to the various pulp filtrates.

The patient (F.D.), from whom the pulp was removed, was re-injected with his own filtrate (F.D. #8) three months later and again gave a very similar (50x60 mm) response in five minutes which was also again completely negative in one hour. This patient appeared only "borderline" Dick-positive in 24 hours as the erythema, although slight (7x7 mm), was quite definite and without any evidence of edema or induration. In actuality, this reaction must be construed as positive for "the slightest erythema, no matter how faint, constitutes a positive reaction if it measures as much as 10 mm in any direction" (123). Having used a half skin test dose, the 7 mm is virtually comparable to a 10 mm reaction when we invoke the "law of diminishing returns" as applied to skin reactions.

Case History and Findings

Date	February 9, 1949
Patient	F.D.
Age	18
Sex	Male
General History	Pneumococcal pneumonia one year ago. This patient's father, a physician, reports that his son has not had a septic sore throat or scarlet fever. The remainder of the history seems quite insignificant.
Dental History	Right maxillary central incisor (8) was very sensitive to hot and cold. A deep silicate restoration probably was the etiologic factor. A patent suppurative fistula was present. Radiographically, there was disclosed a diffuse para-apical myelitis situated interapically between the maxillary central incisors. The pulp in this tooth was partially decomposed and non-vital. This material was essentially purulent, the substance having a greenish-blue appearance, and was fouly putrescent.
Viable Bacteria	Streptococcus hemolyticus.
pH of Suspension	7.50
Suspension Smear	Numerous Gram-variable diplococci and long chains of Gram-positive streptococci.

Intracutaneous tests with filtrate F.D. #8

1. Rheumatoid arthritic patient (P.S.)

	Saline control	Filtrate
Initial reaction	Neg.	Neg.
6 hours	Neg.	Neg.
12 hours	Neg.	Neg.
24 hours	Neg.	Neg.

2. Patient from whom pulp removed (F.D.)

	Saline control	Filtrate	Dick Test
Initial reaction	Neg.	55x65 mm*	Neg.
6 hours	Neg.	Neg.	6x6 mm
12 hours	Neg.	Neg.	7x7 mm
24 hours	Neg.	Neg.	7x7 mm

* 5 minute reaction; disappeared completely in one hour.

3. Normal control (B.J.)

	Saline control	Filtrate	Dick Test
Initial reaction	15x15 mm	15x15 mm	10x12 mm
6 hours	Neg.	Neg.	Neg.
12 hours	Neg.	Neg.	10x12 mm
24 hours	Neg.	Neg.	12x14 mm

The normal control (B.J.) was definitely Dick-positive. However, the reaction of the filtrate in this control was completely negative, when discounting the response to physiologic saline solution. The highly positive homologous reaction, in contrast, constituted a very important observation in view of the fact that hemolytic streptococci were present in the pulp canal. The control (B.J.) was a 20 year old female having a delicate skin-reactive capacity and no known dental or systemic disease.

The rheumatoid arthritic patient was a well developed female of 15 years of age. Her chief complaint was that she had "trouble with her joints". About two and a half years previously this patient recalled having had a "very bad sore throat". Just six months later she noticed an ache in her right hip joint which lasted for about two days; then intermittant short episodes of pain followed. At this time there was no residual loss of motion and no deformity. However, the pain next appeared, a few months later, in the proximal interphalangeal articulation of the third finger of the right hand. At this time the joint did not appear inflammatory but there was definite edema, tenderness and residual deformity. Two months later, pain appeared in both wrists with much edema, tenderness, but no erythema. The motion in these joints was quite limited. About a year later there was pain in many joints with considerable edema and tenderness. A definite active synovitis was particularly manifested in the left elbow. However, there was no concurrent weight loss, or other constitutional manifestation, associated with the joint symptoms. At this time a series of treatments with gold salts proved ineffectual.

At the time that the intracutaneous tests were performed on this patient, she was taking 2.4 gm of acetylsalicylic acid daily. The proximal interphalangeal joint of the third finger of her right hand was markedly enlarged with an advanced degree of flexion contraction in evidence. The left wrist was now quite fixed and crepitant. The right wrist exhibited a fair degree of motion although it was quite enlarged with the overlying skin appearing taut and swollen but with no erythema. All of her teeth were adequately restored with no evidence of any pulp disease. However, some teeth had been previously extracted. Roentgenographically, generalized osteoporosis of the hands and wrists was evident. There was bilateral joint destruction between the carpals and metacarpals. The lytic and cystic osteoporosis, as well as the destructive changes, were classically characteristic of advanced rheumatoid arthritis. Corroboratively, there was an elevated blood sedimentation rate and a significantly high agglutinin titer. The average agglutinin titers, using five different strains of group A hemolytic streptococci, were 1:80, 1:80, 1:320, 1:160, and 1:160 (Olmstead-Lipman technique).

It will be noticed in this case presentation that the dental patient was 18 years of age, the rheumatoid arthritic patient was 15 and the normal control 20. Whenever practicable, all the injectees within a single case investigation, such as this, were chosen as close to the same age level as possible. This consideration was important during the first phase of this investigation while performing concomitant Dick tests. It is well known that with each decade of increase in age within a group, there is a progressive increase in the number of Dick-negative

persons. Another important consideration was the fact that, although the reactive filtrates appeared quite stable, the homologous reaction was performed first, and ordinarily within a day or two, after preparing the filtrates. The tests were then performed just a few days later on the rheumatoid arthritic group and then perhaps a day or two later on the normal controls. Because of the early indication, after the first 26 cases (Table 1), of heightened reactivity among the homologous group over the arthritic group and, in turn, the arthritic group over the control group, it was bel'sved possible that the potency of the filtrates may have waned. Hence, this sequence of performing the injections was reversed for the next 39 cases but there was no apparent change in the previous trend.

This filtrate (F.D. #8) was unlike any other filtrate, with but one exception, due to its high potency and the extremely rapid positive skin reaction which it produced. In addition, it was remarkably specific for this particular patient from whom it was removed. The author believes that a positive Prausnitz-Küstner reaction may have been demonstrable with this filtrate. It seems that it is quite probable that the potent skin-reactive substance in this filtrate was elaborated by the hemolytic streptococcus. As a possible exotoxin, it seems reasonable that it may have particularly sensitized this patient by having left the tooth and by having entered the general circulation. This filtrate (F.D. #8), although unrelated to the erythrogenic toxin of Dick, gave about half the degree of response when tested on a very sensitive reactor who was very strongly positive to the Dick test (Table 5). The homologous skin reaction

to this filtrate may be seen in Fig. 13a and Fig. 13b.

