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EPIDEMIC KERATO-CONJUNCTIVITIS IN THE MIDDLE EAST*

Clinical and Experimental Study

BY

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THE following is our experience of so-called keratitis superficialis punctata epidemica during several years in the Middle East, particularly in Palestine. The report consists of a clinical and an experimental part.

(A) Clinical part

In 1937 and 1938 sporadic cases of so-called keratitis superficialis punctata began to appear and later the disease assumed epidemic proportions. Colleagues working in the coastal area of Palestine were the first to see these cases in numbers, while those in the hinterland began to see a few cases only in 1938, and these came with extraordinary consistency from the coastal areas.

The classical keratitis superficialis punctata (Fuchs, 1889) which is usually sporadic and without striking conjunctival changes has been observed here as elsewhere for a long time. It is, however, sometimes forgotten that Fuchs described acute conjunctival

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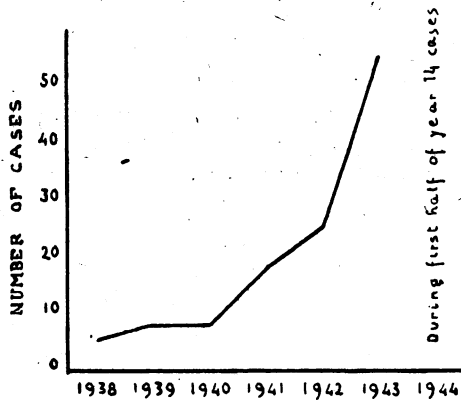
changes in this disease which at that time also seems to have appeared epidemically. It is noteworthy that three months before Fuchs' publication this keratitis arising from conjunctivitis had been described and named more correctly keratitis subepithelialis by H. Adler of Vienna (1889) who thus is the author to be credited with the first description of the disease.

In 1939 the first report appeared of an epidemic occurring in Palestine (Feigenbaum). It identified the condition with the epidemic kerato-conjunctivitis which had occurred during previous decades in the coastal towns of India: Bombay, Madras, Calcutta and in Batavia, as reported by Herbert (1901), Kirkpatrick (1920), Wright (1930), Zachariah (1930), Kirwan (1934), Westhoff (1912), and further expressed the opinion that the condition was in some way associated with the climate of the sea coast (and possibly with some kind of hypovitaminosis as a predisposing factor). The first two patients of A.F., who did not come from the coastal plain, were two children from Jerusalem whose father had brought them a pail of sand from Jaffa.

In the meantime a series of reports have appeared in Europe and North America in which there is a notable consensus of opinion that the condition is aetiologically associated with proximity to the sea. This opinion is expressed in the description "Shipyard-conjunctivitis" which has been applied to it (Sanders, 1942). In 1941 J. B. Hamilton described an epidemic of the disease in Tasmania where the majority of the cases also came from the coastal region.

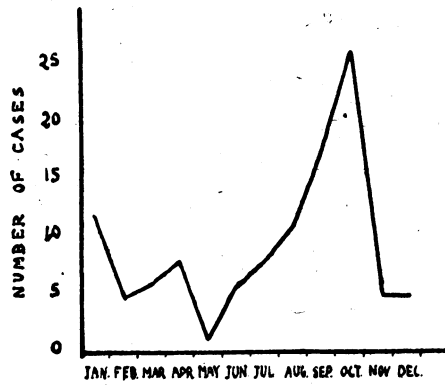
The increasing incidence of the condition in this part of the world as well as a great number of cases among Polish cadets stationed

GRAPH 1 - YEARLY INCIDENCE



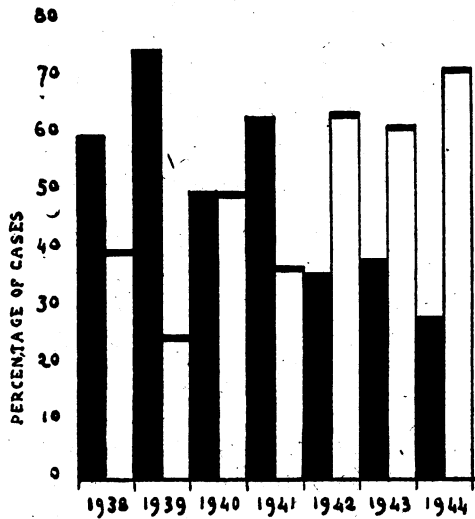
Yearly incidence of cases belonging to Group I. Note the rise.

