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EXPERIMENTAL STUDIES ON THE PATHOGENESIS AND TREATMENT OF OCULAR TUBERCULOSIS*

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THE investigations which it is my privilege to summarize here have extended over a twelve-year period. Since the more recent studies on the action of antibiotics are as yet in press, it is an additional pleasure to present them here in England, the home of Fleming's and Florey's great and fundamental work, before their actual publication.

A number of my colleagues have participated in this work and are co-authors of the various reports. To these gentlemen I extend

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full acknowledgment for the major part they have played in these investigations.

These studies were primarily stimulated by Rich's demonstration in 1929 of the independence of hypersensitivity and immunity, and his explanation then given of the pathogenesis of the tubercular lesion. Prior to Rich's contribution, it was generally supposed that the phenomena of hypersensitivity and immunity were interdependent. Since the time of Koch, it had been clearly recognized that, to comparable doses of the tubercle bacillus, the normal animal reacts in a manner quite different from the animal previously infected with tuberculosis. When the normal animal is infected by the local injection of living bacilli, there is an insignificant reaction at the site of the injection. Thereafter there ensues a slow propagation of the bacilli, involvement of the regional lymph nodes and the formation of hard tubercles. As the infection progresses beyond the local lymph nodes, there follows a widespread systemic infection, first with hard tubercles, which may either become fibrosed, or else necrotic, caseous, and softened. On the other hand, when the previously tuberculous animal receives a comparable injection of bacilli, a sharp inflammatory reaction occurs at the site of the injection. This is characterized clinically by oedema, erythema, induration, and sometimes even by necrosis and sloughing. Histologically this reaction is characterized by a primary outpouring of serum and leucocytes, and later a mobilization of macrophagic cells. The bacilli tend to become fixed at the site of the injection, and, if the infecting dose were small, they may even be destroyed.

The natural deduction from this observation was that the initial inflammatory reaction, which was correctly interpreted as a hypersensitive phenomenon, was intimately related, or indeed responsible for, the local fixation and destruction of the bacilli. In other words, allergy was responsible for immunity. This idea Rich challenged in 1929, pointing out that while this dictum was widely accepted, there was no scintilla of experimental evidence to support it. In a series of brilliant experiments, he and his co-workers demonstrated that there was no relation between hypersentivity and resistance, and that the previously tuberculous animal could be completely desensitized to the extent that not only the soluble bacterial products, but even living bacilli injected in the skin, caused no local inflammatory reaction. At the same time the resistance to dissemination of the bacilli, or immunity to reinfection, was totally undisturbed. As a result of these and other investigations, Rich stated that the factors governing the development and course of a tuberculous lesion could be expressed in an equation, now known as Rich's law. This equation is :—

$$\text{Lesion} \propto \frac{\text{No. and virulence of bacilli} \times \text{Allergy}}{\text{Resistance}}$$

Thus following infection with a moderate or large dose of virulent bacilli, if there is a high tissue allergy and a low immunity, an inflammatory, caseating, necrotizing lesion results. On the other extreme, if the number of infecting bacilli is small, and if there is a low degree of tissue allergy and a high resistance, there will be little inflammation or tissue destruction. Rapid encapsulation and healing occur, and there may even be complete destruction of the bacilli. Varying degrees of infection, allergy and resistance, between these two extremes, explain the various intermediate types of the tubercular lesion.

This concept of the pathogenesis of a tubercular lesion offered obvious therapeutic points of attack. These were, first, an enhancement of resistance which had long been the cornerstone in the treatment of tuberculosis. The second point of attack would be the removal of the fatal tissue allergy, or desensitization. This was something of a new idea, tuberculin previously having been used somewhat disastrously and fruitlessly on the old peri-focal concept, *i.e.*, that since hypersensitivity was assumed to be responsible for immunity, hypersensitive reactions should be encouraged. Thus tuberculin therapy fell somewhat into disrepute. The third point would be a direct attack on the tubercle bacilli, a therapeutic measure which up to that time had been unsuccessful, there being no chemo-therapeutic or antibiotic agent yet demonstrated with any real deterrent action on the tubercle bacillus.

It was the purpose of the investigations which I summarize today to investigate experimentally first, the validity for ocular tuberculosis of Rich's law, and secondly, the therapeutic possibilities suggested thereby.

At the beginning of these studies, there was already considerable reason to believe that Rich's law, or some modification thereof, governed the lesions of tuberculosis in the eye. Primarily, it had been known for some years that experimental ocular tuberculosis ran quite a different course in the normal animal and in the animal previously infected with tuberculosis (the immune-allergic animal). This had been clearly shown as early as 1924 by Henri Lagrange. Secondly, the most striking characteristic of clinical ocular tuberculosis is its amazing pleomorphism. Certainly some extrinsic factors are responsible for the widely varying manifestations of the disease in the eye. The obvious factors are those enumerated by Rich—the dose and virulence of the infecting organism, the degree of tissue allergy present, and the degree of

systemic immunity to infection. It was not known, however, to what extent these factors would be effective on a localized tuberculous lesion in the tightly enclosed scleral and corneal envelope. One troublesome point in the acceptance of this law for ocular tuberculosis was the clear fact that certain experimental animals and humans with ocular tuberculosis showed a high degree of acute inflammation and even caseation in the eye, indicating a high degree of local tissue hypersensitivity, while the cutaneous reactivity of these animals and humans to tuberculin was low or absent.

The immediate objectives of these investigations may therefore be listed as follows:—

I. *Pathogenesis.* Does Rich's law for the pathogenesis of the tuberculous lesion hold true in localized ocular tuberculosis? This was explored by determining: A. The effect of the number and virulence of the infecting organism on the resulting ocular lesion. B. The influence of local tissue sensitivity. C. The influence of systemic immunity. D. The relation of cutaneous and ocular sensitivity.

II. *Therapy.* A. Is enhancement of local resistance or immunity possible? B. What is the effect of desensitization on the local ocular lesion? C. What are the possibilities of sulfone and antibiotic treatment in ocular tuberculosis?

This was an over-ambitious programme. On some points, notably the stimulation of an artificial local immunity, we have not even scratched the surface. On other points, while the work is incomplete and fragmentary, some information has been adduced which confirms the validity of Rich's law on the pathogenesis of the tuberculous lesion in the eye, and strengthens the suggestions for therapy. Some observations have been made on the therapy of experimental ocular tuberculosis which appear to have a bearing on the clinical attack on the disease. These experiments may be summarized as follows:—

THE PATHOGENESIS OF OCULAR TUBERCULOSIS

A. *The influence of the number and virulence of organisms.* The first problem was the determination of the proper strain of tubercle bacilli to be used in the experimental animals and the proper dosage. This was largely a trial-and-error procedure, and it is unnecessary here to go into the various details. Suffice to say that avian and bovine strains proved unsuitable for use in the rabbit on account of the malignant course of the ocular disease

when the organisms were injected into the eye, and the speedy death of the rabbits from generalized tuberculosis when the organisms were injected systemically. A virulent human strain was finally used. Injected into the eye of a normal or immune-allergic rabbit in proper dose, satisfactory lesions developed, and injected systemically, the animals rarely developed widespread tuberculosis, but as a rule developed a self-limiting disease with inconspicuous histological findings, from which they recovered spontaneously, retaining a well developed hypersensitivity to tuberculin, and a definite acquired resistance to re-inoculation—an immune-allergic status. The ocular inoculations were all made in the anterior chamber. Intra-carotid inoculations were unsatisfactory for such a prolonged study as this, because the development of ocular tuberculosis in such animals was a chance and inconstant affair.

When a small dose of the bacilli was injected into the anterior chamber of a normal rabbit, there was a minimal or no local reaction to the injection. About the fourteenth day after injection, these rabbits developed slight peri-corneal injection, slight steaminess of the cornea, and hard tubercles over the iris. About the fourth week this indolent reaction became aggravated, vascularization of the cornea became evident, and acute inflammation, and later, evidences of necrosis and caseation developed. About the sixth week, or thereafter, some of the eyes perforated. In the remainder, beginning about the eighth or tenth week, the acute inflammation began to subside, and the eyes entered the stage of beginning fibrosis. By the twenty-third or twenty-fourth week the disease was usually inactive, leaving blind, scarred and sometimes perforated eyes (Study 2).

When a larger dose of the same virulent organisms was given, this picture was greatly accelerated, the degree of acceleration depending upon the amount of bacilli present in the inoculum. The incubation period before the development of symptoms was shortened to a week or less, the acute inflammatory phase developed rapidly, and perforation of the eyes within four to six weeks was the general rule.

When the eyes of normal rabbits were inoculated with a attenuated strain of human organisms, the results were minimal. The inflammatory symptoms were slow in appearance, and of low degree. Many eyes showed little or no reaction, and those that did show disease usually healed within three months with minimal damage and scarring.

In the immune-allergic rabbit—the animal recovered from a previous systemic infection—the course of the ocular disease after

anterior chamber inoculation was quite different. Primarily in order to produce any tuberculous disease in the eyes of these rabbits, it was found necessary to give a much larger dose of the same virulent bacilli, the minimum dose required to produce low grade tuberculous disease in the eyes of these rabbits being usually fifty times that required for the normal rabbit. When this dose was given in the anterior chamber, there developed within 24 hours a marked inflammatory reaction which subsided within a few days. This was similar to the reaction caused by the anterior chamber injection of tuberculin, and was obviously a reaction to the tuberculo-protein in the inoculum, the eyes, like the other body tissues, having become sensitized by the prior systemic infection. After the subsidence of this tuberculin reaction, the eyes remained asymptomatic for a period of two weeks or longer. Then low-grade ciliary congestion developed, and sometimes discrete tubercles appeared in the cornea and iris. Thereafter the eyes ran a restrained course of chronic inflammation, showing moderate secondary iridic and corneal changes with vascularization. The inflammatory reaction slowly increased, reached a low maximum about the fourth week, continued to the tenth or fourteenth week, and then gradually subsided. The maximum degree of inflammation resulting from the minimal dose capable of producing lesions was decidedly less than that eventually developing in the eyes of the normal rabbits affected with their minimal dose, and perforation practically never occurred (Study IV). In fact, the course of the disease in its various corneal and iris manifestations simulated amazingly ocular tuberculosis in the human adult.

