EXPERIMENTAL TUBERCULOSIS OF THE CORNEA.*[1]

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When an attempt is made to study the tissue reactions against the tubercle bacillus in either the normal or the immune animal, difficulties of a technical character are at once encountered. The final picture in an animal infected with tuberculosis is extremely complicated, and it is impossible to resolve it into its functional elements. Methods of examination during life are practically limited to such general conditions as changes in activity, body-weight, and temperature. The great help derived in the study of other infections from the investigation of changes in either serum or cells of the blood does not touch the problem.

In the belief that it would be of great advantage to be able to study a discrete tuberculous lesion which could at the same time be kept under constant observation without the necessity of resorting, in each experiment, to the tedious technical procedures involved in microscopic examination, we have undertaken a renewed study of experimental corneal tuberculosis.

The work is still in progress. This paper is intended to present primarily the technical phases of the subject, and to indicate briefly the limitations and possibilities of this line of experimentation. Secondarily, there will be presented some facts to show that the corneal lesion is susceptible of influence in an appreciable degree by both local and systemic measures and conditions.

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[1] The experiments here published were used as the basis of a paper read before the Lænnec Society of Johns Hopkins University in April, 1912. Extraneous circumstances have interfered with the prompt publication of the matter. In the meantime the experiments have for the most part been repeated on a large scale in connection with chemotherapeutic studies. The results of the later experiments conform in all essential respects to those here described.

The general features of the lesion of the cornea following inoculation with tubercle bacilli or tuberculous matter were described by Cohnheim and Salomonsen (1) in 1877. Their inoculations were made into the anterior chamber, and consequently their lesion did not develop in detail according to our description.

Other observers have used the corneal experiment to study microscopically the early development of the tubercle, but this is a matter with which we are not here immediately concerned.

Hänsell (2) in 1879 inoculated the cornea, conjunctiva, and anterior chamber with tuberculous matter. He described the corneal lesion in its essentials with accuracy, both in its gross and microscopic features.

Krusius (3) has presented interesting data on experimental tuberculosis of the various parts of the eye. He has developed the fact that the cornea is less susceptible to infection than the anterior chamber and the vitreous humor. It is more susceptible than the lens. Krusius states that the incubation period of the lesion in the cornea is inversely proportional to the dose of culture used. This author notes that in order to get consistent results it is important to use a culture of known virulence and rabbits of equal resistance (same weight, age, etc.). In a general way our results agree with those of Krusius entirely, although we have developed them and have considered the subject from an entirely different point of view. In one or two points there is an apparent contradiction between our results and his. He states that generalization does not occur from the eye lesion even when a culture of bovine type is used. He also found that the lesions showed frequently a distinct tendency to heal. Our animals when they have been kept for a sufficient length of time have always shown tubercles in the internal organs. In but few instances have the corneal lesions shown any definite tendency to heal. These differences indicate that we have used cultures of higher virulence than those employed by Krusius.

The observations on the use of iodin locally have been indicated by the clinical use of this substance more or less regularly since the time of Lugol for the purpose of influencing tuberculosis in one or another of its manifestations.

In using calcium lactate we have followed the observations of Chiari and Januschke (4) on the inhibitory influence of soluble calcium salts administered generally on simple inflammations.

Our observations on benzol have been made under the influence of the experimental work of Selling (5), in which the pronounced leucotoxic action of this substance was developed.

TECHNIQUE.

In all the experiments reported, we have used rabbits. The animals have varied in weight from 1,500 to 3,000 grams, have been of various colors, and purchased from various sources. They were of no especial breed. For most of the experiments we have used a culture of bovine type, isolated from cervical glands in 1907. The culture retains a high degree of virulence, as 0.01 of a milligram intravenously injected will kill a rabbit of 2,000 grams in from

twenty to thirty days, with generalized tuberculosis. The culture was grown on glycerin agar, and the growth was used within fourteen days of the time of transfer. The culture was suspended in a salt solution to make a suspension as homogeneous as possible, and this was centrifuged at high speed for a short time to remove the macroscopic clumps. The suspensions varied in weight, but were, as a rule, made by suspending one glycerin agar tube in ten cubic centimeters of salt solution. The completed preparation was usually of sufficient density to be just completely opaque when contained in the ordinary twenty millimeter bacteriological test-tube.