GRAPH 2 - MONTHLY INCIDENCE.



Monthly incidence of cases belonging to Group I. Note the peak in the beginning of wet season.

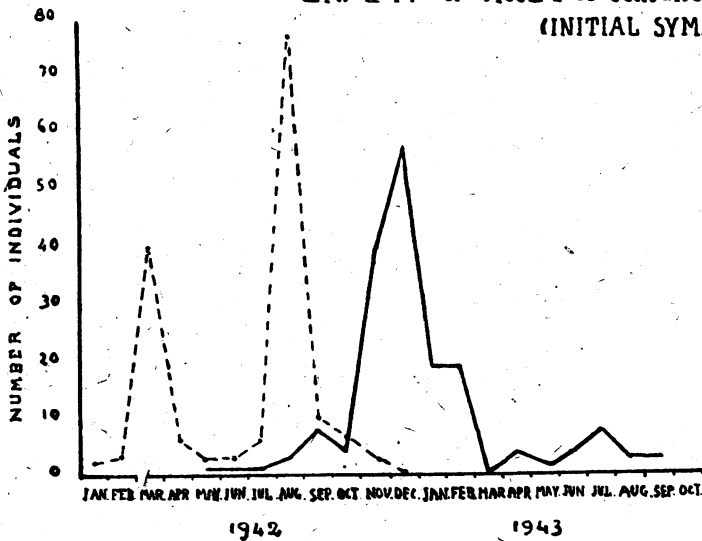
GRAPH 3 - ORIGIN OF PATIENTS
(WITH REGARD TO SEA COAST)



■ PATIENTS FROM COASTAL REGIONS
□ PATIENTS FROM INTERIOR OF COUNTRY

Distribution of cases belonging to Group I according to their origin—with regard to coastal regions and inland (whether from the sea coast or from the interior of the country). Note the rise in percentage of the cases coming from the interior.

GRAPH 4 - --- GRAPH OF EMIGRATION FROM RUSSIA
 — GRAPH OF ONSET OF CONJUNCTIVITIS
 (INITIAL SYMPTOMS)



Relation of different periods of emigration from Russia of cases belonging to Group III and the incidence of the disease (initial symptoms among them. Note the peak of incidence in (November and December 1942), while the second peak of emigration falls on August (three to four months before).

in the Middle East, gave us the opportunity of studying the problem from epidemiological, clinical, experimental and histological standpoints.

Sources of cases investigated :—Our 381 patients consisted of :—

- (1) Patients from the Ophthalmological department, the Rothschild Hadassah University Hospital, Jerusalem, and private practice, total 133 cases;
- (2) Fifty patients from the British Army; and
- (3) One hundred and ninety-eight Polish cadets already mentioned.

Epidemiology :—Graphs 1-3 show the cases mentioned in Group 1 and give incidence during the years of observation, the seasonal distribution, and the association with the sea-coast.

Graph 4 gives the seasonal incidence of the cases mentioned in Group 3. It can be seen from Graphs 2 and 4 that the beginning of the winter (in this part of the world the rainy season) is the special period for the epidemic incidence, while only sporadic cases were observed during the rest of the year. This is in complete agreement with the Indian and recent American reports which are very detailed in clinical observation (Hogan and Crawford, 1942).

While the source of the "first case" is not known, there is no

doubt that each case constituted a source of infection for susceptible contacts. Frequently members of one family were affected simultaneously.

The rôle of trauma in aetiology.—In our experience trauma such as corneal epithelial lesion, hordeolum or operation on the globe, plays a not inconsiderable rôle. A very slight epithelial trauma which can easily occur may give an opportunity for the entry of an ubiquitous virus which may also be conveyed from one patient to another by medical or nursing staff. In this connection mention may be made of two small house epidemics which occurred in patients on whom operations had been performed for cataract, glaucoma, etc. These patients complained of a feeling of pricking and of the sensation of a foreign body in the eye. During the change of dressing one observed a number of fibrinous threads between the lid margins. Smears and cultures did not show any bacteria. The lids and conjunctiva were swollen and in several of these cases the conjunctivitis was followed by a typical punctate keratitis. Bandaging did not interfere much with the healing of the condition. We particularly mention one case where a paracentesis of the cornea had to be done because of profuse bleeding into the anterior chamber with secondary glaucoma although a conjunctivitis, of the type just described, was present: no harm to the eye resulted despite the paracentesis and the repeated subsequent re-opening of the anterior chamber. This case was in hospital during the house epidemic already mentioned.