If a larger dose of the virulent bacilli was given to the immune-allergic rabbits, the entire picture was reversed. There was an early violent reaction which never subsided, the eyes rapidly developed a spreading destructive inflammation, with necrosis and caseation, and there was a high percentage of rupture within 6 weeks. The process was even more acute and destructive than the disease in the normal rabbit (Study VI).

When avirulent organisms were given to immune-allergic rabbits, local tuberculous disease did not result, and there was no reaction other than the immediate one to the tuberculo-protein in the inoculum.

From the experiments, it is clearly apparent that the importance of the dose and virulence of the infecting organisms holds true in ocular tuberculosis. In the normal animal with a minimal dose of virulent bacilli, there is a slowly spreading infection, which becomes acute as the organisms propagate and spread through the eye. With a larger dose of the same organisms this reaction is violently accelerated. With avirulent organisms there is little

or no reaction. In the immune-allergic animal, where the propagation and spread of the organisms is restrained by the acquired systemic resistance, after the reaction to tuberculo-protein subsides, the subsequent inflammation is of low degree, and parallels the slow propagation of the bacilli in the eye. However, with a larger dose of bacilli, there is a violent immediate inflammatory reaction, the immunity is overwhelmed, the bacilli propagate rapidly, and necrosis, caseation and rupture occur early.

In the subsequent experiments, in both the normal and immune-allergic rabbit, the minimum dose capable of producing lesions with fair constancy, was uniformly used.

B. *The influence of hypersensitivity on the ocular lesion,* It has already been noted that acute inflammation with necrosis and caseation develops in the normal rabbit as the bacilli propagate and spread through the eye. In the immune-allergic rabbit after the immediate hypersensitive reaction subsides, acute inflammation develops only as the bacilli propagate, is of lower degree than in the normal, and there is minimal necrosis and caseation. What is the relation of this acute inflammation to hypersensitivity of the eye?

The ocular sensitivity can be accurately gauged by the injection of tuberculin* into the anterior chamber, estimating the clinical reaction, and then enucleating these eyes and evaluating the histological reaction. This was done at weekly intervals in sample pairs of rabbits throughout the course of several experiments. Thus a graph could be prepared illustrating the development and the course of ocular sensitivity during any period of observation. Likewise, by clinical estimation on a numerical scale, the degree of ocular inflammation resulting from infection could be estimated in the remaining animals of the group, graphed, and compared with the degree of ocular hypersensitivity as measured by the intraocular tuberculin test in the sample rabbits. This was done for both normal rabbits (Study II) and for immune-allergic rabbits (Study IV). The validity of graphs was checked statistically (Study IV).

The results of this graphic study for normal rabbits are shown in Fig. 1, and for immune-allergic rabbits in Fig. 2. The ordinates represent the degree of ocular inflammation and sensitivity and the abscissae the time in weeks. Thus it is evident that in both the normal and immune-allergic animals with ocular tuberculosis, the degree of ocular inflammation resulting from infection closely parallels the degree of ocular sensitivity. The ocular inflammation

* The tuberculin used for the determination of ocular sensitivity was the purified protein derivative of Seibert, known as P.P.D.

resulting from infection increases as the degree of ocular reactivity to tuberculin (P.P.D.) increases, and in general decreases as this ocular reactivity wanes,

The influence of sensitivity on the ocular lesion was further investigated in two other experiments. In the first experiment, advantage was taken of the fact that when rabbits are inoculated

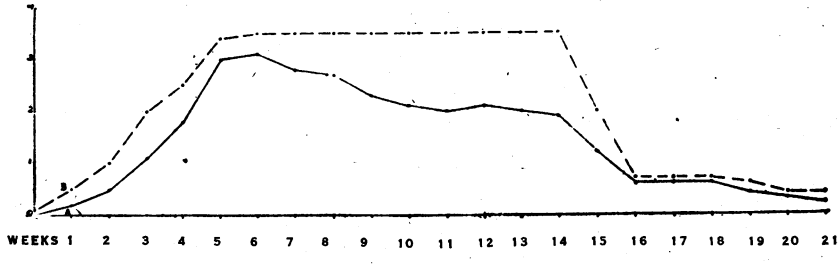


FIG. 1.

Curves showing the relation of ocular inflammation to ocular sensitivity. Curve A represents the ocular inflammation and curve B the ocular sensitivity.

systemically with virulent human tubercle bacilli, they do not all develop a uniform amount of allergy. Thus by initially using a large number of rabbits, when the appropriate period has elapsed after systemic inoculation, certain individual animals showing marked cutaneous hypersensitivity and others showing a low

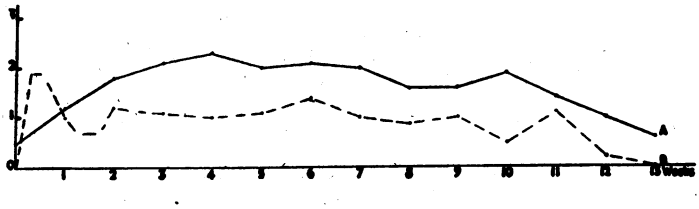


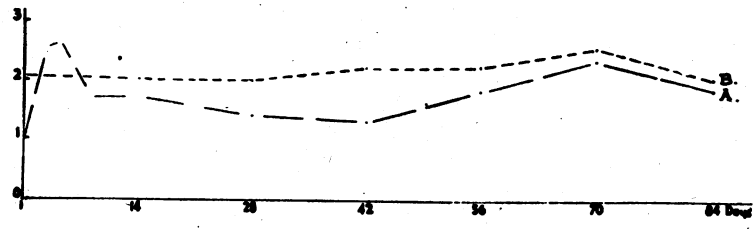
FIG. 2.

Relation of ocular sensitivity (A) to ocular activity (B) after intraocular injection of tubercle bacilli in immune-allergic rabbits.

degree of cutaneous hypersensitivity can be selected. As will later be pointed out, in immune-allergic rabbits without ocular disease, the ocular sensitivity parallels the cutaneous sensitivity. Thus two sets of rabbits, one with high ocular and cutaneous sensitivity, and the other with low ocular and cutaneous sensitivity, were made available for inoculations of the eye and study of the resulting inflammation. During the progress of this experiment,

specimen pairs of animals were sacrificed at weekly intervals to determine the course of the ocular sensitivity.

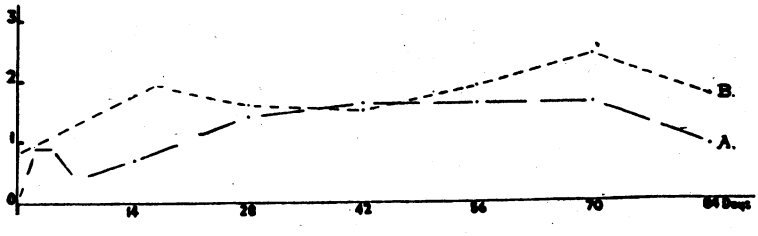
Fig. 3 shows the course of the ocular inflammation and ocular sensitivity in the rabbits with initial high sensitivity, the ordinates again representing the degree of ocular sensitivity and inflammation, and the abscissae the time in days. Thus it is apparent that



A. Ocular Inflammation.
B. Ocular Sensitivity.

FIG. 3.

Course of ocular activity and of ocular sensitivity in rabbits with initial high cutaneous sensitivity. A, ocular activity, and B, ocular sensitivity.



A. Ocular Inflammation.
B. Ocular Sensitivity

FIG. 4.

Course of ocular activity and ocular sensitivity in rabbits with initial low cutaneous sensitivity. A, ocular activity, and B, ocular sensitivity.

rabbits with a high degree of sensitivity showed an immediate high degree of reaction to the tuberculo-protein in the inoculum, and a high degree of persisting ocular inflammation. Again the degree of ocular inflammation resulting from the infection paralleled the degree of ocular sensitivity to tuberculin.

Fig. 4 shows the same findings in rabbits with an initial low sensitivity. The primary reaction to the tuberculo-protein in the inoculum was low, and the early inflammatory symptoms slight. However, as the bacilli spread slowly through the eye, the ocular sensitivity to tuberculin increased, and ocular inflammation

resulting from the infection likewise increased and paralleled the ocular sensitivity to tuberculin.

In the second experiment (Study VI), a group of immune-allergic rabbits was divided into three sub-groups A, A^1, and B. Prior to inoculation of the eyes, the A rabbits were desensitized with tuberculin, and in order to maintain desensitization, the tuberculin treatment was continued after the anterior chamber inoculation. The A^1 rabbits were partially desensitized, and were given no tuberculin after ocular inoculation. The B rabbits were not treated with tuberculin at any time.

The course of the ocular inflammation in these three groups of rabbits is shown in Fig. 5, the ordinates expressing the degree of inflammation, and the abscissae the elapsed time in weeks. The desensitized A rabbits showed no initial reaction in the eyes to the tuberculo-protein in the inoculum, and later developed a

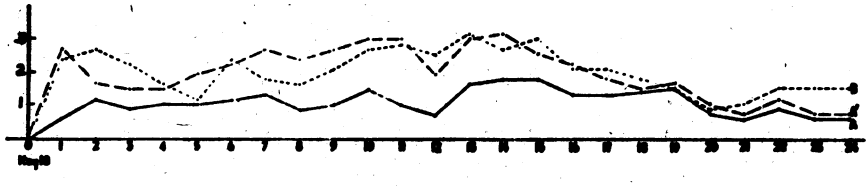


FIG. 5.