On only one other occasion was a hemolytic streptococcus recovered (Table 1, 15. E.K. #9). Contrary to filtrate (F.D. #8) it was not only profoundly positive (70x75 mm) homologously, but was moderately positive for the rheumatoid arthritic patient (22x24 mm), and mildly positive (12x12 mm) for the normal control person. The physiologic saline control injection in all three of these injectees was completely negative. The rheumatoid arthritic patient was Dick-positive and the normal control person was Dick-negative. Of interest is the positive skin reaction in this rheumatoid arthritic patient who demonstrated a low average agglutinin titer with the five different strains of group A hemolytic streptococci. These respective agglutinin titers were 1:40, 1:40, 1:80, 1:40, and 1:80. Titers less than 1:160 are usually considered of little significance. The positive reactions to filtrate E.K. #9 also occurred rapidly, i.e., within 5 to 10 minutes, and this filtrate, as may be seen in Table 5, was also thermolabile. Filtrates E.K. #9 and F.D. #8 were remarkably similar if not virtually identical.

If these toxins were <u>directly</u> responsible, in an allergic sense, for the symptoms of rheumatoid arthritis, one would expect at least some slight exacerbation to have occured immediately, in the rheumatoid arthritic patients, upon injection. This, however, did not manifestly occur in either rheumatoid arthritic patient. Of course it may be that the toxin was bound (and thus inactivated) by the sensitizing and, hence, "fixing" antibodies of the skin. However, one rheumatoid arthritic patient showed no reaction. If we should postulate an inter-

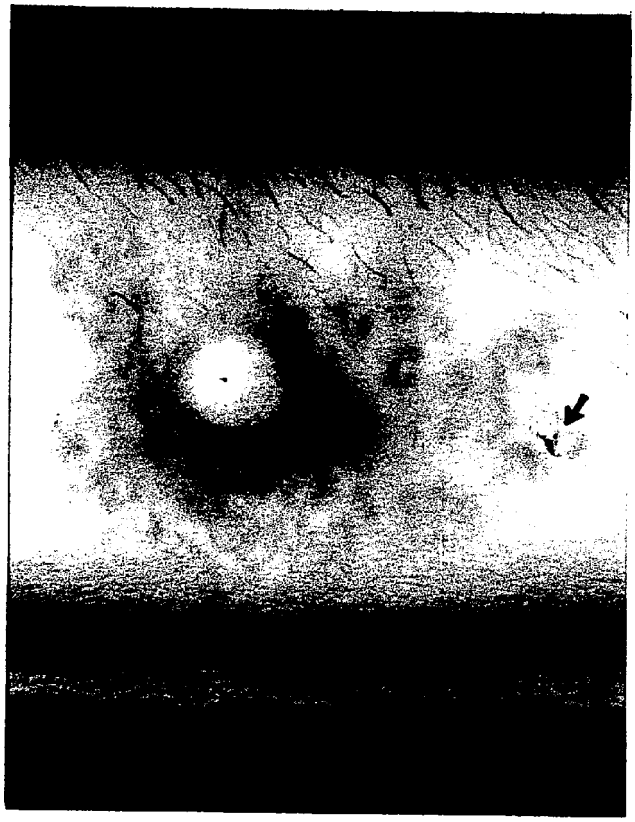

Fig. 13a. This drawing depicts the virtual "half life" of the homologous reaction obtained upon injecting 0.05 ml of filtrate F.D. #8 prepared from the pulp from which a hemolytic streptococcus was recovered. The degree of erythema was approximately twice as great as shown within 15 to 20 minutes after injection at which time the wheal-flare ratio was 1:4 or 15:60 mm respectively. This drawing represents the extent of erythema, and its approximate intensity, one half hour after injection. The control injection (indicated by arrow) was performed with 0.05 ml of sterile physiologic saline solution. The actual photograph of this skin reaction is shown in Fig. 13b.

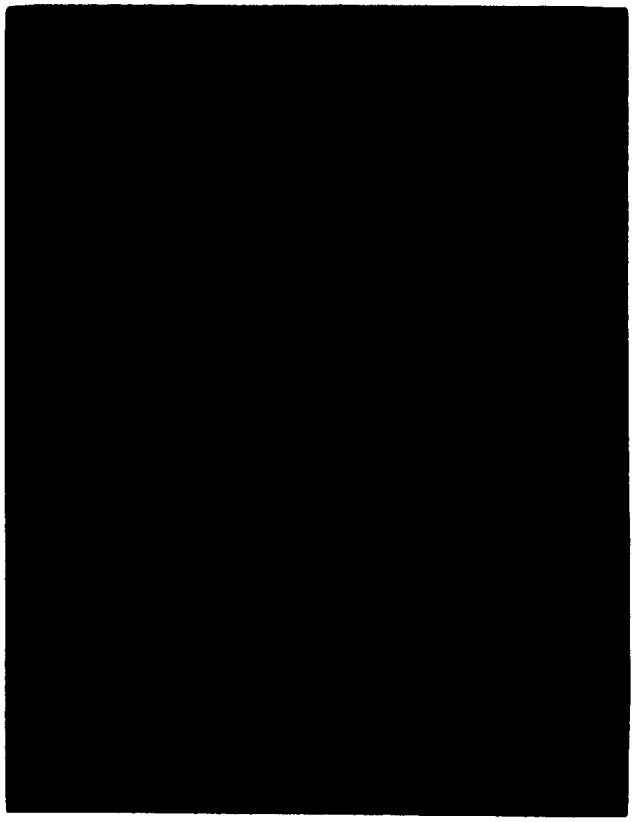

Fig. 13b. The actual photograph of the reaction depicted in Fig. 13a. In color prints, much contrast and color intensity is frequently lost, yet there remains a certain authenticity to other features of the reaction which are significant. The position of the site of the needle puncture over the wheal indicates that the direction of the needle insertion was made from left to right (wrist to elbow direction). It also indicates that the needle was inserted only a "bevel's" depth as the site of needle puncture is only slightly eccentric. The wheal is taut, slightly blanched, and has a regular periphery. The marginal stippling of the flare has already made considerable centripetal progress. There was slight hemorrhage in the line of needle puncture in the control. These color prints should be viewed at about 24 inches, or at full arms length, from the observers eyes in order to obtain the maximum general effect.

mediate shock organ, e.g., the pituitary or adrenal glands, then the manifestation may be quite latent and so slow in producing an "exacerbation" that it could easily be confused with the spontaneous progress of the disease. The pituitary gland is mentioned only because of the remarkable results that have been obtained with the pituitary adrenocorticotropic hormone (ACTH), in the treatment of rheumatoid arthritis, as reported by Markson (124) and several other investigators even more recently. The Shwartzman phenomenon has also been invoked as "the mechanism underlying the production of a variety of spontaneous diseases and syndromes of known and unknown etiology, in which vascular lesions are a predominant feature" (125). In the aforementioned investigation, it has just recently been demonstrated that the adrenocorticotropic hormone is capable of inhibiting the Shwartzman phenomenon by preventing hemorrhagic necrosis in the reactive skin sites following the provocative injections. They believe that this demonstration "may afford a new experimental approach for the elucidation of the beneficial therapeutic effect of the adrenocorticotropic hormone in various diseases affecting the collagenous and vascular systems."