It is no wonder then that the reports from the United States indicate a strong incidence among factory workers, while the rest of the population in the same neighbourhood was only slightly affected. This indicates that slight lesions of the conjunctiva increase the susceptibility to the disease, and this justifies the ruling of the California State Industrial Accident Commission that disability resulting from the disease is subject to compensation (M. J. Hogan in discussion of paper of Bedell, 1943).

The incidence of the condition is affected by neither age (the age incidence of our cases varied from 4 months to 68 years), sex (both sexes were almost equally represented) nor social class. Certain conclusions regarding epidemiology may also be drawn from the experimental part of this report.

No indisputable instance of recurrence was observed in any of our cases, although exacerbations were seen especially in the very chronic cases found in Group 3.

Symptomatology.—It is difficult to estimate the incubation period which in our cases appeared to be between 5 and 14 days. As in all descriptions of this condition the subjective symptoms were prominent. The general condition of the patient was unaffected in the great majority of cases. The symptoms consisted

of pricking, itching, and/or the sensation of a foreign body in the eye and later sometimes of a slightly blurred vision indicating corneal involvement.

The objective findings were: lacrimation; swelling of the lids accompanied by pseudoptosis which involved mainly the outer 2/3 of the lid in 85 per cent. of Group 2; typical glistening of the conjunctiva ("cellophane surface appearance"), marked swellings of the conjunctiva of the lower lid which sometimes reached cocks-comblike dimensions, swellings of the plica semilunaris and caruncle, and painful swelling of the pre-auricular gland often accompanied by swelling of the sub-maxillary gland. The pre-auricular glands were palpable in 90 per cent. of the cases in Group 2, but in some there was no accompanying tenderness, the gland appearing to remain palpable after the tenderness had gone. Especially notable was a delicate fibrinous thread-like secretion, such as is commonly found in spring catarrh. The secretion contained a few monocytes. No inclusion bodies were found in epithelial scrapings.

The condition began bilaterally (50.3 per cent. of our cases) or unilaterally (49.7 per cent.). In the latter there was no difference in the frequencies of affection of either eye (25.5 per cent. showed right eye affection and 24.2 per cent. showed left eye affection). In the course of a period varying from four days to several weeks the second eye frequently became affected. In it the conjunctival changes were usually less severe. In Group 3 where the condition was extremely chronic only two out of 198 cases remained unilateral.

The conjunctival changes described above must be considered as the basic manifestation of the disease. They are characteristic enough to allow a certain diagnosis even in the absence of corneal lesions, especially when occurring in epidemics and when conjunctival smears and cultures are negative. This applies to our cases where, moreover, conjunctival scrapings in an overwhelming proportion showed mononuclear cells and where no inclusion bodies were found.

The corneal lesions enter the picture very frequently as a kind of complication and are specific to such a degree that for quite a long period the name of the disease was derived from them.

The corneal changes presented themselves in three or four different forms:—(1) Ill-defined sub-epithelial dots ranging from tiny spots to small maculae (macular keratitis). This was the most frequent form. The number and distribution of these dots varied. These lesions in the cornea were located either mainly in the centre or in the periphery, and there were also mixed types.

(2) Superficial epithelial infiltrates some of which were vesicular and some of which stained with fluorescein.

(3) Disciform keratitis. This is the rarest form but is definitely a manifestation of the disease, as it may appear simultaneously with the typical dots in one or the other eye of the patient. The disciform infiltration accompanied by thickening of the cornea, with which there may be marked folding of Descemet's membrane and often keratic precipitates, recedes leaving several dots of opacity at various levels of the cornea which gradually fade away. 5.3 per cent. of our cases showed precipitates in Descemet's membrane.