Average ocular activity of reacting rabbits of groups A, A^1 and B.

restrained ocular tuberculosis of low degree. Only at the end of the eighteenth week, when the disease was beginning to subside in all three groups, did the clinical inflammation of the desensitized A rabbits approximate that of the other two groups. On the other hand, both the partially desensitized A^1 rabbits, and the hypersensitive B rabbits showed a marked degree of reaction to the tuberculo-protein in the inoculum, and both groups showed more severe inflammatory reactions in the eyes than did the desensitized rabbits.

Thus it appears that the influence of tissue allergy on the resulting tuberculous lesion is substantiated for ocular tuberculosis. In both normal and immune-allergic rabbits, infected in the eye with the minimal dose of bacilli capable of producing constant symptoms, the ocular inflammation, necrosis and caseation, parallel the degree of ocular sensitivity. In immune-allergic rabbits with an initial high ocular sensitivity, the immediate ocular inflammation is severe, and so remains. In similar rabbits with an initial low ocular sensitivity, the ocular inflammation is low at first, and only develops as the ocular sensitivity increases. Desensitization prior to inoculation abolishes the primary ocular reaction to

tuberculo-protein, and the ocular inflammation resulting from inoculation of the eyes is of low degree when the desensitized status is maintained.

C. *The influence of immunity on the ocular lesion.* The degree of immunity existing in the various immune-allergic animals was not uniformly quantitated by the injection of living bacilli in the skin, and clinical and histological examination of the resulting reaction. In the early experiments this was done in a number of rabbits, but no noteworthy differences in the reaction were observed, and the procedure introduced the element of possible changes produced by a second infection. The information on the rôle of immunity on the ocular lesion, as evidenced by these experiments, may be summarized as follows.

First, the minimal dose of bacilli which produces marked ocular disease in the normal rabbit, has no effect when introduced in the eye of a rabbit recovered from a prior systemic infection (Study III). The rabbit has developed a resistance to re-inoculation. Second, it requires approximately fifty times this dose to produce tuberculous disease in the eyes of immune-allergic rabbits, the amount varying in different animals. In a small percentage of immune-allergic rabbits, no ocular disease resulted from the standard fifty-fold dose. Third, this resistance to re-inoculation is only relative. If enormous doses are given, it is immediately overwhelmed, and violent inflammatory symptoms develop (Study VI). Fourth, this resistance to re-inoculation is totally undisturbed by complete desensitization of the rabbits prior to inoculation (Study VI). It still requires the same increased dose to produce ocular lesions, as compared with the normal rabbit. In fact, in this experiment, the desensitization appeared actually to favour the action of the immunity, the desensitized group showing a slightly higher incidence of complete immunity, and a decided increase in the incubation period—seven weeks as compared to two weeks in the sensitive controls.

There is therefore considerable confirmation for the influence of immunity on the tuberculous lesion in the eye. The course of the ocular disease is radically different in normal animals with no immunity, and in immune-allergic animals with immunity from a prior infection. While the degree of immunity resulting from a prior infection may be variable, it always requires a much higher dose of bacilli to produce tuberculous lesions in the previously infected animal than it does in the normal animal. Immunity functions entirely independently of sensitivity, and indeed in these experiments appeared somewhat enhanced when sensitivity was removed.

D. There remains one last point to be discussed in this connection—the puzzling question of why certain experimental animals, and likewise clinical patients with ocular tuberculosis, show a low degree of cutaneous reactivity to tuberculin, when the clinical appearance of the eye would indicate a high degree of tissue sensitivity.

This was investigated by simultaneous estimations of the cutaneous and ocular reactivity to tuberculin (*a*) in systemically infected animals without ocular disease, (*b*) in normal animals infected with tubercle bacilli in the eye, and (*c*) in immune-allergic animals secondarily infected in the eye.

Fig. 6 illustrates the general parallelism between ocular and cutaneous sensitivity in systematically infected animals without ocular disease (Study I). While there were wide fluctuations, in

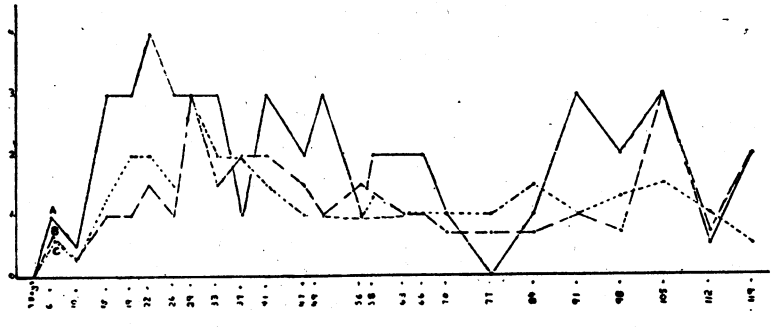


FIG. 6.

Curves showing the parallelism of ocular and cutaneous sensitivity in the systemically infected rabbits. Curve A represents the cutaneous sensitivity; curve B the clinical ocular sensitivity, and curve C the histological ocular sensitivity.

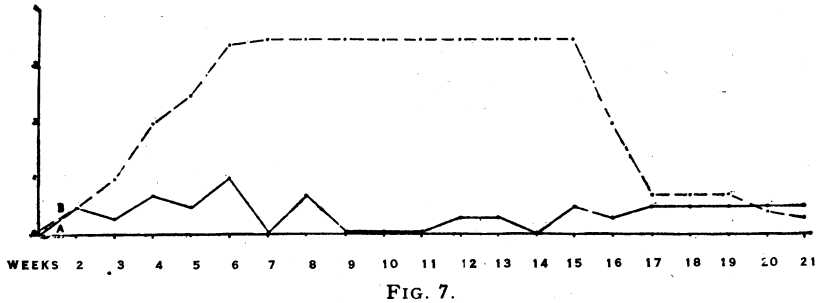


FIG. 7.

Curves showing the relation of the ocular sensitivity to cutaneous sensitivity in the normal rabbit after inoculation of the anterior chamber with tubercle bacilli. Curve A represents the cutaneous sensitivity, and curve B the ocular sensitivity.

general the cutaneous and ocular sensitivity paralleled each other. The most marked fluctuations occurred in the cutaneous sensitivity, when hot weather appeared to increase the cutaneous reactivity.

Fig. 7 represents the relation of ocular reactivity to tuberculin to cutaneous sensitivity in normal animals inoculated in the eye with tubercle bacilli (Study III). As the bacilli propagate and spread through the eye, intense local reactivity to tuberculin develops which only subsides when the process burns out. The cutaneous reactivity to tuberculin is only slightly stimulated by the local disease process in the eye. In short, in the otherwise

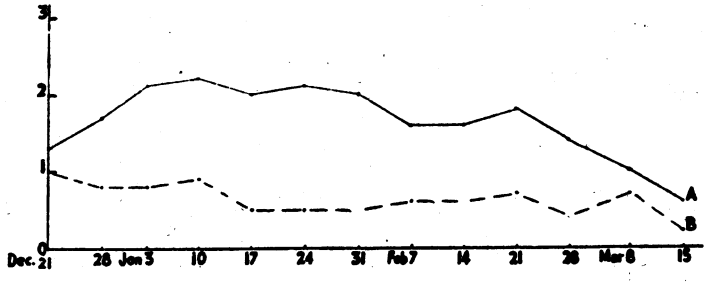


FIG. 8.

(Experiment 1)—Relation of ocular sensitivity (A) and cutaneous sensitivity (B) after intra-ocular injection of tubercle bacilli in immune-allergic rabbits.

normal animal with local ocular tuberculosis, the cutaneous reactivity to tuberculin is no index of the ocular reactivity.

Fig. 8 shows the relative ocular and cutaneous reactivity to tuberculin in immune-allergic rabbits infected in the eye with tubercle bacilli (Study IV). At the time of inoculation the ocular and cutaneous sensitivity were about the same, as would be expected. As the bacilli spread slowly throughout the eye, there was a concomitant slow rise in the local ocular reactivity or sensitivity. The ocular sensitivity began to decline slowly at the end of the ninth week, as the local disease subsided. The cutaneous sensitivity, however, was not affected by the local ocular disease, and over a three-month period slowly declined and faded.

These experiments offer an explanation for the relative cutaneous anergy sometimes observed in ocular tuberculosis with acute inflammation. If there is local tuberculous disease in the eye there is no necessary relation between the ocular reaction to the products of the tubercle bacillus and the cutaneous sensitivity. If the cutaneous sensitivity is high, the ocular sensitivity is also high,

for the eye partakes of the general systemic sensitivity. However, local disease in the eye may produce a high degree of ocular sensitivity to tuberculin, while it does not influence or affect a normally low cutaneous sensitivity. In other words, if the cutaneous sensitivity is low, it does not necessarily imply that the ocular sensitivity is also low. The latter may be high or low, depending on the presence and extent of an ocular tuberculosis. Whether the increased ocular reactivity to tuberculin is the result of an increased vascularity produced by the local disease, or is a true selective local sensitization, is largely an academic question which need not concern us here.

It seems reasonable to conclude from these experiments that the principles involved in Rich's law, laid down for systemic tuberculous lesions, also hold good in localized ocular tuberculosis without active systemic lesions. It is further evident that the determination of the cutaneous reactivity to tuberculin does not always give an accurate picture of the local ocular reactivity. If cutaneous sensitivity is low or absent, and there is tuberculous disease in the eye, the ocular reactivity to the products of the tubercle bacillus may still be high.