The eyes before inoculation have been carefully anesthetized with cocain. When this is done, the injection can be made without restraining the animal in any way, and it gives no pain or discomfort. In the beginning we tried to fix the eye with a pair of forceps. We found later that the injection could be made equally well without it. The injury done by the fixation forceps frequently gave rise to secondary foci of infection which should be avoided. We have used a small record syringe, but we have found it better to use special needles that we have had made for the purpose. The most satisfactory are of iridoplatinum, of about gauge 26, and ground to a very short bevel, with a sharp point. The advantage of the platinum needle is that it retains a smooth surface free from rust, even after being sharpened repeatedly. This seems to be very important in making a successful injection. The ordinary steel needles can be used with success when they are new, but they cannot be used after sharpening or repeated boiling.

The injection is made by placing the point of the needle against the cornea in the position desired, holding the syringe in a plane parallel to the cornea, considered as a flat surface. The needle is then inserted by a succession of short, jerky pushes, in which only slightly more force is used than that exerted by allowing the weight of the syringe to bear on the needle point. By this means, the eye is rotated, and between each push is allowed to come back almost to its normal position. When the aperture of the needle is entirely covered by the corneal epithelium, pressure is put on the piston, and the suspension forced into the cornea. Considerable pressure is required. The fluid raises a small vesicle which is at once opaque.

We have not found it practicable to measure the amount of fluid injected, but a fairly uniform series of inoculations can be secured by watching the size of the opacity produced. We have had most uniform results when this measured about three or four millimeters in diameter.

It cannot be said that this technique is perfect. Occasionally, instead of raising a definite circumscribed vesicle, the fluid flows into the cornea along radiating lines, spreading widely from the point of puncture. In a certain number of instances the needle penetrated the anterior chamber. This accident, however, has grown much less frequent as we have gained experience. Moreover, it seems probable that the ultimate result varies, to a certain extent, according to whether the injection mass remains localized close beneath the corneal epithelium, or flows more deeply into the tissue. When the larger amounts suggested are injected, there seems to be less variation due to this factor. In certain instances, instead of injecting the material we have made the inoculation simply by pricking the cornea with the needle wet with the culture. The results of this procedure will be explained later.

In our examinations made subsequent to inoculation we have confined ourselves to the use of oblique illumination. We have found it advantageous to use artificial light because of the greater constancy, and have adopted a 40-watt Tungsten lamp with a ground glass bulb held about sixteen inches distant from the eye. With this we have used a small hand lens.[2]

We have, of course, made careful notes of the development of the lesion, but have found that frequently we save much time and secure, on the whole, more useful records by charting the conditions from day to day.

[2] Dr. Thomas B. Holloway has recently been over the methods of examination of the eye with us and has pointed out the possible usefulness of the corneal loop and the ophthalmoscope. We have not used these instruments extensively, so far as the results at present recorded are concerned, but they will probably add to the accuracy of our examinations in the future. It is likely that the use of these more accurate methods of examination would modify our statements only as they concern the time of appearance of the earliest vascularization of the cornea.

DEVELOPMENT OF THE CORNEAL LESION.