Corneal changes were found in 60.4 per cent. of cases in Group 1. Of these, 22.5 per cent. had both eyes, 37.5 per cent. the right eye and 40 per cent. the left eye affected. In Group 3 the cornea was affected in 65.6 per cent. of cases. Of these 59.2 per cent. had both eyes, 13.8 per cent. the right eye and 27 per cent. the left eye affected. As far as one could judge the interval between the conjunctival changes and the keratitis was 7-17 days. There was no direct association between the severity of the conjunctivitis and the appearance of the keratitis.

The conjunctival changes gradually diminished after a few weeks while the corneal complications in most cases lasted a longer period, usually from a month to 2½ years. Blurring of vision caused by the subepithelial dots may be ascribed to two factors: the opacity as such and the irregular astigmatism caused by the dots even when they are subepithelial, as some of them elevate the epithelium to a varying degree. This can be seen at the slit-lamp where the anterior corneal band of the optical section appears in places slightly broken when the light is moved over the corneal surface and, still more distinctly, at the ophthalmometer where the images are distorted as usual in irregular astigmatism.

The gradual diminution in number and density of these opacities can be followed graphically. During the stage of subsidence the larger dots frequently presented the appearance of a well defined linear rim with a pale centre. In cases that have been over-treated the conjunctival changes may not only remain for a long time but can be aggravated in a striking fashion. This occurred among our cases of Group 3 as will be described later.

It is noteworthy that a certain number of our patients easily developed inflammatory changes in the palpebral skin (sometimes extending even to the cheeks) during the course of the disease. This dermatitis had the character of an allergic lesion provoked either by the tears or by some drug used.

Peculiarities of Group 3.—At the beginning of 1943 it came to the knowledge of one of us (A.F.) that there was a great number of trachoma cases among the Polish cadets, boys and girls between 12-18 years, stationed in Palestine. These cases constituted 10 per cent. of the total number of the 3,150 youths of Barbara Camp.

They had spent the end of 1941 and the whole of 1942 partly in Russia, partly in Persia at the shores of the Caspian Sea where they had gone (as can be seen by the data of Graph 4) after leaving Russia. They spent a variable time in Pahlevi and Teheran, whence they travelled via Iraq to Palestine. In many of the cases the precise date of onset of the disease is uncertain, but it is definite that the onset was not at the Caspian Sea but either in Teheran, Iraq or Palestine.

It appeared to us very doubtful whether these cases were true trachoma. The 198 cases detailed in this report (out of a total of 323 cases treated at Barbara Camp) were thoroughly re-examined with the help of the slit-lamp.

The clinical picture in the majority of the cases showed a great resemblance to trachoma as far as chronic gross papillary hypertrophy of the conjunctiva was concerned, but as one of the essential signs of the disease, namely pannus, was absent, the diagnosis had to be dismissed. There was in fact not a single case of trachoma in this group. The fibrinous discharge of the conjunctiva suggested a disease somewhat related to spring catarrh, but as only one case was found to be a true spring catarrh (with one typical limbal nodule), which was of course eliminated, and as a great proportion of the remaining cases showed the corneal changes of so-called epidemic superficial keratitis, it seemed most probable that this disease was the cause of the pathological changes in these cases.

The most striking feature of the cases in Barbara Camp was the great hypertrophy of the conjunctiva of both lids which was often very gross. This was frequently accompanied by a severe mucopurulent discharge which on bacterial examination gave a negative result. This unusual feature can be explained by the very drastic treatment given to these cases which as already mentioned were considered to be trachomatous. The treatment consisted of silver nitrate, copper sulphate, sublimate massage and in the majority of cases even expression which left very definite scarring. In this way the disease was maintained and healing prevented. A third of the patients showed chronic ptosis. More than three-quarters of the material used for experimental purposes was obtained from these patients.

Therapy.—There is more to be said negatively than positively under this heading. It seems clear that energetic treatment aggravates the condition. In this connection silver preparations are chiefly at fault as ophthalmologists so very enthusiastically turn to them in acute catarrh of the conjunctiva, but even other drugs used in conjunctivitis do more harm than good. It is not easy to say whether or not the heavy salts increase the disposition to keratitis as indicated in a personal communication by Dr. Levy-

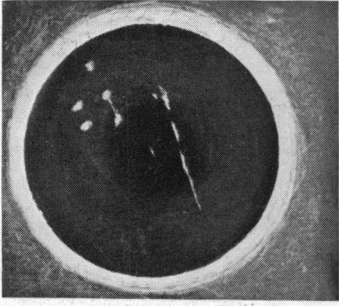


FIG. 1.