II.—THE TREATMENT OF EXPERIMENTAL OCULAR TUBERCULOSIS

A. *The Enhancement of Immunity.* In 1893 Trudeau stated that the achievement of a complete artificial immunity in tuberculosis was an ideal never likely to be attained. Unfortunately that is still largely true today. It has already been pointed out that as a result of a prior infection, the rabbit acquires a well marked resistance to re-inoculation. This well known fact has been employed in the mass inoculation of children with the attenuated B.C.G. organism, and the results have been somewhat gratifying in the lessened incidence of tuberculosis in the inoculated group. This lengthy procedure is, however, a far cry from the artificial stimulation of an immunity calculated to benefit an already existing infection. While we have done little work on this problem, and the little we have done has been quite barren of any results, one observation has been made which may be of some academic interest.

It was noticed that in immune-allergic rabbits with a secondary ocular tuberculosis, the clinical evidences of inflammation and activity might entirely subside while there was still a low degree of demonstrable sensitivity in the affected eye, and that these eyes did not develop further tuberculous disease when given a second injection of bacilli shortly after the subsidence of symptoms (Study VI). It was thought at first that this might be due to an exhaustion

of the reactive capacity of the eye, but this supposition was proven untrue, since the eyes reacted quite briskly to other non-tuberculous stimuli and infections. It was then supposed that this phenomenon might be due to an increase in the general immunity brought about by the recent ocular disease from which the animal had recovered. This supposition was also untenable, because it was found that the second undiseased eyes of these same rabbits reacted quite promptly to the injection of the proper dose of bacilli (Study VI). Another possible explanation was that this healing and immunity to re-inoculation might be due to the increased vascularity produced by the ocular disease, thus rendering the humoral element of the general immunity more efficacious. Experiments (Study VIII) in immune-allergic rabbits with eyes vascularized by other non-tuberculous infections, revealed that increased vascularity was not in itself the dominant factor in this resistant state, and indicated some mechanism other than humoral immunity.

Continued study of these apparently immune eyes revealed the fact that the local immunity was only transitory, that spontaneous recurrences of the inflammation occurred in 25 per cent. of such rabbits within a year, and within this same time the remaining 75 per cent. lost their immunity, and these eyes developed a further attack of ocular tuberculosis on re-inoculation (Study VIII). Further, the histological examination of eyes recently recovered from an attack of tuberculosis, and presumably immune to re-inoculation, revealed definite sub-clinical areas of infection with epithelioid cells and macrophages. Examination of similar eyes a year later, when they were again theoretically susceptible to re-inoculation, showed that the epithelioid cells and macrophages had largely disappeared.

On the basis of these observations it was concluded that the transient immunity to re-inoculation shown by eyes recently clinically recovered from tuberculosis was due not to a humoral immunity, but to the persisting mobilization of macrophagic cells. In short, whatever might be the rôle of the circulating anti-bodies in immobilizing and fixing the bacilli, the *sine qua non* in this local resistant state appeared to be the presence of macrophagic cells.

The obvious method of testing this hypothesis was the premobilization of macrophagic cells in the eye prior to the introduction of the infecting bacilli. This we attempted to do by the local injection of tuberculo-phosphatides in the anterior chamber, and finally in the stroma of the ciliary body.

This experiment, previously unreported, was completely inconclusive. When phosphatides were introduced into the anterior

chamber, they were apparently immediately excreted, and the eyes on section showed no cellular response. When the phosphatide was injected in the ciliary stroma, there ensued a clinical inflammatory reaction, and histologically an outpouring of epithelioid cells and macrophages. When such eyes were later injected with tubercle bacilli and compared with controls, it was difficult to differentiate with any certainty the tuberculoid lesions caused by the phosphatides and true tubercular lesions produced by the living bacilli. The intra-ocular injection of phosphatides did not appear to be a happy experimental approach to the problem. Thus these efforts to enhance the local resistant state have been fruitless.

B. *The Effect of Desensitization on Experimental Ocular Tuberculosis.* Prior to Rich's paper in 1929, it was generally supposed that allergy and immunity were inter-dependent, and that by evoking an allergic reaction, immunity might be stimulated. This was the idea underlying the therapeutic use of tuberculin in localized tuberculosis—namely to evoke sub-clinical allergic reactions and thus stimulate immunity. This was known as the perifocal concept. Rich's demonstration of the independence of allergy and immunity introduced at once the new therapeutic concept of removing the fatal tissue hypersensitivity by desensitization with tuberculin. Thus tuberculin would be given sub-cutaneously with the idea of avoiding all clinical or sub-clinical focal reactions, and the dose would be increased only as the point of reactivity receded. By this means tissue desensitization would be finally accomplished. However, it is quite true that either clinical or sub-clinical reactions will produce some desensitization, in that they deplete the local antibody reservoir. The value of avoiding focal reactions is therefore to obviate the local destructive effect such focal reactions necessarily entail.

In experimental work with rabbits, it is impracticable to employ the long-drawn-out process of desensitization with the small doses of tuberculin one would use in humans. Rabbits are not the happiest animals to use for desensitization experiments with tuberculin. They do not become hypersensitive as readily as guinea-pigs, they tolerate huge doses of tuberculin somewhat better than guinea-pigs and vastly better than humans. Lastly, since the ocular disease naturally runs a self-limiting course of from three to six months, it is necessary to accomplish desensitization rapidly in order to evaluate any observed therapeutic results. In the experiment to test the effect of desensitization on an already existing ocular tuberculosis in the immune-allergic rabbit, tuberculin was therefore administered subcutaneously in the large dose of 100 mgms., or (0·1 c.c.) of old tuberculin twice weekly. While a

focal reaction was observed following the first dose, this reaction was evanescent and did not produce any ocular damage. That this focal reaction was not responsible for the therapeutic effect was demonstrated by the observation that a precisely similar focal reaction had no effect on the hypersensitive controls which were not maintained in a state of desensitization.

The essence of this experiment (Study VII) was to prepare a large series of immune-allergic rabbits with secondary ocular tuberculosis. As soon as the ocular disease was established clinically, the rabbits were divided into two groups of equal severity. One group was untreated, while the second group was treated with tuberculin. The clinical course of the two groups was followed, and various minor tests were made during the course of the experiment to determine the progress of desensitization.

The results of this experiment are shown graphically in Fig. 9. After the inoculation of the eyes with tubercle bacilli there was an immediate reaction in the eyes to the tuberculo-protein in the inoculum. This rapidly subsided, and well-marked tuberculous lesions in the eyes were present by the third week, when the rabbits were divided into two groups of equal severity. Tuberculin was then started in one group. There was an immediate focal reaction in the eyes which subsided within one week. Thereafter the severity of the disease in the treated group, which continued to receive the desensitizing injections, rapidly subsided as compared with the untreated group, and at the end of the twelfth week, this difference in the two groups was marked. As the experiment progressed to the twenty-fourth week and the ocular disease in the control group naturally burned out, the picture in the two groups approximated each other. Various minor tests, the determination of ocular reactivity in the control group, the determination of the relative ocular sensitivity of sample pairs from the two series, the estimation of the ocular and cutaneous sensitivity at the end of the experiment, all indicated that the observed clinical improvement in the treated group paralleled their desensitization to tuberculin. Indeed, there was some evidence that there was a selective desensitization of the eyes, which might be expected, since it is known that tuberculin is a fine colloid and tends to filter out at the site of local inflammation.

The ultimate fate of the desensitized rabbits is interesting, and may be of importance in planning the method in which tuberculin should be used clinically. At the completion of the experiment the treated rabbits showed a degree of cutaneous sensitivity so low that the animals were practically insensitive. One year later, after cessation of treatment, the sensitivity had returned, and the animals showed a high degree of both ocular and cutaneous sensitivity.

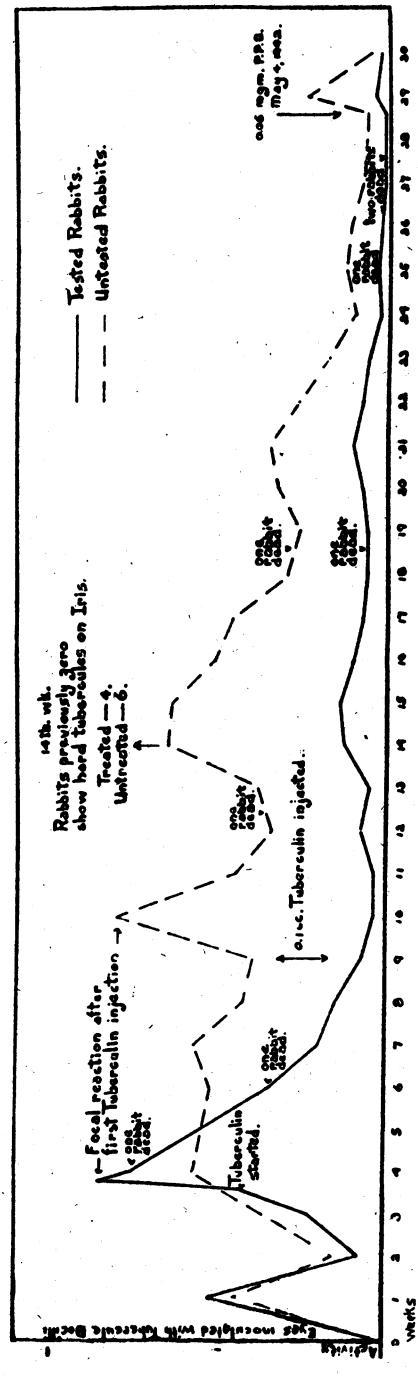


FIG. 9.