The Effect of Large Doses of Tubercle Bacilli.—When a heavy suspension of tubercle bacilli is injected into the cornea in such a way as to make a distinctly circumscribed infiltrated area of three to four millimeters in diameter, the first result, as has been said, is a circumscribed opacity, which probably marks fairly well the limits of the infiltration. At the end of twenty-four hours this opacity has usually almost disappeared. At this period one is able by careful examination to see the defect in the cornea that marks the point where the needle penetrated, and extending from this defect a very short way into the cornea is a very fine, whitish or brownish white line. At this period, the sclera and bulbar conjunctiva will have their normal appearance, and there will be no evidence of any irritation of the eye. In from three to seven days, depending on various factors, there is a noticeable change. A small increasing opacity of grayish white color develops at the point of inoculation on the cornea, and the conjunctiva of the bulb becomes increasingly injected from day to day. On the seventh day, for the average, the opacity at the point of inoculation has a diameter of about one millimeter. The relation of the size and rapidity of growth of the opacity to the appearance and development of the injection of the bulbar conjunctiva is not perfectly regular, although it is fairly so. As a rule, the conjunctival congestion begins at about the time that the first visible increase in the exudate at the inoculation point shows itself. After several more days, the conjunctival congestion having in the meantime become very intense, loops of newly formed capillaries appear in the cornea, pushing out from the margin towards the inoculation point, and the exudate at the inoculation point continues to increase. The loops of vessels extend further and further into the cornea, until finally they come into contact with the exudate. The congestion of the bulbar conjunctiva becomes complicated by congestion of the conjunctiva of the lids. The lids swell and there is more or less of an increased secretion, which is at times white, at times yellowish. It is usually sufficiently fluid to remove itself spontaneously, but occasionally it becomes sticky and glues the lids together. This does not often occur

before the third week after inoculation. At about the time that the corneal vascularization or pannus comes into contact with the exudate at the point of inoculation, the corneal epithelium over the exudate usually breaks through leaving an open ulcer.

The formation of the pannus takes place in a regular way. The first vessels formed are capillaries and an advancing margin is maintained as a capillary zone. Behind the advancing margin there is a tendency for the pannus to thicken to a definitely raised rather pale zone of granulation tissue. Between this area and the corneoscleral junction the vessels are gradually reduced in number and increased in size until there are formed several main trunks of supply to the more central lesion. These supply trunks run through a cornea which has become partially clear again. In the later stages, with the advance of the exudate and the invasion of the pannus by it, the peripheral clear zone again becomes obscured and filled with granulation tissue.

When large doses are used, the pannus never tends to invade, in any marked degree, the mass of exudation. On the contrary, the exudate gradually extends and infiltrates the pannus. Most of our experiments have terminated at this point. The exceptional instances which have been kept longer under observation will be commented on in later paragraphs in connection with special points. With the large dose in the untreated eye, the vascularization of the cornea usually begins between the eighth and twelfth days.

The Effect of Small Doses of Tubercle Bacilli.—With the results following large doses, we have contrasted the effects of a minimal inoculation. They have been obtained, as before stated, by simply wetting the needle with the culture and pricking the cornea. In making the inoculation in this way it is essential that the epithelium be pierced. If the epithelium be merely superficially scratched, the inoculation is not effective. With the small dose, there come into play the same factors that have been described for the large dose. Very essential differences, however, are to be noted. The rate of progress of the lesion is much less uniform. The ultimate appearance of the lesion, in the majority of cases, is much the same, but in details of the first importance there are variations. It is especially by the use of this method of inoculation that one can deter-

mine differences in individual resistance of the animal. In old rabbits the changes occur much more slowly than in young rabbits. It has occasionally happened, in using this method on old rabbits, that the pannus has completely infiltrated the mass of exudation at the point of inoculation. In the young rabbit it has happened conversely that the pannus has not only been invaded by the mass of exudation, but that it has actually been involved extensively in the ulcer, and has been almost completely eaten away. Various intermediate conditions are encountered.

INFLUENCE OF VIRULENCE OF CULTURE.

We have not so far studied extensively the action of different cultures on the cornea. However, some inoculations were made with cultures of human type which are, of course, non-virulent for rabbits. The large dose before described produced the same end result that the large dose of the virulent culture did. Smaller doses or doses of less virulent human culture produce a lesion which is smaller and which shows a great tendency to heal spontaneously. The lesion in its development follows the general course of that described for the bovine type culture with some differences which serve to place it between the bovine lesion and that about to be described for the non-virulent acid-fast bacilli. The immediate reaction to the human type is somewhat more rapid as a rule. The blood vessels begin to form more quickly but the progress of the lesion is less rapid. While the virulent culture generalizes from the eye as a focus, the human type of culture does not generalize. We hope later to develop this phase of the subject.