Subepithelial corneal changes six days after inoculation (Rabbit No. 193).

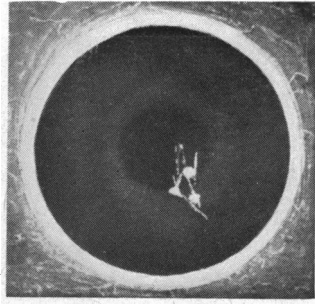


FIG. 2.

Corneal changes after four days (Rabbit No. 605).

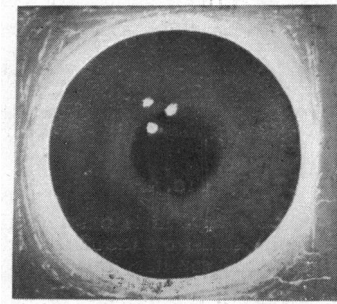


FIG. 3.

The same rabbit as in Fig. 2 six days after.

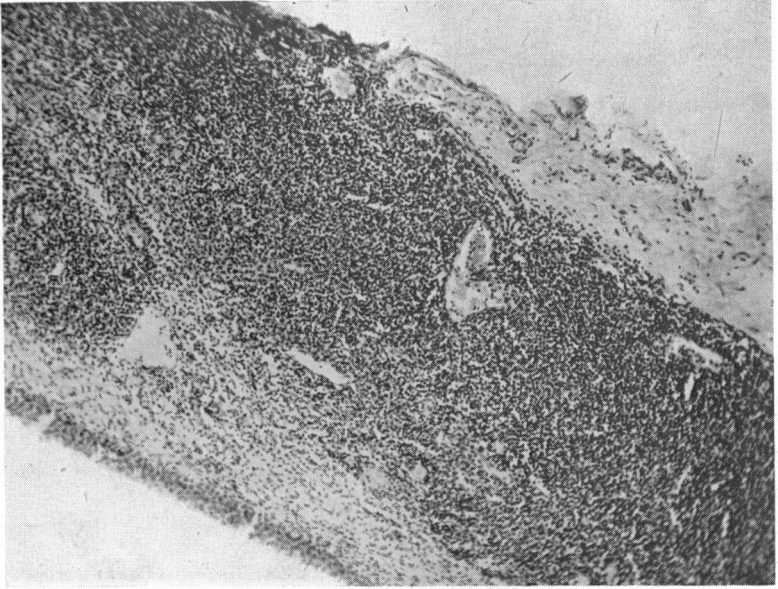


FIG. 4.

Section of conjunctiva *two days* after onset of the disease. Note oedema separating the epithelium from the marked subepithelial diffuse infiltration where pronounced capillary dilatation exists (Case 177; X32).

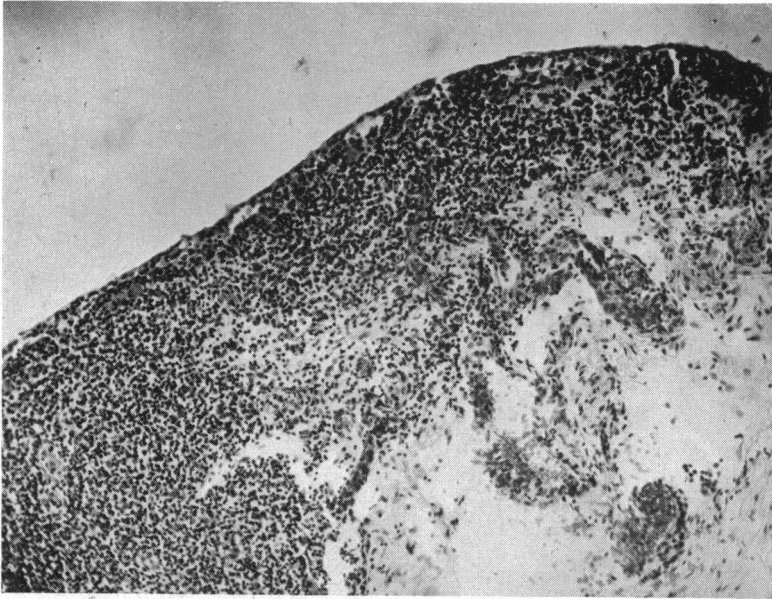


FIG. 5.