Effect of treatment on ocular tuberculosis in immune-allergic rabbits.

Synchronous with the return in sensitivity, there had been a disastrous ocular recurrence in 36 per cent. of the rabbits.

The conclusions to be drawn from this experiment appear clear. Desensitization with tuberculin is accompanied by a marked decrease in the clinical inflammatory manifestations of the disease, and exerts a thoroughly beneficial effect. It does not cure the disease in the sense of destruction of the bacilli, or even of bacteriostasis. It merely removes the factor responsible for the destructive phases of the lesion. If tuberculin treatment is terminated, the fatal tissue sensitivity recurs, and coincident with its return in a large percentage of the animals, there is a recurrence of the ocular inflammation.

C. *The effect of chemotherapy and antibiotics in ocular tuberculosis.* Prior to 1940, no therapeutic agent had been found with any marked specific deterrent action on the tubercle bacillus. It is true that Koch had observed the inhibitory action of colloid of gold salts on the *in vitro* growth of tubercle bacilli, but their clinical use had given no spectacular results, and the untoward effects were so severe that their use has been almost completely abandoned. Efforts had been made to treat tuberculosis with various dyes combined with metallic salts of bactericidal action. The thought behind such treatment was the known ability of such dyes to penetrate tubercles. The clinical results were, however, not encouraging. Some hope was later aroused by the demonstration that sulphanilamide used prior to inoculation had an inhibitory effect on the later development of tuberculosis in guinea-pigs. However, sulphanilamide had no effect in tuberculosis when used clinically. It was not until 1940, when Feldman and his co-workers showed the deterrent action of certain diamino-diphenyl sulphones in experimental tuberculous lesions, that there was any real demonstration of an effective chemo-therapeutic agent in tuberculosis. These sulphones were known as diasone, promin and promizole. Diasone was relatively toxic and therefore of little value. Promin was moderately toxic, while promizole was relatively non-toxic. In fact, promizole can be administered to humans up to 12-15 grams daily with comparative safety.

The first step in the study of chemo-therapy of ocular tuberculosis was to determine the effects of promin and promizole in both normal and immune-allergic rabbits with ocular tuberculosis. The drugs were administered in food in 1·0 per cent. concentration over a four-month period. The daily dose was approximately 1·5 gm., which produced a blood level ranging around 2·0 mgm. per cent. The results of this treatment in normal rabbits (Study XI) are shown in Fig. 10, where the course of the disease in the treated animals is compared with untreated controls. There was

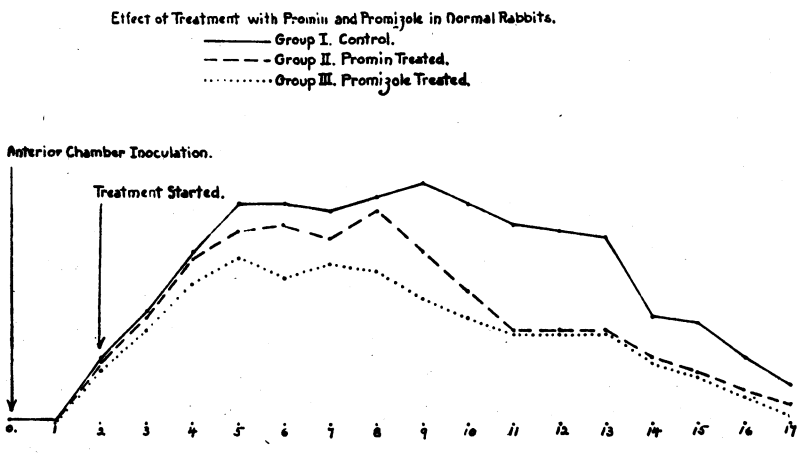


FIG. 10.

Effect of treatment with promin and promizole in normal rabbits.

a slight, but statistically insignificant, relative decrease in the disease in the treated animals at the end of the second week, and a slightly more significant decrease at the end of the eighth week. However, at the end of the twelfth week, the majority of the eyes in both the control and treated groups had gone into buphthalmos and ruptured. There was only a slight advantage in favour of the treated groups. There was little difference in the histological picture, and on transmission experiments from the diseased eyes the uveal tracts of both the treated and untreated groups were all infectious. It was concluded therefore that promin and promizole had only a very slight deterrent action on ocular tuberculosis in the normal animal.

The results of treatment in immune-allergic rabbits were much more striking. These results are shown in Fig. 11. After three

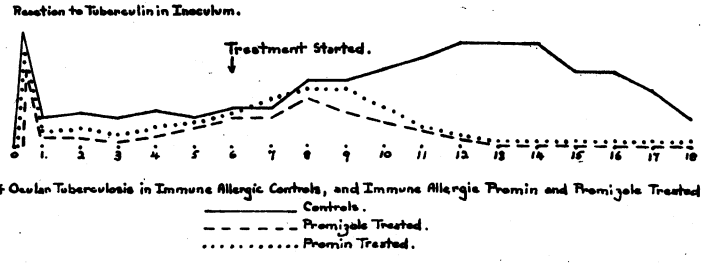


FIG. 11.

Course of ocular tuberculosis in immune-allergic controls and immune-allergic promin and promizole treated rabbits.

weeks' treatment, the treated animals began to improve, while the disease became more severe in the untreated controls. At the end of the fourteenth week, when the disease in the controls was at its maximum, the eyes of the treated rabbits were almost completely inactive. At the end of the sixteenth week, the eyes of all the treated animals were entirely quiet on clinical examination, while 80 per cent. of the controls still showed active inflammation. The histological examination, however, was not so conclusive. Of the treated rabbits sacrificed for histological study, approximately 50 per cent. showed small, persisting areas of active tuberculosis, while the remainder showed only scarring. There was no statistical difference between the promin- and the promizole-treated animals. On transmission experiments, under the technique used in this experiment, only one of the treated animals showed an infectious uveal tract, while all the controls were positive.

It was concluded from this experiment that both promin and promizole had a marked deterrent action on ocular tuberculosis in the immune-allergic rabbit, but this action was not absolute in the sense of producing a complete destruction of the tubercle bacilli. In the light of the negative experiment in normal rabbits it was suggested that this deterrent action might be due either to a degradation or attenuation of the virulence of the organisms, allowing the resistance of the host to become more active, or to a partial bacteriocidal action, bringing the infection within the range of the host's resistance.

The next step in the search for agents with a deterrent action on the lesions of ocular tuberculosis was obviously to explore the effect of the antibiotics. Although Abraham, Chase and Florey had reported as a result of their *in vitro* experiments that the tubercle bacillus was insensitive to penicillin, nevertheless the literature was singularly barren of conclusive evidence that this was true *in vivo*. Such experiments as were reported were all open to the criticisms that the dosage was insufficient, or the number of animals too few to validate any conclusion. It seemed, therefore, worth-while to investigate the question further.

Normal rabbits, injected in the eye by the usual anterior chamber injection, were used (Study IX). This was admittedly a severe test. Sixteen rabbits were treated with penicillin, 200,000 units per diem, in divided doses, for a period of 45 days. An equal number of similarly infected, but untreated, rabbits served as controls. The results of this experiment are shown in Fig. 12. The clinical course of the treated and untreated rabbits was identical. Penicillin had no effect whatsoever on the clinical lesions. Histological examination of the treated and untreated eyes showed slightly less tuberculous disease in the treated eyes,

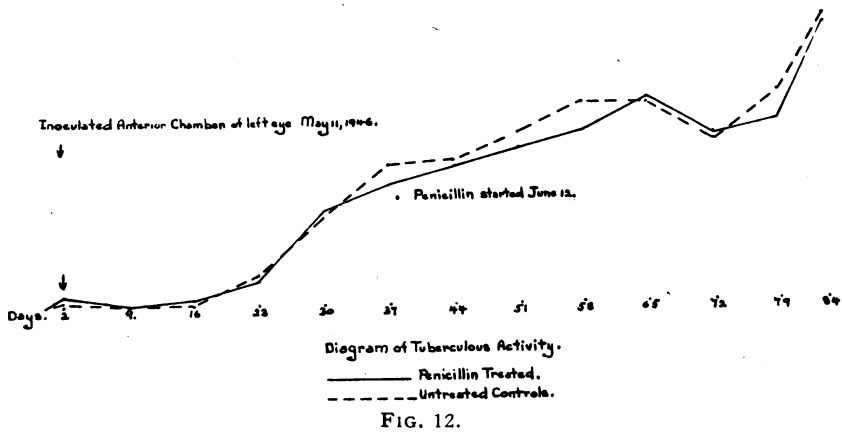


FIG. 12.

Diagram of tuberculous activity in controls and penicillin treated rabbits.

but when this was subjected to statistical analysis it was found to be without significance. It was concluded that penicillin was without action in ocular tuberculosis under the conditions of this experiment.

The last experiment performed was on the action of streptomycin alone, and of streptomycin combined with promizole (Study XII). This experiment was done in immune-allergic rabbits. Inasmuch as the same untreated group served as controls in both the streptomycin alone and the streptomycin plus promizole treated rabbits, these two experiments are shown together. Streptomycin was given in both the treated groups in the dosage of 50 mgms. per kilo of bodyweight per diem, in one dose, and promizole in the dose before outlined, about 1·5 gms. per diem. These dosages gave blood plasma levels up to 7·6 mgms. per cent. for streptomycin, and an average of 1·7 mgms. per cent. for promizole. The period of treatment was two and a half months. The number of rabbits in each group (controls, streptomycin-treated, and streptomycin-plus-promizole-treated) was approximately 20 each.