We may wonder just where the allergic concept of rheumatoid arthritis fits into the total picture. In this respect it appears that most people with diseased pulps, even if some substance from such a focus should find its way into the general circulation, would probably be unaffected by it in an allergic sense. Nevertheless, now that physiologically active substances have been unequivocally demonstrated as being formed in many diseased teeth, it is possible that such substances may

act as allergens in certain individuals predisposed to the "allergic state".

It now appears that streptococci, even those of the hemolytic variety, do not necessarily have anything to do with the reason for the increased agglutinin titers occasionally observed in the blood serum of rheumatoid arthritic patients. However, we cannot completely overlook the fact that rheumatoid arthritis has followed in the wake of certain originally local diseases due to hemolytic streptococci, whether these may have been a septic sore throat or puerperal fever. The reason only a few patients succumb to the secondary arthritic manifestations may simply be due to the fact that an inherent predisposition or "diathesis" must first exist. In attempting to adduce any meaningful correlation from these known facts, the author believes that we must first understand what is cause and what is effect. To cite an example, rheumatoid arthritis may follow, in a certain percentage of cases, a septic sore throat due to hemolytic streptococci. On the other hand, this pharyngitis may have led, in other patients, to acute rheumatic fever. Quite earlier it was pointed out that about five percent of all cases of acute rheumatic fever may lead to rheumatoid arthritis. If any allergic mechanism is to be incriminated, then it is important to know at what time or during which episode did sensitization take place, and at what time or during which episode did the "shocking" effect take place. It seems illogical, in view of what is now known, to assume that the joints are the directly sensitized or shocked tissue; if, indeed, they are sensitized at all.

Krogh (126) demonstrated that reactive hyperemia may be due to the absence of a pituitary hormone which is responsible for normal capillary tone. Contingent upon this finding, Lewis (127) states that "it is supposed that when the circulation is brought to a standstill, the normal pituitary [perhaps, actually, Cortisone-like?] content of the blood locked up in the arm becomes reduced, and that the vessels of the part thereupon lose their tone and are ready to expand. It is known that an extract of pituitary gland, when injected intravenously or intradermally, exercises a conspicuous effect on human skin capillaries, producing general or local blanching." It is also known that the anterior lobe of the pituitary gland is the site of the production of those hormones which directly or indirectly, influence skeletal growth and that the adrenocorticotropic hormone is antagonistic, i.e., inhibiting normal growth (128). As such, it is not surprising that the primary lack of this hormone could be indirectly responsible for the joint symptoms of rheumatoid arthritis.

In Fig. 14a and b the other types of reactions that may result from the intracutaneous injection of filtrates of a slightly different reactive character are shown. These positive skin reactions, and that shown in Fig. 13a and b, constitute the three essentially different types encountered in this investigation.

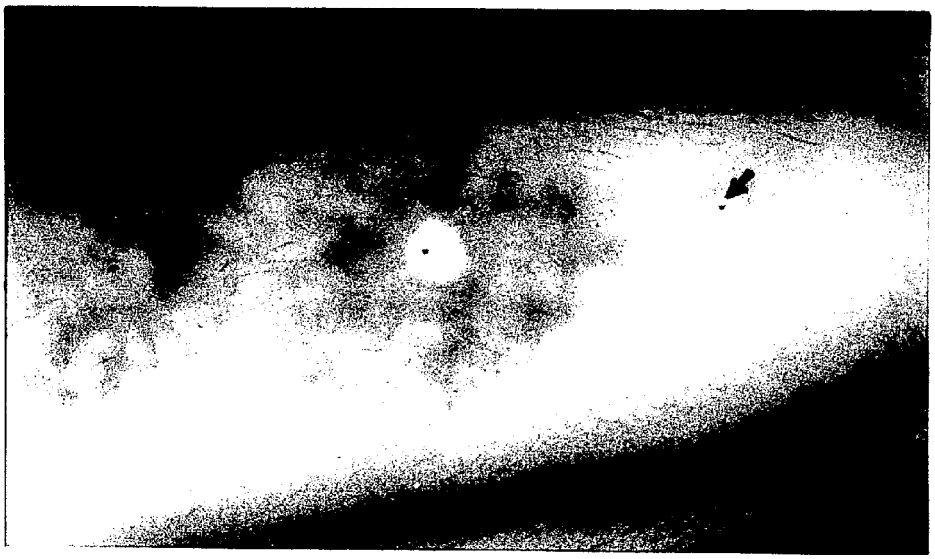

Fig. 14a. This drawing depicts two additional types of positive skin reactions which were most commonly encountered in this investigation. All of these injections were performed with 0.025 ml quantities. In this procedure a normal pulp filtrate (P.D. #7, Table 3) control (indicated by arrow) was completely negative. The reaction on the left was elicited by filtrate L.B. #9 (Table 2), and the reaction to the right by filtrate G.L. #24 (Table 2). These reactions are shown at their height, i.e., about 15 to 20 minutes after the injections were made. Hence, no appreciable marginal stippling is as yet evident. The intensity of erythema, as represented here, falls between the light-moderate and light-bright. At least a two inch spacing between the injection sites (the drawings and photographs are approximately three-quarters actual size) usually assures the avoidance of two adjacent positive reactions becoming confluent. The actual photograph of these reactions is shown in Fig. 14b.