Section of conjunctiva *six days* after onset of the disease. The massive subepithelial round cell infiltration is everywhere in contact with the epithelium (Case 179; $\times 58$).

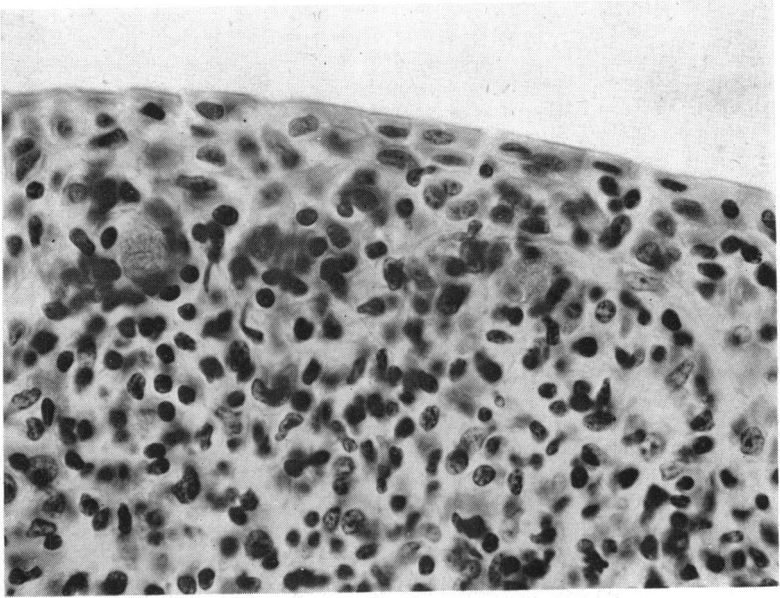


FIG. 6.

The same section as in Fig. 5 ($\times 650$) showing flattening of the epithelium.

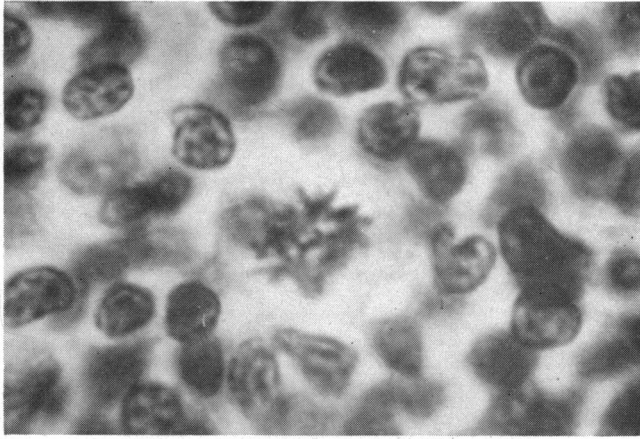


FIG. 7.

The same section as in Figs. 5 and 6 showing round cell infiltrate with one cell undergoing mitosis ($\times 1800$).

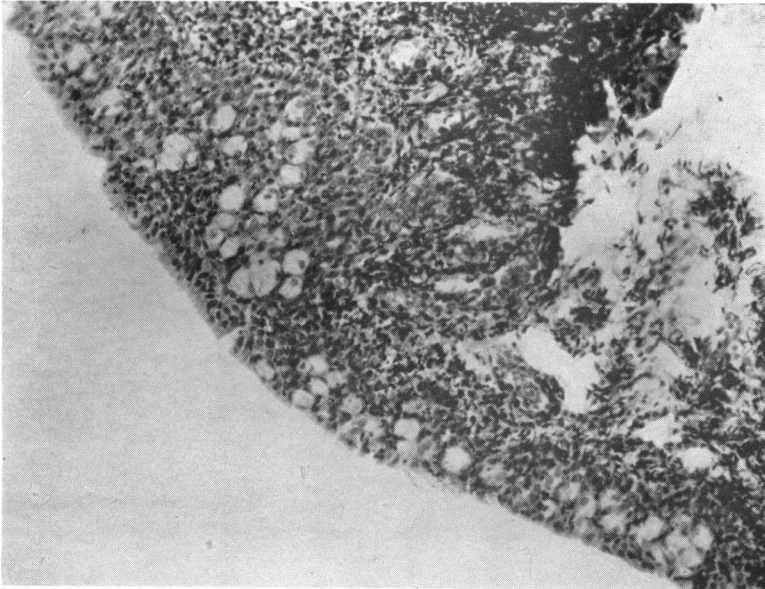


FIG. 8.