The results of this experiment are shown graphically in Fig. 13. In both treated groups the results were dramatic. At the end of the second week of treatment, there was a marked difference in favour of the treated groups. At the end of the fourth week this change was striking. The control group had a level of " 2 " for the ocular inflammations, while the treated groups were almost quiescent clinically, the average being 0·25. While the charted averages of activity are practically identical in the two treated groups, the improvement was much more marked in the group

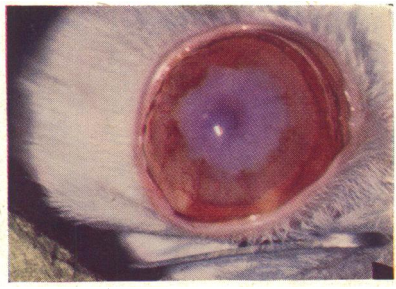


FIG. 14.

Control—February 25—maximum reaction

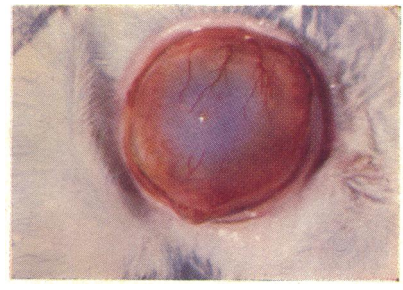


FIG. 15.

Control—April 28—maximum reaction.

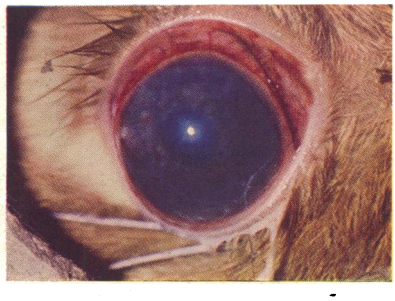


FIG. 16.

Control—February 25—minimum reaction.

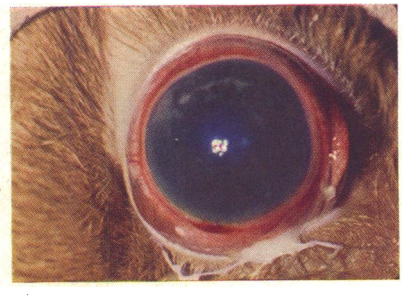


FIG. 17.

Control—April 28—minimum reaction.

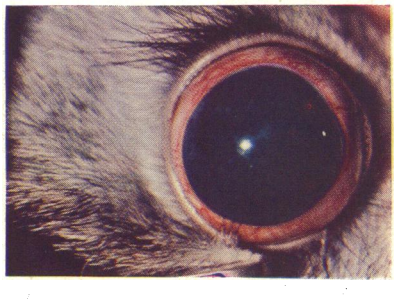


FIG. 22.

Streptomycin-treated—before treatment.

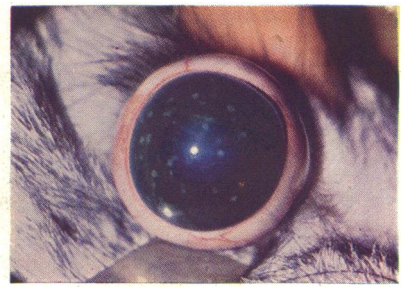


FIG. 23.

Streptomycin-treated—after treatment.

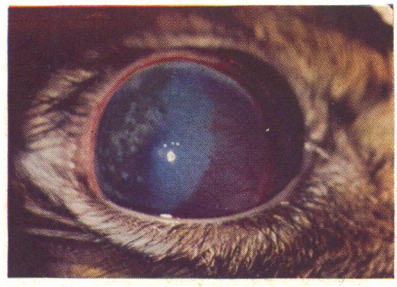


FIG. 24.

Streptomycin-treated—before treatment.

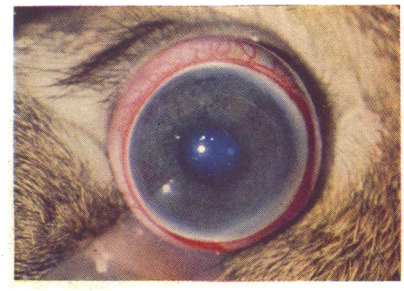


FIG. 25.

Streptomycin-treated—after treatment.

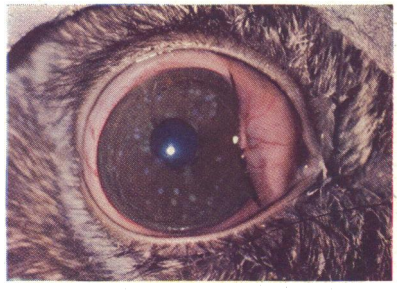


FIG. 30.

Streptomycin-plus-promizole-treated rabbit—before treatment.

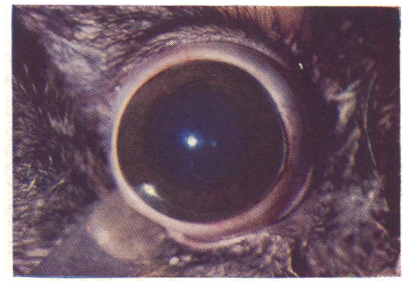


FIG. 31.

Streptomycin-plus-promizole-treated rabbit—after treatment.

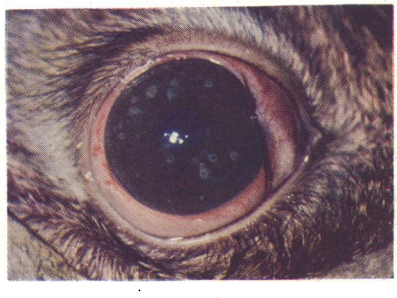


FIG. 32.

Streptomycin-plus-promizole-treated rabbit—before treatment.

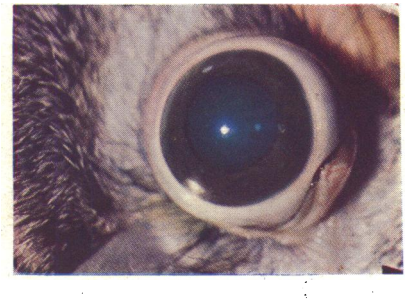


FIG. 33.

Streptomycin-plus-promizole-treated rabbit—after treatment.

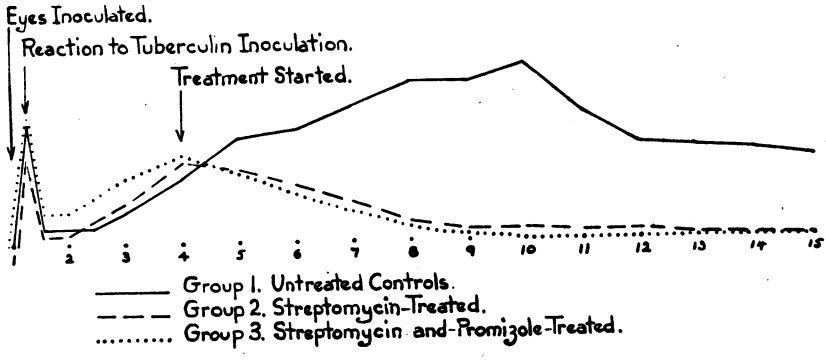


FIG. 13.

The effect of streptomycin alone and streptomycin plus promizole in the immune-allergic rabbit.

treated with the combination of streptomycin and promizole. One apparently non-immune rabbit with a severe and resistant ocular tuberculosis accounted for the greater portion of the activity in the streptomycin-promizole group. The lesions in this rabbit subsided only after twelve weeks of treatment.

The differences in the histological pictures were equally striking. The rabbits of the control group sacrificed for histological study all showed numerous hard tubercles throughout the cornea, iris and ciliary body together with monoculear and epithelioid cell infiltration. The rabbits treated with streptomycin alone showed minimal lesions only. The rabbits treated with a combination of streptomycin and promizole showed no active lesions, the sections showing only scarring, an occasional encapsulated tubercle, and persistence of wandering cells in the iris and ciliary body.

The different clinical course and histological pictures can well be demonstrated pictorially. Thus Figs. 14 and 15, and Figs. 16 and 17, show the high and low extremes in the control group, at the onset of inflammation and at the end of the experiment. Figs. 18-19 show the typical histological picture of untreated tuberculosis in these same control immune-allergic rabbits. Figs. 18-19 illustrate the maximum histological reaction, while Figs. 20-21 illustrate the minimum histological reaction. Figs. 22-23, and Figs. 24 and 25, show the before and after clinical pictures of typical rabbits treated with streptomycin alone, and Figs. 26-29 show the minimal histological lesions in these same rabbits. Figs. 30-31, and Figs. 32-33, show again the before and after clinical pictures of typical rabbits treated with the combination of streptomycin and promizole, and Figs. 34-38, show the total absence of any active histological lesions in these same animals, the evidences of tuberculosis being limited to an encapsulated tubercle and the persistence of wandering cells.

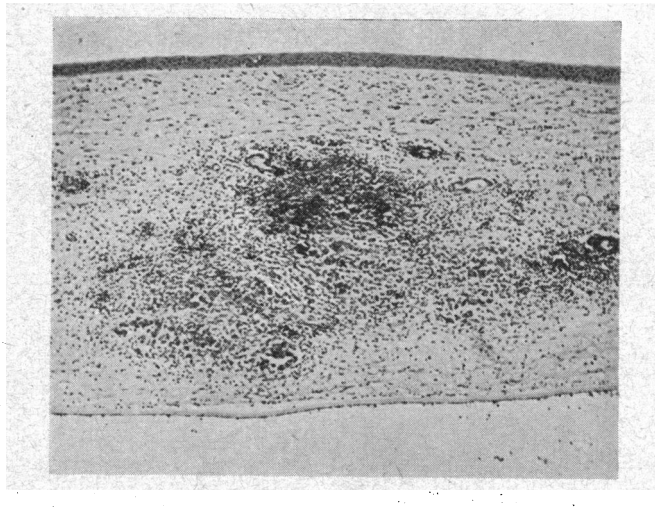


FIG. 18.