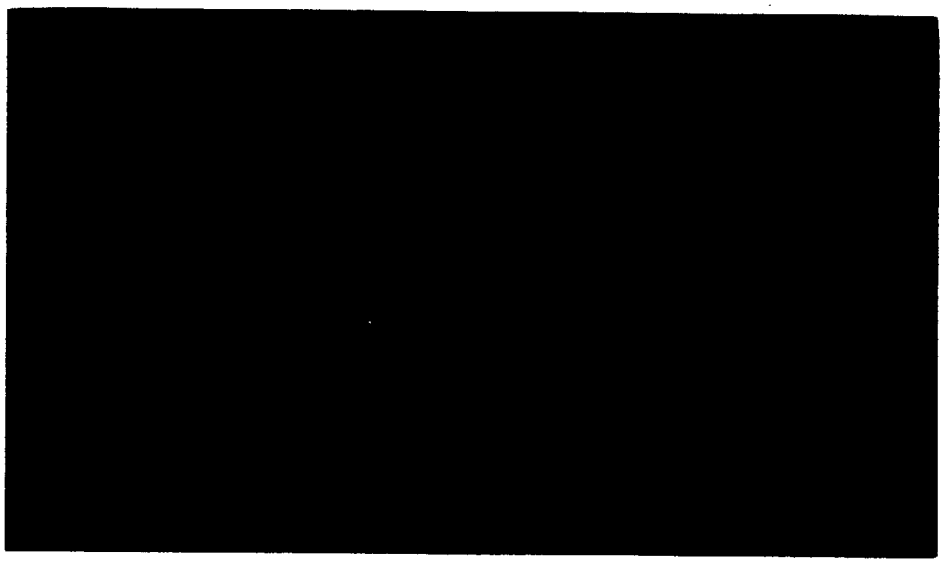

Fig. 14b. The actual photograph of the reactions depicted in Fig. 14a. The reddish hues of the erythematous areas were especially lost in the printing process. However, both flares in the two positive reactions are quite comparable. All original injection "blebs", due to the volumetric displacement incident to the quantity of filtrate injected, in the three sites, were absolutely equivalent. However, in the reaction to the left, the resultant wheal was negligible and it assumed almost the same degree of erythema as did the flare. This is not unusual when using filtrates prepared from completely decomposed pulps. The reaction to the right shows a moderately developed wheal which is fairly ischemic. This type of reaction is most common when using filtrates prepared from incipient pathologic pulps exhibiting fair or good structural integrity. The slight elevation of the control site is only the residual effect due to the injection "bleb".

DISCUSSION

The primary purpose of this investigation was to determine whether or not toxic, or at least physiologically active, substances are formed within pulp-involved teeth. After the occasional presence of such substances had been incontrovertibly established, certain procedures were undertaken to determine whether or not these substances could, conceivably, play some part in the causation of, or in some way be related to, certain cases of rheumatoid arthritis. Some attempts were also made to characterize these substances biochemically.

To isolate bacteria from the pulps of teeth, to culture them artificially in abundance, and then to skin test certain individuals with these bacteria, various fractions thereof, or their products, is obviously a spurious procedure. Such a mass of bacterial protoplasm, or even their products, is in no wise comparable to the total substances as produced in a diseased pulp. It is common knowledge that the most elaborate bacteriological laboratory methods frequently fall far short of absolutely reproducing the exact conditions existing in body fluids and tissues. It was the author's primary purpose to remove, *in toto*, all of the material within a pulp canal (including bacteria, bacterial products, and tissue degradation products) and to develop a procedure whereby these substances could be expeditiously extracted. Immediately after extraction it was imperative that the bacteria be removed by filtration. In this way, no substance appearing in the filtrate could possibly have been construed as something other than that which had actually been present within the tooth. Ordinarily, the time that elapsed between the removal of the pulp canal contents and filtration varied between two

and three hours. This length of time was required only because, on occasions, as many as two, three, or even four specimens were procured and would be carried through the total procedure simultaneously. A two to three hour interval before filtration was found to have had no influence on the specimen suspension as the lag phase of most bacteria under the most ideal conditions is ordinarily this long. In addition, the first two or three specimens obtained, at any one time, were immediately placed in a refrigerator (12°C) for the period of an hour or two after having procured them. This only slightly cooled the suspensions until the laboratory procedures were begun.

Certain deviations from accepted procedures in the clinical approach to the pulp canal, and in the removal of the contents therefrom, are wholly peculiar to this investigation. Unless assiduously and expertly executed, the removal of the total pulp canal contents in one operation, without the advantage of previous therapeutic protection, may lead to a high incidence of severe exacerbations. It has been pointed out (129), that "during the canal cleaning we invariably push particles of the necrotic pulp beyond the apical foramen where they normally produce inflammations..." However, the severe exacerbations that may ensue are due to the inoculation of the periodontium and alveolar bone when instruments are inadvertantly exteriorized beyond the confines of the pulp canal. The author, in his preliminary experiments, always found normal pulps to be sterile as determined by culture. If these pulp canals are not conferred therapeutic protection after pulpectomy, with an agent such as camphorated para-mono-

chlorophenol, but only a dry sterile paper point is inserted, a mild to severe tissue response may occur. Although no growth was obtained from the initial inoculum from the pulp canals, streptococci have occasionally been cultured from them just 24 hours later. Once these streptococci established a periapical infection, the teeth demonstrated only a mild pericementitis in a few days. If staphylococci alone, or in conjunction with streptococci, established the infection, a severe exacerbation occasionally occurred only a day or two later. The symptoms were, essentially, considerable edema and cellulitis of the adjacent tissues, a mild, sometimes throbbing pain, and, upon re-entering the canal, considerable pus usually spurted forth. It has recently been reported (130) that staphylococci have been recovered from all subacute and acute periapical exacerbations. These findings are contrary to the author's experience. Streptococci alone have often been recovered from subacute and acute periapical exacerbations. However, the presence of staphylococci have occasionally been observed to probably enhance the rapidity and severity of exacerbations. As early as 1915, Ulrich (131) pointed out that hematogenous infection is the most plausible explanation for all apical abscesses with the probable exception of teeth exposed or affected by deep carious lesions. It may be during a transient bacteremia that bacteria settle in the periapical area and find this environment quite propitious in which to multiply. It seems not unlikely that the so-called hematogenous route may terminally occur via the capillaries supplying the periodontal membrane. Bacteria may, however, in most instances, be originally derived from the flora of the gingival crevice.

Pollia (132) states that "radiographic studies of periapical lesions do not substantiate the opinion that periapical infections are under pressure and are therefore being 'forced' into the system." This is only true to the extent that when periapical infections of an acute nature occur, the greatest evidence that such areas are under pressure is clinical. Severe induration of the gingival tissues overlying the apical region in chronic cases, and extensive regional edema in the acute cases, frequently attest to this. Further proof is the fact that, upon establishing a patent canal in an affected tooth, pus not infrequently spurts forth. In chronic necrotic pulps, gases may be evolved upon entering the pulp canal, and although we ordinarily think in terms of the expansion of gases, those gases that are under pressure usually go into solution. In other diseased pulps that remain clinically and subjectively negative, the periapical changes that are revealed upon radiographic examination also, it is true, do not show that they are under pressure or that anything is "forced" into the system. The mere fact that proper obturation alone (Fig. 1), results in the ultimate return to normal structure (Fig. 2), is ample evidence that the causative substance is at least initially derived from the pulp canal. If only apical pachymenia, or the so-called mere thickening of the periodontal membrane in the immediate vicinity of the root apex, is in evidence, there exists a good possibility of an interchange of fluids from the canal to the alveolar bone and vice versa. Although the periodontium is not a true membrane, it is structurally capable of acting as such. In view of this fact, at least an exosmosis, or the passage of fluids from