Section of conjunctiva *eighteen days* after onset of the disease. Epithelial changes with formation of goblet cells. Subepithelial round cell infiltration with admixture of polymorphonuclear leucocytes (secondary infection) as well as a moderate number of fibroblasts (Case 183; $\times 160$).

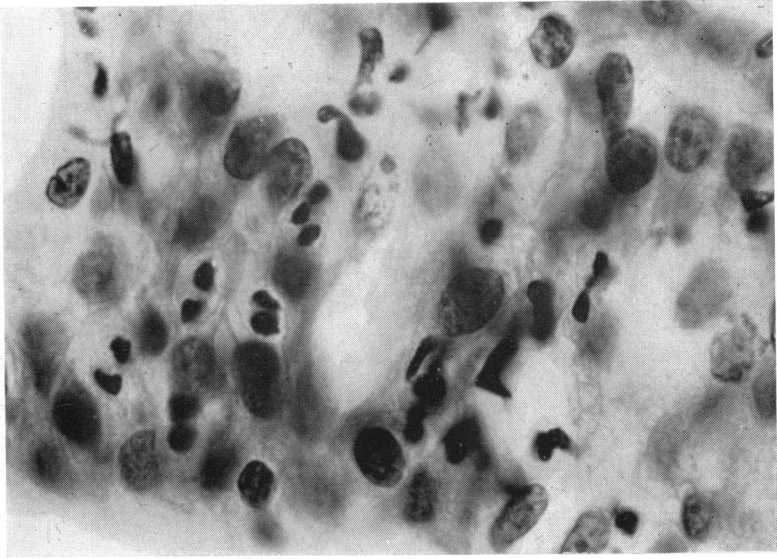


FIG. 9.

Same section as in Fig. 8 ($\times 1350$) also showing polymorphs (secondary infection).

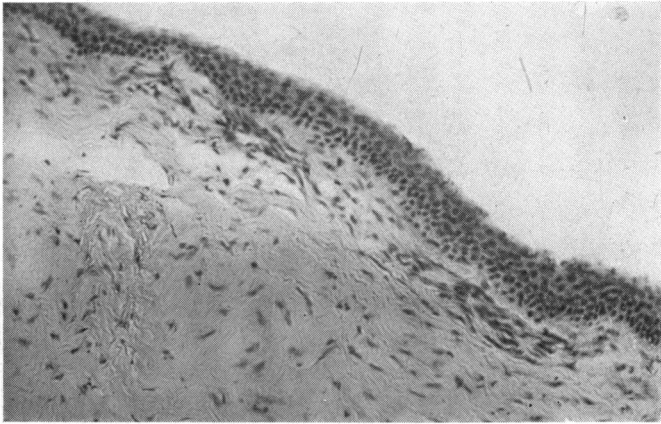


FIG. 10.

Section of a rabbit's cornea five days after successful inoculation. Portion containing the subepithelial macular lesions. Note the subepithelial bundles of elongated cellular elements. At one place these cells seem to intrude into the thinned epithelial cover (Rabbit No. 176, $\times 120$).

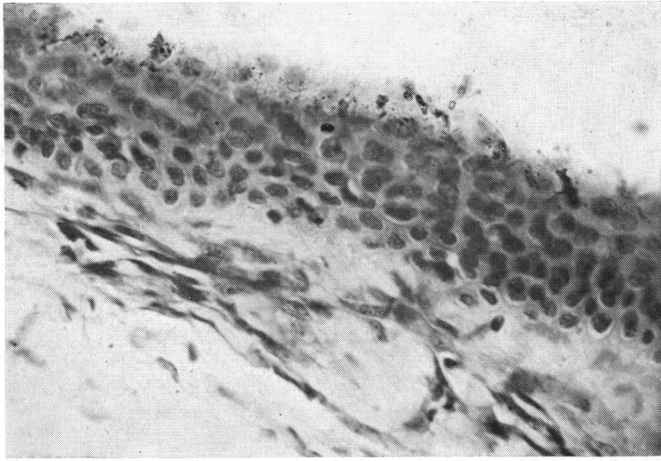


FIG. 11.