CONSEQUENCES OF PERFORATION.

If the cornea is perforated at the time of inoculation or, as has often happened in our series, the ulceration of the cornea occurs internally as well as externally, an infection of the anterior chamber results. The consequences of such infection have been sufficiently described by others. We wish to note, however, one or two points which are of interest by contrast with the pure corneal infection, and which chiefly concern the pannus. The typical pannus, which

is formed as a result of the infection of the cornea, begins at a single point on the corneoscleral junction, extending centrally and broadening out from this point. If the injection is so placed that it is somewhat above central, the pannus always begins at the upper margin of the cornea. When the lesion has become quite extensive, a second pannus may appear starting from below. Placing the injection forward or back of central throws the point of beginning of the pannus either somewhat forward or back, as the case may be. It is an interesting point that when the injection is either precisely central or slightly below central, the first pannus will still form above. From the moment that the cornea perforates internally, however, a circular pannus begins to form. We have controlled this point by injecting the anterior chamber in such a way that the cornea was not injured; that is, by passing the needle through the sclera from behind. Here the pannus is also circular, and no difference between the upper and lower margins can be noted. These differences presumably depend on the circulatory channels in the cornea, but we have at present no more precise explanation for them.

THE EFFECT OF KILLED CULTURES OF THE TUBERCLE BACILLUS.

When we had proceeded with this work for some way, we were led to consider how much of the effect produced by the inoculation was specific for the tubercle bacillus, and how much might depend on the non-specific factors. Before trying anything more remote, we used the same culture killed by heating for one half hour to 60° C. When such a killed culture is injected in about the quantity of a large dose of living culture, the first effect is the same. At the end of twenty-four hours a difference appears, in that the bulbar conjunctiva is usually pronouncedly injected, and the eye appears to be definitely more irritated than by the injection of the living culture. The inoculation point becomes marked out by an exudate in about the same way as with the living culture, but this exudate seldom reaches more than pinhead size. On the fourth or fifth day, as contrasted with the eighth to the twelfth day for the living culture, loops of vessels appear in the cornea. They appear

at the situation favored by the vessels appearing in response to the living culture. They extend very rapidly into the cornea as a thin leash which on the sixth or the seventh day reaches nearly to the point of inoculation. The base of the leash never broadens; it is usually about three to five millimeters in width. From the seventh day on these vessels seem rapidly to disappear. By the tenth day nothing more of them can be seen in the cornea by our methods of examination. No permanent pannus is formed. The exudate at the inoculation site may be entirely reabsorbed, or may persist for months as a pinhead-sized scar. The vessels do not, however, really disappear, at least not within a period of two months. They cease to functionate. This can be shown by making a fresh inoculation with either dead or living cultures near the old scar. Under these circumstances at the time when the congestion of the sclera becomes well marked, the old corneal vessels suddenly fill and the new formation of vessels in consequence of the second inoculation takes place from the end of the old leash.

For obvious reasons we have compared the lesions so far described with the corneal lesion produced by other bacteria. The strains of *Staphylococcus aureus* and *Bacillus typhosus* used, when inoculated on the cornea near its center, produce a rapid local exudation. There is, however, no tendency to vascularize the cornea and the exudate finally resolves more or less completely leaving a relatively small opacity at the inoculation site.