the pulp canal, at least to, and possibly through the "membrane", must account for the hypertrophic condition of the periodontium. Certain blood elements, e.g., the globulins, albumins, electrolytes and other soluble principles, may be capable of endosmosis, and as a result a mutually functioning biosmosis, may be established. Such an interchange would only assume greater importance after the blood and lymph flow has become static. In advanced periapical lesions, with the destruction of the periodontium over the apex, there can, of course, be no "osmosis" but there is abundant clinical and histopathological evidence of a bidirectional exchange. If only a unidirectional movement, namely an egress of the pulp substance, its degradation products, bacteria, and all else occurred, the pulp canal would become a perfect void. The author has encountered but few instances in which this probably has occurred. This condition is most obvious in <u>necrosis sicca</u> or the so-called "dry gangrene" of the pulp, which occurs quite infrequently. In one of the preliminary experiments in which 30 freshly extracted "normal" anterior teeth were each placed within the peritoneal cavity of a guinea pig, there was incontestable evidence of a bidirectional exchange between pulp substance and the peritoneal fluid. Filtrates were prepared from these pulp canals at approximately five day intervals for a duration of 100 days. These filtrates, upon intracutaneous injection, became increasingly reactive for the author and less so for the guinea pigs. The most plausible explanation for this phenomenon is the exchange of foreign protein. This exchange occurred through the granulation tissue which proliferated over the apices of all teeth

and arose from the hyperemic mesenteries. Leukocytes and bacteria were also readily demonstrated to make their ingress through apical foramina under such conditions. The evidence that substances may leave a tooth through the apical foramen is irrefutable. The likely ingress of blood serum elements, and certainly of bacteria and leukocytes, is probably most common even with teeth *in situ*.

Various species of streptococci have been incubated in human blood media, under both aerobic and anaerobic conditions, for as long as 90 days. Despite the fact that frequent pH determinations were made throughout this period, the original pH of 7.30 was maximally depressed only about an average of 0.5 of a pH unit, for all cultures, in 48 hours. The cultures remained at this pH for 88 days and all of the streptococci in all of the cultures remained viable. No putrefactive odor was evolved from any of the cultures and growth was not macroscopically ascertainable. This bears out the fact that streptococci have long been regarded as very weakly proteolytic and that they alone do not seem to possess the qualitative or quantitative enzyme systems to effect putrefactive proteolyses which may be readily detected by the evolution of a very offensive odor. This is especially interesting in view of the fact that the three species, *Str. viridans*, *Str. hemolyticus*, and *Str. anhemolyticus*, were obtained from root canals which were extremely malodorous. Autolysis, to some degree, may occur as a result of stagnation within the pulp canal. Clinically, and histopathologically, it seems that leukocytes may play an important role (in conjunction with streptococci) in pulp degradation with the liberation of putrefactive odors. This would probably be accomplished

by their peptic, autolytic, or tryptic enzymes (133). All frankly purulent pulps that evolved an obviously putrescent odor, varied from pH 7.10 to 7.70 (Tables 1 and 2) with an average pH of 7.45. On only two occasions when the pulp chamber was entered, and considerable pus actually spurted forth, was there no noticeable putrescent odor. Radiographically, one of these teeth showed evidence of a rarefying osteitis almost a centimeter in diameter mesiodistally, and also in its vertical diameter. The other tooth evidenced a large 2x2.5 cm radicular cyst. Oddly enough, both of these pulp canal specimens tested pH 6.90. This may be of significance in respect to Menkin's findings (134) that when the pH falls below the level of 6.90 or 6.80, macrophages and polymorphonuclear cells no longer survive and frank suppuration ensues. Although it has been demonstrated that leucocytes contain appreciable quantities of histamine (135), (136), many of the filtrates prepared from purulent specimens were not found to contain skin-reactive substances.

In a series of early investigations, it was found that filtrates of putrefied whole saliva caused burning upon being injected and that the entire area of erythema was light and slightly edematous. Other attempts to cause human blood serum to putrefy, when heavily inoculated with various species of streptococci, were just as futile as the attempts undertaken with blood. A few skin tests performed with filtrates obtained from fistulous aspirates and trans-canal aspirates were occasionally non-reactive even if the filtrate prepared from the canal should be substantially reactive. It seems that the pulp canal may

indeed be a unique environment. It is known that "most pathogenic aerobes are facultative anaerobes or facultative microaerophiles", and that, "parasitic species may prefer tissues and cavities with low oxygen tension" (137). The occasional recovery of strictly anaerobic streptococci may attest to the fact that the pulp canal can be just such an environment.

Relative to the approximate 40 percent positivity in skin reactions (Tables 1 and 2) that occurred in the group comprised of the patient from whom the specimen was removed and another patient with periapical disease, it must be admitted that this observation is virtually identical to that made by Price (138). Using his filtrates of crushed extracted teeth, he found that many patients were "sensitized to the toxic substance as extracted from infected teeth of other individuals, and in other instances, evidences of a marked specificity to the infection of their own teeth." The rheumatoid arthritic group, having demonstrated about a 20 percent positivity to the filtrates of pathologic pulps, just borders on the point of being statistically valid. The group comprised of persons with no known systemic or dental disease, which showed approximately a 10 percent positivity, imparts high statistical validity to the first group.

It seems only reasonable that the first, or "pulp-involved" group must, at some time, have become sensitized to the identical or very nearly identical antigenic substance or substances. It would be entirely conjectural to maintain that the sensitizing substances were solely derived from the diseased tooth, or other previously diseased teeth, but it is indeed possible that such may be the case. This sen-

sitivity does not seem to be a true bacterial allergy for, as Cooke (139) states, "...the majority of the clinical evidence of bacterial allergy belong in that group we have designated as the tuberculin type. The reason for this is a rather negative one, namely, patients with bacterial allergy, with rare exceptions, do not react by immediate wheal reaction on test with bacterial filtrates or heat-killed vaccines...Non-protein drugs may give immediate reactions...but the skin test is negative in all cases in which an immediate urticaria is not a part of the clinical picture." Cooke also points out that "allergy to chemicals may be similar to true protein allergy despite the fact that the earlier objections to this theory were based chiefly on the non-protein character of the drugs concerned and the failure to elicit positive skin tests. The newer knowledge of the importance of haptens in hypersensitivity supplies the missing link." Cooke recognizes the existence of non-reaginic substances and even suggests the term "reactin or reactogen...to indicate substances, e.g. tuberculin, that react with antibody but do not stimulate antibody formation."