Cornea of control rabbit—maximum reaction.

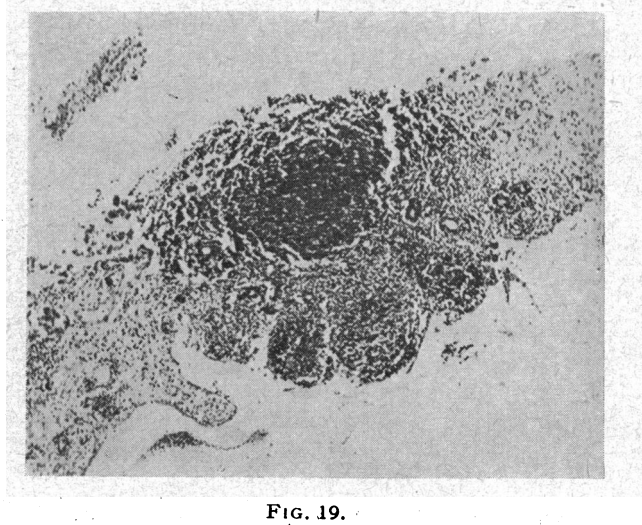


FIG. 19.

Iris of control—maximum reaction.

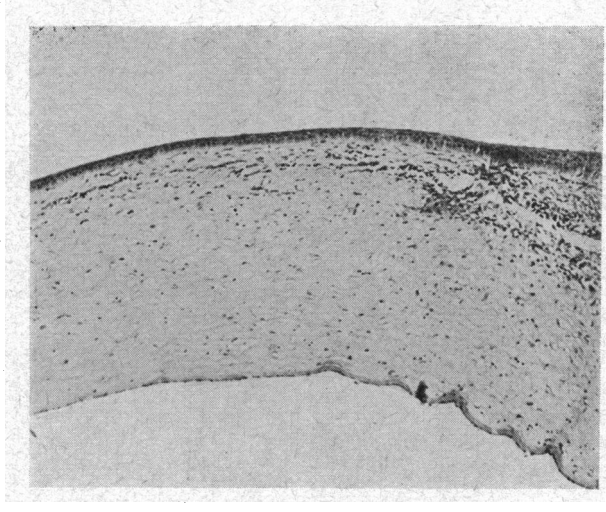


FIG. 20.

Cornea of control—minimum reaction.

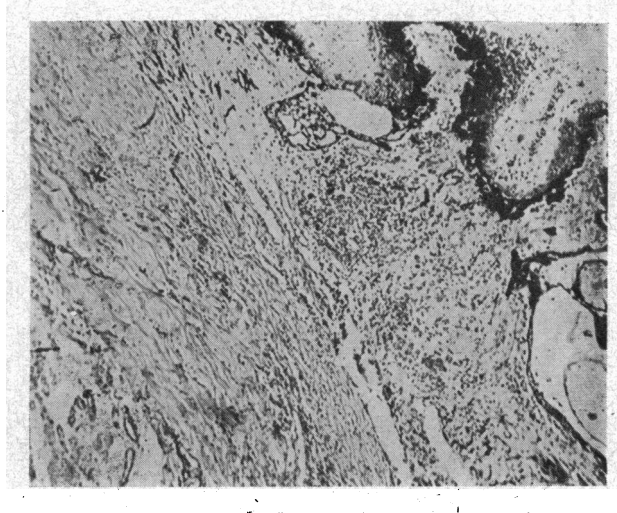


FIG. 21.

Ciliary region of control—minimum reaction.

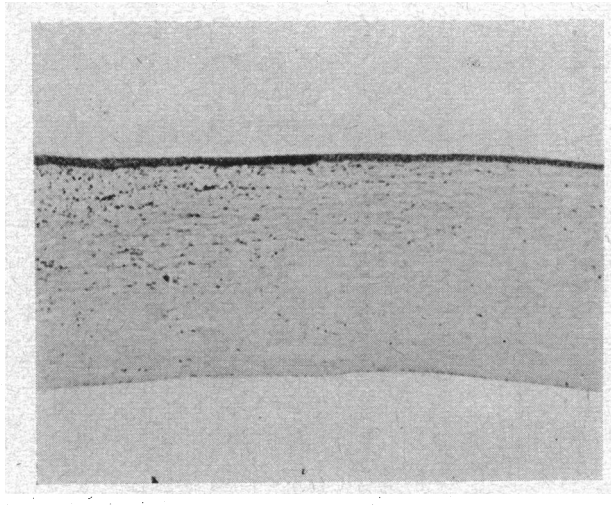


FIG. 26.

Cornea of streptomycin-treated rabbit—minimal infiltration.

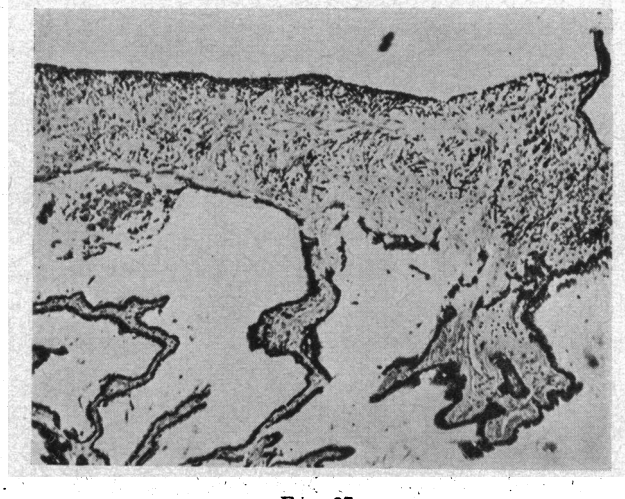


FIG. 27.

Iris of streptomycin-treated rabbit—small tubercle on posterior surface. Healed tubercle in stroma.

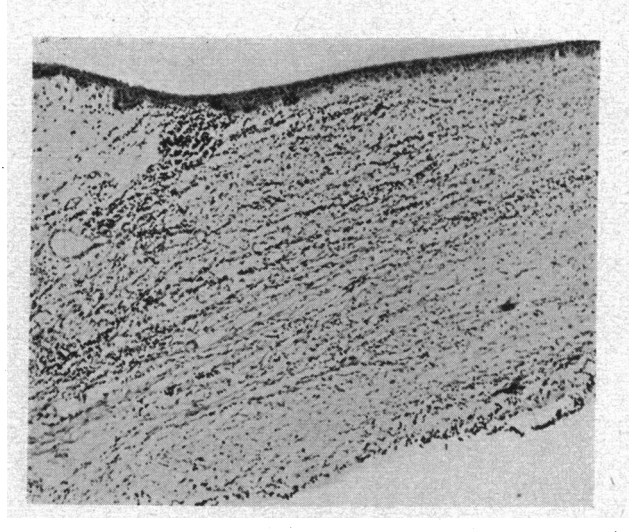


FIG. 28.

Cornea and root of iris of streptomycin-treated rabbit—minimal infiltration.

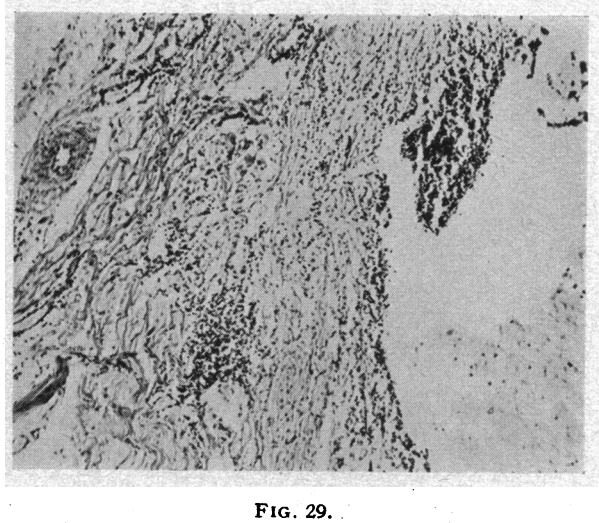


FIG. 29.

Root of iris and ciliary body of streptomycin-treated rabbit—moderately intense infiltration.

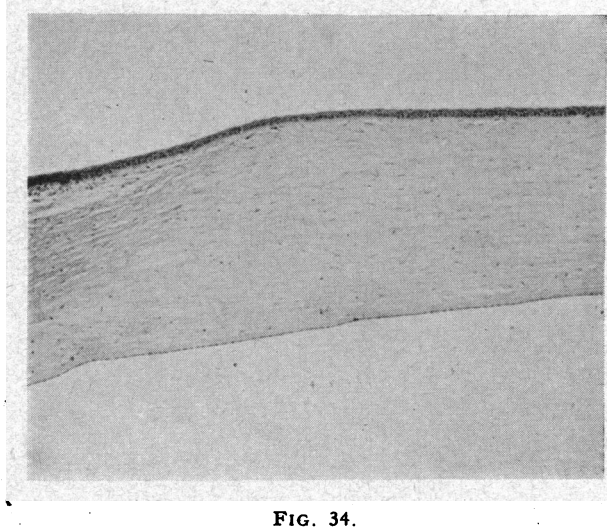


FIG. 34.

Streptomycin-plus-promizole-treated rabbit—minimal scarring.