The acid-fast bacteria other than *Bacillus tuberculosis* give a lesion which in severity and time relation develops much more like the lesion caused by the dead tubercle bacilli than by the living. The exudation is small, the vascularization commences early, and the vessels cease to functionate after a short time. The lesion usually resolves, leaving only a small scar. We have used in this connection the bacillus of timothy, the butter bacillus of Grassberger, Korn's grass bacillus D., the bacilli of frog and fish tuberculosis, an old isolation of the bacillus of avian tuberculosis, and the chromogenic *Bacillus leprœ* of Duval. The last of these cultures has sometimes shown a more progressive and persistent lesion than the others. The difference between *Staphylococcus aureus* and *Bacillus typhosus* on the one hand, and *Bacillus tuberculosis* and the acid-

fast bacteria on the other, in capacity to stimulate the formation of vascular tissue in the cornea is striking. It is, however, a purely quantitative difference or one which is not manifest under more general conditions. This is shown when *Bacillus typhosus* or *Staphylococcus aureus* are inoculated, avoiding the cornea, into the anterior chamber through the sclera. As a consequence of the anterior chamber infection a vascular, circular pannus quickly forms on the cornea.

As described, the corneal lesion due to the inoculation with the tubercle bacillus proceeds in an orderly fashion through certain clearly defined stages and presents in some degree an opportunity for an analysis of the processes involved. Certain experiments were now undertaken to determine whether or not the course of the lesion could be influenced by the local and general administration of various substances. As we hope to develop this phase of the subject in greater detail in the future we shall now merely present our results in outline in so far as they show that the lesion can be modified.

Iodin, Locally Applied as Lugol's Solution.—If the conjunctival sac be washed daily with Lugol's solution the early development of the lesion is in no way influenced. In many instances the pannus when well formed thickens to an unusual degree. The secondary thinning out of the vascular tissue near the corneoscleral margin does not take place. In order to attain this effect the concentration of the iodin in the solution and the duration of the exposure must be sufficient to produce a distinct inflammation when applied to the healthy control eye. The effect may be in large measure an additive one. We have never seen the pannus invade the central exudate under the influence of iodin.

Tuberculin.—Koch's old tuberculin given intravenously in large or small doses or instilled into the eye is without effect on the early development of the lesion. We have not tried its effect later when panophthalmia exists, and our results in no way contradict those of previous observers who have made out a favorable influence from the treatment of experimental and clinical tuberculosis of the eye with this preparation.

Calcium Lactate.—Calcium lactate administered intravenously or subcutaneously in doses sufficient to intoxicate the animal severely is able to inhibit the corneal process in considerable degree. The central exudation perhaps increases more slowly than usual, although this is not perfectly clear. The vascularization of the cornea is somewhat delayed in point of time and proceeds more slowly and much less vigorously than usual.

Benzol.—The subcutaneous administration of benzol in olive oil to the point of severe intoxication decreases the amount of exudation to the inoculation point and causes the vascularization of the cornea to develop slowly and feebly. This experiment like that with calcium lactate cannot be carried to a satisfactory conclusion because it is necessary to give the substance in doses certainly although slowly fatal in order to get demonstrable results. The control blood counts have shown that only in those cases in which the leucocytes were reduced very low has there been an appreciable influence on the development of the lesion.

Intercurrent Disease.—In several instances we have inoculated recently purchased, supposedly normal animals which in the first few days developed an acute illness followed by a period of extreme emaciation. In these animals the development of the corneal lesion was also slow and without vigor. The exudation at the inoculation point was inhibited and until this was well developed there was no vascularization of the cornea.

DISCUSSION.

The foregoing experiments taken in a general sense can hardly be said to differ radically from certain of those previously performed by others. Moreover, when we consider them in detail and attempt to formulate their meaning it is often difficult to be sure that what we have learned is not already fairly well understood by those closely familiar with the pathology of tuberculosis. In spite of a degree of uncertainty as to the final value of such considerations, it is our purpose to state in our own language the point of view at which we have arrived by way of this work and to indicate some of the problems which are defined by it.