In deference to the "hapten hypothesis" which has been invoked as an explanation for certain positive skin reactions, this possible phenomenon could not be ignored. Some consternation was caused by the fact that the rheumatoid arthritic patient, from whom the unusually thermostable skin-reactive factor appeared in the pulp filtrate (C.T. #7, Table 4), was consuming various quantities of sodium salicylate daily. On first impression, one is inclined to believe that the salicylate, per se, or as a conjugate, may have found its way into the pulp

canal and that this may have been the reactive substance or the substance responsible for the positive reaction. Most patients on salicylate therapy demonstrate an average blood level of 5 to 25 mg percent of this drug. Sodium salicylate, in physiologic saline solution, was prepared in 5, 10, 15, 20, and 25 mg percent dilutions. These dilutions were Seitz-filtered and injected intracutaneously into both the rheumatoid arthritic patient (C.T.) and into a normal control. All of these injections were completely negative. This does not, however, eliminate the possibility of some conjugate of the salicylate in the blood from acting antigenically. It was decided to seek such a factor in the blood serum of the rheumatoid arthritic patient. In a number of instances it had been found that old serum, or serum heated to 56°C for one half hour was usually capable of eliciting some degree of skin reactivity even when it did not do so when fresh. Heated fresh serum, homologously injected, may also demonstrate this phenomenon. Hence, it was necessary to differentiate the skin-reactive factors, found in the various pulp filtrates, from those normally occurring blood elements which may have been potentiated to skin-reactive substances upon heating. The differentiation of such substances also from possible salicylate conjugates is essential.

A one percent solution of the blood serum of a rheumatoid arthritic patient (C.T.) was prepared. This concentration is well in excess of the concentration of the amount of blood elements that may be ordinarily obtained from a normal pulp in the preparation of a filtrate. This one percent serum solution elicited a 10x12 mm skin reaction, before

it was heated, when injected into a normal control. When injected into the rheumatoid arthritic patient (homologous injection), it elicited only an 8x10 mm skin reaction. Both of these were only negligibly, and hence, insignificantly, greater than the physiologic saline control injections. After heating the one percent serum filtrate at 56°C for one half hour, it showed no visible turbidity, but the skin reactions increased to 20x22 mm and 10x15 mm, in the normal control and rheumatoid arthritic patient, respectively. This reactivity was completely destroyed, i.e., the skin reactions were equivalent to the control injections upon autoclaving the serum and then again testing it upon both injectees. This procedure was repeated, on one other occasion, with practically comparable results when using the blood serum of the normal injectee. These procedures quite conclusively indicated that the highly thermostable substance was not a potentiated blood component and that it was unrelated to the salicylate content of the serum of the rheumatoid arthritic patient. Neither is there any demonstrable difference when normal blood serum, also similarly treated, is injected into a normal or rheumatoid arthritic patient. Obviously, no significance, in this respect, can be ascribed to the thermoduric filtrate obtained from a diseased pulp in the rheumatoid arthritic patient.

Although not a single positive culture was demonstrable from the four pulp-involved teeth of the rheumatoid arthritic patients, it is not to be unexpected that sometime during the degradation bacteria were present. The noxious degradation products within pulp canals of diseased teeth hardly constitute a favorable substrate in which bacteria

can remain viable. This is not, however, particularly true for the relatively resistant streptococci. It seems most plausible that bacteria would abound most luxuriantly at that ill-defined junction between the normal tissue and that which is frankly pathologic. This area is, in advanced pulp disease, somewhere within the periapical area. Because of the necessity of remaining just within the confines of the tooth in obtaining the various pulp canal specimens, it is quite possible that many otherwise positive cultures may have been missed which would not have occurred had the conventional method of obtaining a culture from the pulp canal been employed. The bacteria recovered in this investigation should therefore be construed as representing the "bacteriology of diseased pulp canals" and not the usual concept which also includes the bacteria recovered from the periapical region. In checking against the growth obtained with the conventional procedure of procuring an inoculum from each canal of all teeth in Table 2, about a 15 percent higher incidence of positive cultures were obtained. A revaluation of the data regarding these cases as positive for bacteria, did not materially account for many of the positive skin reactions and this was not, therefore, incorporated into the data. All cases appearing in Tables 1 and 2 are listed in the order in which they were performed. It was especially interesting that case 46 through case 60, barring the likely single contamination, gave no growth. This may reflect, in part, an unsuspected situation. Many of the pH values in this group are quite low and the periapical lesions in most patients in this group were quite extensive although they were not ordinarily designated as such in the table. Dur-

ing the two-month period that these specimens were obtained, the number of patients available to the author were quite abundant. As a result, only those patients exhibiting the most extensive periapical involvements, radiographically, were ordinarily selected. Most of these teeth generally represented quite chronic periapical lesions and could not, with the single instance of probable contamination, possibly have been invaded by bacteria from the saliva. In addition, the author was extremely careful, among this group, not to propel material beyond the apical foramen when obtaining these pulp canal specimens. In the author's experience, it is in many of these chronic situations that injudicious instrumental intervention can readily disturb a quiescent focus by superimposing a rather intractable local sub-acute exacerbation. All of these factors must be considered as possibly contributory to the number of consecutive negative cultures in this group. Many of these specimens were quite putrescently odoriferous, and in these instances bacterial growth was not obtained by this experimental procedure for procuring an inoculum or by the conventional method. This certainly bore out the fact that a putrescent odor is no indication that viable bacteria are present. In fact, if a "pulp" was completely decomposed and quite malodorous, the likelihood of demonstrating viable bacteria just <u>within the pulp canal</u> was rather infrequent. The bacteria will have invariably succumbed to their own obnoxious stench and end-products. How completely erroneous was the method of root canal therapists in the past who regarded putrefactive odor as indicative of infection.

We cannot, as yet, wholly disregard a possible important role

on the part of the Hemolytic streptococci growing, and producing a highly skin-reactive substance, within the root canals of diseased teeth. This is true even if we should doubt the fact that the elevated agglutinin titers found in certain rheumatoid arthritic patients which, according to Holman (140), "indicates that there has been intimate and rather prolonged contact between the streptococci and the interior tissues of the body." Although only two filtrates were obtained from pulp canals in which hemolytic streptococci were found, the results therewith seemed to substantiate certain observations of other investigators. Williams (141), in this respect, has shown that "the skin test properly controlled has a wide application in demonstrating the presence of exotoxins of hemolytic streptococci...", and that, "...the hemolytic streptococcus toxin is very complex and that its range in specificity is...much wider than that claimed by the Dicks, Birkhaug and others."