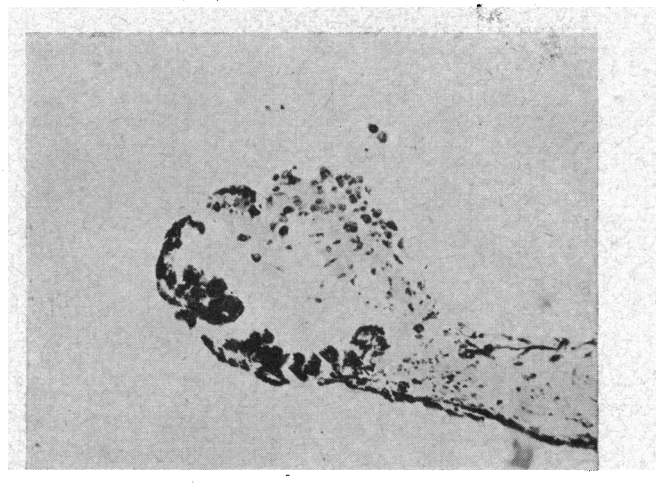


FIG. 35.

Streptomycin-plus-promizole-treated rabbit—healed tubercle at tip of iris.

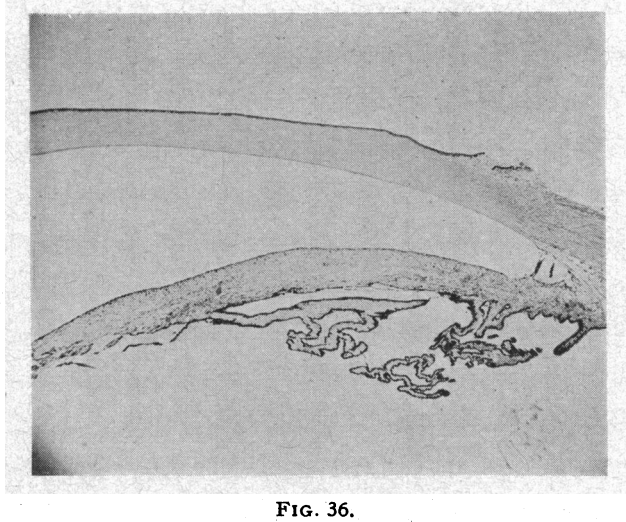


FIG. 36.

Streptomycin-plus-promizole-treated rabbit — normal anterior ocular segment.

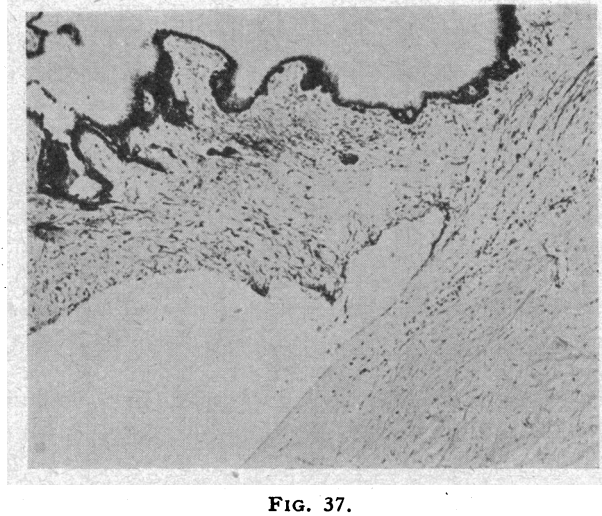


FIG. 37.

Streptomycin-plus-promizole-treated rabbit—minimal scarring of iris, persistence of wandering cells.

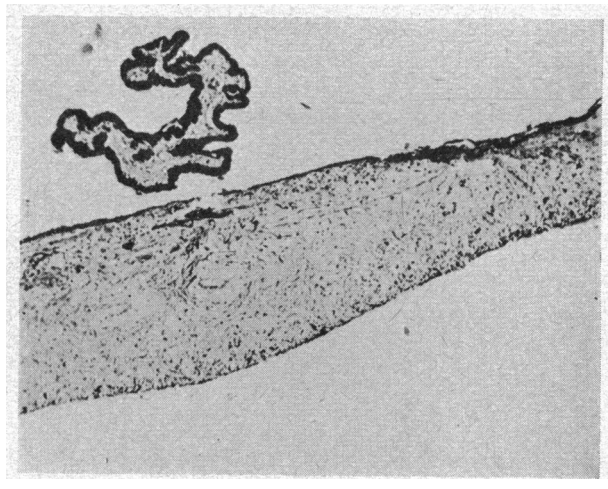


FIG. 38.

Streptomycin-plus-promizole-treated rabbit — showing persistence of wandering cells in iris.

At the conclusion of the therapy, six rabbits in each group were sacrificed for histological study, six others for culture of the dissected-out uveal tracts and for transmission experiments, and the remainder kept under observation for any recurrences of inflammation. The transmission experiment in these rabbits was a much more severe test than that employed in the previous experiment for rabbits treated with promin and promizole alone. The dissected uveas were macerated in only 1·5 c.c. of salt solution, whereas 5·0 c.c. of dilutant had been used in the experiment with promin and promizole alone. The results of these culture transmission experiments, and the incidence of recurrences in the surviving rabbits are shown in Table I. Thus the cultures of all controls were positive, showing from 15 to innumerable colonies in each slant. In the rabbits treated with streptomycin alone, three cultures were positive with 1-3 colonies only, while three were negative. In the rabbits treated with streptomycin and promizole, five cultures were entirely negative, while the sixth culture showed no macroscopic colonies, but bacilli were found on microscopic examination.

On transmission experiments, all the uveal tracts of the control group were infectious. In both the streptomycin-treated rabbits and the streptomycin-promizole-treated rabbits, 50 per cent. of the uveal tracts were infectious, and 50 per cent. non-infectious.

Recurrences occurred in three out of nine surviving rabbits treated with streptomycin alone, the average remission period

	Transfer		Cultures of Uveal Extracts		Recurrences	
	Results	Incubation Period	Results	Incubation Period	Results	Remission Period
Group I (untreated controls)	100% positive.	17 days	100% positive	Heavy growth in 4 weeks.	gradually waning activity.	
Group II (Streptomycin alone)	50% (3) positive. 50% (3) non-infectious.	26 days	50% (3) positive for tubercle bacilli 50% (3) neg	1-2 colonies in 5 weeks.	3 out of eight (37%)	12 days (average)
Group III (Streptomycin + Promizole)	50% (3) positive. 50% (3) non-infectious.	33 days	83% (5) negative. 17% (1) positive.	microscopic growth in one culture only in 8 weeks.	1 out of nine (11%) (minimum reaction)	34 days

TABLE I.

Results of transfer experiments and cultures and incidence of recurrences.

being 12 days. In the streptomycin-promizole treated rabbits, there was one recurrence in nine rabbits, the remission period being 34 days. Resistance experiments on the recovered organisms are as yet incomplete.

From these studies on the action of sulphones and antibiotics in ocular tuberculosis, it is conservative to conclude that a relatively non-toxic sulphone, promizole, has a deterrent action on the local tuberculous lesion in the immune-allergic rabbit. The deterrent action is far from absolute, and at best is scarcely more than sufficient to restrain the growth of the bacilli, or attenuate their virulence, to within the bacteriostatic or bactericidal range of a fairly well-developed immunity. Streptomycin has a much more powerful therapeutic action, undoubtedly a true bacteriocidal action, but again, as administered in these experiments, this action is not always absolute. The combined action of streptomycin and promizole is much more pronounced, and appears to eradicate the bacilli from the infected tissues in a large percentage of the treated cases, and certainly to curtail their growth and virulence in the remaining cases.

SUMMARY

These experimental findings may be summarized as follows: It appears that Rich's law for the pathogenesis of tuberculous lesions holds true in localized ocular tuberculosis. The factors governing the course and character of the lesion are the number and virulence of the infecting organisms, the degree of tissue hypersensitivity present, and the amount of the resistance established by the host. These studies also offer an explanation for the

relative cutaneous anergy often present in animals and man with inflammatory tuberculous lesions of the eye.

This concept of the pathogenesis of a tuberculous lesion offers obvious points for therapeutic attack on the disease. The first of these is enhancement of immunity, and nothing specific has been done as yet to stimulate local resistance artificially. The second is removal of the fatal tissue hypersensitivity, and this can usually be accomplished by the use of tuberculin as a desensitizing agent, and has a distinctly beneficial effect on the clinical course of the lesion. The third is the direct attack on the tubercle bacilli, and in streptomycin and promizole combined, we have a powerful weapon to this end. Doubtless other better antibiotics and sulphones will be found, but at present this combination appears the best available.

THE VASCULAR ACTION OF PILOCARPINE, ESERINE ADRENALINE AND ATROPINE, AND THEIR INFLUENCE IN PRIMARY CHRONIC GLAUCOMA*

BY

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BOLOGNA

THE rationale of the action of miotic and mydriatic drugs in glaucoma is a matter which still retains its importance, not only because our knowledge of the mechanism of the action of these drugs is still incomplete, but also because the discovery of new antiglaucomatous drugs and modern researches on the chemical mediation of the transmission of nerve impulses have amplified the problem without offering any substantial explanation. Moreover, the interpretation of the mechanism of these drugs—still an unsolved problem when they act upon the normal eye—becomes more complicated when they are considered in relation to the added problems involved in the development of a raised intraocular pressure in glaucoma.

In physiological literature we find the most diverse views regarding their effect on the ocular tension and the vascular system in the eye. Without mentioning Leber's notes in "Graefe Sämisch Handbuch," but reviewing the opinions of recent authors only, we find that in Colombo's opinion eserine and pilocarpine cause vasoconstriction, whereas Bailliart and Bidault attribute to

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