Tuberculosis is classed by systematic pathologists as an infectious

granuloma. The justice of this classification is emphasized by these experiments. The corneal lesion consists of a mass of granulation tissue the various elements of which are present in variable proportions at different stages in the development of the disease. The experiments recorded in which the action of *Bacillus typhosus* or *Staphylococcus aureus* is contrasted with that of the tubercle bacillus on the cornea indicate that the latter much more readily gives rise to the formation of blood vessels. This power of stimulating blood vessel formation is also possessed by other acid-fast bacilli. As shown by the results of anterior chamber inoculation, however, the difference is one of quantity rather than of quality. *Bacillus typhosus* and the pyogenic cocci under proper conditions may likewise stimulate blood vessel formation. As a large exudative lesion produced on the cornea with *Bacillus typhosus* may fail to give rise to the new formation of capillaries, while even a minimal lesion with dead tubercle bacilli or with non-virulent acid-fast organisms of other kinds leads to their rapid formation, we are probably justified in believing that the difference is a large one and perhaps of vital importance in the development of the disease. The corneal lesion develops in an orderly way. The irritation due to the injection is recovered from, is followed by a latent or incubation period, and this in turn is followed by the development of a local exudation. Not until the exudate has become definite does the formation of blood vessels begin. In experiments such as those with benzol and calcium lactate in which the primary exudation is limited or suppressed, the blood vessels fail to develop or develop very feebly. Presumably if it were possible to prevent the exudation entirely, the vessels would never develop. Expressed in terms of the prevention of the disease, these results are of little significance. While it is possible to say in these terms that the development of a tuberculous lesion has been definitely delayed by these agencies, yet it has been by methods that are so severe in their general effects that even the experiments can hardly be continued to a satisfactory conclusion.

From another point of view, however, the matter has interest. In the well developed corneal lesion the tubercle bacillus is present in enormous numbers. Several of these inhibitive experiments have lasted from two to three weeks, and in this time the cultures we

have used on culture medium attain a large growth. If in this time there was a great growth of the organism in the transparent cornea it might be expected to become visible; or if the bacteria did not become visible as a colony, the cornea should develop a local defect. As a matter of fact no visible change occurs in the absence of exudation and it seems to be a fair conclusion that we are dealing with a true condition of latency, one in which the bacillus grows little if at all, and in which the tissues are practically unchanged. Stated in another way, the conclusion is indicated that there is nothing in the fixed tissues of the cornea to provide for food for the bacillus and little or nothing about the living tubercle bacillus which does injury to these tissues. With the coming of the cellular exudate the case is entirely different. The products of cellular degeneration furnish food for the bacillus and the fixed tissues are attacked either by the products of the growth of the bacillus in this pabulum or by the products of cellular activity or cellular degeneration. From this heterogeneous combination also, substances are set free which give a stimulus for blood vessel formation and doubtless for the proliferation of other fixed tissue cells.

If this reasoning is in any measure correct, and we believe that it is largely so, we are forced to agree with those who have held that tuberculosis is primarily an exudative inflammation and secondarily proliferative in its character. More than that, we are led to the belief that the cellular exudate, protective though it may be in purpose, is defective and is destructive in its actual influence. Even the phagocytic activity of the cells of such an exudate may probably serve to distribute living bacilli and thus spread the disease to fresh areas.

BIBLIOGRAPHY.

1. Cohnheim, J., and Salomonsen, Ueber künstliche Tuberculose (Impfung in die vordere Augenkammer), in Cohnheim, Gesammelte Abhandlungen, Berlin, 1885, 591.
2. Hänsell, P., Beiträge zur Lehre von der Tuberkulose der Iris, Cornea und Conjunctiva, *Arch. f. Ophth.,* 1879, xxv, pt. 4, 1.
3. Krusius, F. F., Experimentelle Tuberkulosestudien, *Veröffentlichungen d. Robert Koch-Stiftung z. Bekämpfung d. Tuberkulose,* 1913, Nos. 5–7, 133.
4. Chiari, R., and Januschke, H., Hemmung von Transudat- und Exsudatbildung durch Kalziumsalze, *Arch. f. exper. Path. u. Pharmakol.,* 1911, lxv, 120.
5. Selling, L., Benzol als Leukotoxin, *Beitr. z. path. Anat. u. z. allg. Path.,* 1911, li, 576.