Ordinarily, exotoxins are thermolabile and deteriorate upon standing. Similarly, both filtrates from the canals, from which hemolytic streptococci were isolated, were thermolabile. Of course, the only final criterion of a true exotoxin is its ability to stimulate the production of a specific antitoxin when injected into a suitable animal. Unfortunately, the amount of filtrate is much too small to carry out such a procedure and practically all others of a similar nature.

In taking a broad over-all view of the skin reactions elicited with the various filtrates and being aware of the vast complexity of the reactive substances, only certain generalizations can be made. By exclusion, if one of the skin-reactive factors in a filtrate were strepto-

lysin or even leucocidin it would be inactivated at 56°C for one half hour. This holds true for many other known thermolabile substances. Apparently, far fewer pulp filtrates are thermolabile in all probability. The ptomaines, per se, or perhaps as protein-ptomaine complexes could possibly contribute some little part to a positive skin reaction. It is also well known that the skin-sensitizing antibodies are capable of instantaneously "anchoring" simple foreign substances and that these may in this way, give rise to an antigenic complex. Skin reactions are, indeed, far from infallible, and other than expressing a hyper-activity of the immunologic mechanisms which we look upon as "allergy", they may express a sensitivity on the part of the injectee to reactive substances that are present in certain pulp filtrates. Injectees also vary in their skin-reactive capacities and, therefore, certain secondary features of a reaction may be more intimately associated with the cutaneous vascularity. The reactions to the various pulp filtrates and other substances follow closely certain observations made by Lewis (142) that "...the brighter and more extensive the flare the more certain that whealing will happen," and that "...it is also quite clear that the flare is unessential to whealing." The author particularly demonstrated the latter phenomenon when, in early experiments, a one percent crude trypsin (pancreatin, U.S.P.) solution was injected. With this solution a huge wheal is effected with but negligible erythema. The sustained hypersuffusion probably occurred as a result of tryptic activity within the wheal.

The majority of skin-reactive factors within the various filtrates, obtained from pathologic teeth, seem to be protein, protein-like, or intermediate (proteose) or denatured protein substances in nature.

The evidence obtained from such procedures as, heat inactivation, dialysis, and crystalline trypsin digestion attest most convincingly to this fact. The protein test is, of course, not confirmatory but only serves not to negate this assumption. In virtually all positive reactions, at least some degree of an immediate wheal was observed. This too, it should be pointed out, is characteristic of protein substances (143). A trace of histamine or histamine-like substance seems to be more prevalent in filtrates prepared from diseased pulps, not in an advanced stage of decomposition, but demonstrating fair structural integrity. This fact may introduce another variable which cannot be blithely dismissed. In 1926, Hare (144) demonstrated that a histamine stimulus renders the skin refractory to protein allergens and that the latter stimulus renders the skin refractory to histamine. Whether a trace of histamine and a protein skin-reactive factor co-existing in certain filtrates could possibly neutralize each other is not known. In three tests, in which only a drop of 4-parts-per-million of histamine and phosphate were added to a few drops of reactive filtrates remaining in the bottles, the skin reactions to these solutions were all increased.

The author, in his preliminary animal investigations, has not been able to confirm the lethal potentialities that Price (145) ascribed to his filtrates prepared from crushed diseased teeth. However, certain of Price's statements have been extraordinarily confirmed if it can be assumed that he was working with filtrates in which the skin-reactive substances were primarily derived from pathologic pulps. Price maintained that "in cases where the toxin was extracted from the patient's

own tooth, the test, when positive, developed more rapidly and usually more severely...The boiling or even autoclaving of teeth does not always destroy all the toxic substance...I have shown the results of studies comparing the toxin of dental infections with histamine, and it was shown that there were some factors in common, one being the marked engorgement of the mesenteries, dilatation of the blood vessels of the small intestine, and one sensitized rabbit died within two minutes after receiving its infection [sic]. Further studies of this toxic substance indicated that it had some characteristics differing very distinctly from those of histamine."

In a few preliminary experiments, the author prepared suspensions from the dentin "cuttings" of freshly extracted normal and pathologic teeth. The concentration of dentin filings in the suspensions were adjusted to approximately one percent for each specimen. These suspensions were mechanically agitated and Seitz-filtered. Injections with these filtrates were only made heterologously in a person with no known dental or systemic disease. Three out of six of these filtrates produced a rather moderately intense erythema, although the wheals were quite small. At the time these experiments were performed, 0.1 ml of the filtrate was being used as the quantity injected. The reactions occurred within 15 to 20 minutes and averaged about 17 mm greater in average diameter over the physiologic saline control. Two of the positive reactions occurred in filtrates prepared from suspensions of dentin obtained from pulp-involved teeth and one from a normal tooth. Of particular importance is the fact that these three filtrates were autoclaved and upon reinjection gave a slightly reduced, but nevertheless,

substantially greater reaction (average 12 mm) over the control injection. This highly thermoduric factor is probably related to the collagen which constitutes virtually all of the 30 percent organic matter of the dentin (146). This observation also casts some doubt upon the origin of the skin-reactive substances with which Price was working. In addition, Price probably did not recognize the fact that he may have been working with a variety of skin-reactive substances in any one filtrate, although he did maintain that there were distinct differences among his various filtrates.

In the single instance in which a highly thermoduric factor was encountered (Table 4, C.T. #7), it is not unlikely that it may have been derived from the dentin. Armed with the foreknowledge that an excessive amount of dentin filings in a suspension could possibly cause skin-reactivity, this fact was always kept in mind. As indicated in the previous case, the pulp material was quite sparse. As a result, the instrumental efforts may have been a little too ambitious as it is well remembered that the dentin filings were in excess of the amount usually obtained. It is important in the preparation of a suspension of avascular or degenerated pulps to obtain as much material from the walls of the canal as possible if the amount of material is sparse. However, in view of what is known, it is probably inadvisable to remove too much dentin. Of additional interest, is the fact that this filtrate (C.T. #7) produced only a rather moderate degree of erythema in which the wheals were also very small.

Occasionally, certain feasible explanations for a number of rather abstruse findings have been incorporated throughout the discussion and these should not be construed as absolute proof, but as hypotheses upon which future investigations in this field may be based.

SUMMARY

1. A method has been described whereby the pathologic substances found within the pulp canals of diseased teeth may be aseptically obtained, efficiently extracted and filtered, and skin tests performed therewith.

2. The complete data, for all phases of this investigation, were derived from approximately one thousand intracutaneous injections performed upon 101 human subjects. Exactly 517 injections, excluding controls, were performed with filtrates prepared from both normal and pathologic teeth. Immediate markedly positive skin reactions were often obtained when using the filtrates of pathologic pulps which attested unequivocally, to the presence of a physiologically active substance within these preparations.

3. The reactions to the filtrates from pathologic pulps, in the various groups, were as follows:

(a) The homologous patient, and patient with a pathologic pulp, responded positively 25 times in 65 injections, or about twice in every five injections or almost 40 percent of the time.

(b) The rheumatoid arthritic group responded positively 13 times in 65 injections, or once in every five injections or exactly 20 percent of the time.

(c) The normal control group responded positively 6 times in 65 injections, or almost once in every ten injections or almost 10 percent of the time.

4. Particularly high statistical validity exists in the number of positive reactions observed by the use of the filtrates, prepared from pathologic teeth, when injected, homologously or heterolo-

gously, into patients evidencing periapical diseases as compared with the normal control group.

5. Bacterial products appeared to play an important role in the causation of the positive skin reactions observed among the "pulp-involved" group; but this fact was only evident when compared with the group having no known dental or systemic disease. The mere fact that, within the "pulp-involved" group, almost as many positive skin reactions occurred whether or not bacteria were demonstrably present does not preclude their having been present.

6. The positive skin reactions observed with filtrates from pathologic pulps appeared to be of three distinct varieties, namely:

(a) A bright extensive erythematous area with a well-developed ischemic wheal, which results from the use of filtrates prepared from the contents of pulp canals from which hemolytic streptococci have been recovered.

(b) A moderate intensity of erythema which is accompanied by a moderately developed wheal which is only mildly ischemic. This occurs most frequently in those positive reactions obtained with diseased pulps having fair or good structural integrity.

(c) A moderate intensity of erythema which is accompanied by a slight wheal which is negligibly ischemic. This type of reaction is most frequent when using those filtrates prepared from completely degenerated, purulent, or liquefied pulps.

7. The radiographic diagnosis, gross clinical appearance, vitality, and the presence of a fistula or a putrescent odor, were essentially unrelated to positive skin reactivity on the part of any filtrate.

8. Whereas normal pulp filtrates consistently demonstrated a pH value of 7.35 to 7.40, the pathologic specimens varied from 6.80 to 7.90. In the absence of gross structural changes, it appeared that the following criteria may be considered diagnostic of a pathologic pulp:

(a) If the pH value of a specimen was greater than 7.45.

(b) If the material was adequate and lyophilic and less than pH 7.30.

It appeared that cytochemical changes, determinable by an aberrant pH reading, may ordinarily have preceded structural changes. Relative to the effect of the pH value of any filtrate, from the lowest value obtained (pH 6.80) to the highest (pH 7.90), it appeared to play no known part in skin-reactivity.

9. In a smear made from the various specimen suspensions, the only microorganism that may occasionally have been identified with any degree of assurance were the streptococci. Other organisms would have been easily confused with pulpal and dentinal debris. It seemed likely that streptococci may occasionally be present in diplococcal form. There existed, of course, no proof that the bacteria observed in the smear were those which were recovered upon culture.

10. Of the 65 specimens cultured, 19 were found to be positive. This incidence represented the recovery of bacteria essentially from the pulp canal only. Among the 23 smears recorded as positive, bacteria were cultured on only 7 occasions or less than a third of the time. This may have indicated that when bacteria were definitely ob-

served in the smear, that they may have been non-viable about two-thirds of the time. Streptococci were cultured from all of the 7 specimens in which both the smear and culture were found positive.

11. According to the results of the Prausnitz-Küstner procedures which were performed, it appeared that the majority of filtrates giving positive skin tests contained skin-reactive substances of a non-reaginic nature. Precipitation tests performed concurrently with the above procedure were also negative.

12. The apparent existence of possibly a number of skin-reactive substances, in different quantities in the various filtrates, led to attempts to discover what some of these substances may have been. For this purpose various dilutions of ptomaines and other chemical agents, known to result from putrefaction, were injected intracutaneously into a number of subjects. All of the reactions elicited with these substances could not, by analogy, have been regarded as entirely comparable to those reactions obtained with any of the reactive pulp filtrates. The ptomaines and other chemical substances are, of course, intermediate products of putrefaction which, in the end process, may be lost. Nevertheless, they are formed in abundance upon complete proteolysis and especially in a pulp canal which affords only a low-oxygen tension. As virtually all of the chemical substances tested were quite irritating upon intracutaneous injection it is likely that a mixture of these agents could, at the height of their existence, have contributed materially to the cause of demineralization of the alveolar bone within the periapical region.

13. In an effort to characterize the skin-reactive substances in the various filtrates, as to some of their physical and chemical attributes, the following observations were made on several filtrates in each test:

(a) The skin-reactive substances were not found to be dialyzable.

(b) Various skin-reactive filtrates were inactivated at different levels of heat-treatment.

(c) Protein was usually just adequately present in the filtrates so that it may be demonstrated. This, of course, only proved that protein substances co-existed with the skin-reactive substances and not that they were, necessarily, one and the same.

(d) Attempts to inactivate the skin-reactive substances by ultraviolet irradiation were somewhat erratic, although a few reactive filtrates showed attenuation.

(e) In an effort to effect tryptic digestion of the skin-reactive substance by the use of "salt-free" crystalline trypsin, some of the filtrates demonstrated reduced reactivity.

When evaluating the results of these tests it appeared that perhaps most of the skin-reactive substances were, and probably the majority of these filtrates contained, skin-reactive substances of a protein or protein-like nature.

14. There is some evidence that a trace of histamine or histamine-like substance may have been present in certain filtrates. This seemed especially true of those filtrates prepared from pathologic pulps which possessed a fair degree of structural integrity.

15. A very potent skin-reactive substance, entirely unrelated to the erythrogenic toxin, was obtained from two pulps infected with hemolytic streptococci. Contingent upon this finding, the role of such a skin-reactive substance should be further investigated in relation to the agglutinin titers of rheumatoid arthritic patients to certain strains of group A hemolytic streptococci.

16. Now that the occasional existence of skin-reactive substances occurring within diseased pulps has been definitely established, we should not assume that periapical disease is of greater significance than periodontal disease until similar tests using a comparable amount of diseased gingival tissue in the preparation of filtrates are undertaken. Although the positive skin reactions were indicative of these filtrates acting antigenically, there is, as yet, no evidence that any of them were capable of eliciting an "allergic" response in any other tissue. Neither is there any evidence, at present, that by any other mechanism any of these filtrates were capable of producing any systemic or constitutional manifestations.

17. The preparation of a pulp filtrate and the performance of skin-tests therewith, should not, in the present stage of development, be construed as possessing any clinical diagnostic or prognostic value. This procedure, for investigative purposes, may prove to be an excellent method for probing other ramifications of this odontosomatic problem upon which further research may be based.

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