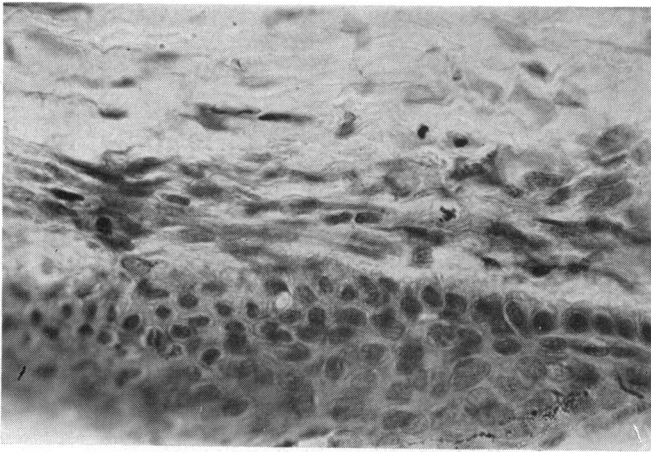


FIG. 12.

Sections of the same cornea as in Fig. 10. In the subepithelial layer the elongated mobilized cells form bundles. The cellular architecture of the epithelium is somewhat disorganised. The basal cells have lost their palisadelike arrangement and their nuclei—the normal perpendicular position. Note the detritus over the surface of the epithelium ($\times 350$).



FIG. 13.

Normal portion of the same cornea. Note the normal arrangement of the epithelial cells ($\times 350$).

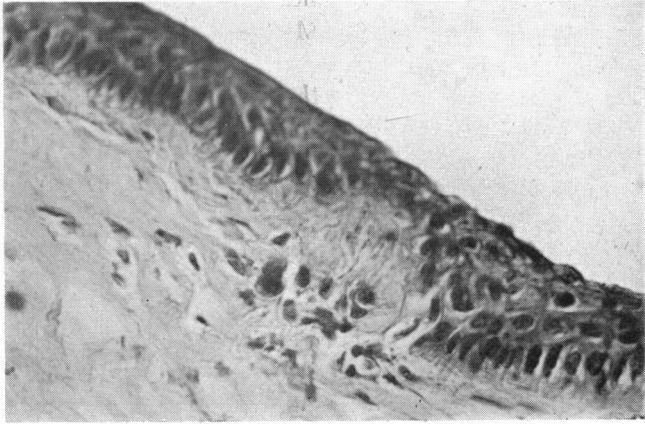


FIG. 14.

The mobilized cellular elements at one place seem to intrude into the epithelium where the latter is thinned and reduced to about 2-3 layers ($\times 350$).

Wolff. These should in any case not be used as they undoubtedly aggravate the subjective and objective signs. The best remedies are the frequent instillation of concentrated adrenalin solution (1 in 4,000) and douchings with cool water. Cocaine is often useful and atropine should be used for the keratitis when ciliary injection is present. After the complete disappearance of the conjunctival changes 2 per cent. yellow oxide of mercury ointment, with 5 per cent. dionine, appear to be of use for the corneal infiltrations. Iontophoresis with sodium iodide was found to be of use in those cases of keratitis where the changes were confined to the epithelium, but had no effect where they were sub-epithelial. Treatment with immune serum is of theoretical interest and has been used by Braley and Sanders (1943) with good results in a small number of cases. Sulphonamide given locally or by mouth does not, in our experience, help the condition, except in cases with mixed infection where only the conjunctival condition may improve.

In a small series (6 cases) penicillin was tried, using 250 units to the c.c. as drops 2-hourly day and night for 3-4 days, without any effect whatsoever.

In a few cases of disciform keratitis intravenous injection of 1-3 c.c. of a 10 per cent. solution of sodium iodide seems to give rapid improvement and is recommended for such cases.

Differential diagnosis.—In the Middle East the following conditions must be considered:—

(1) *Acute conjunctivitis of the type of swimming bath conjunctivitis.*—In this condition there are likewise considerable conjunctival hypertrophy and swelling of the pre-auricular gland, but the sparse secretion contains polynuclear leucocytes and scrapings of the conjunctiva show inclusion bodies with varying frequency. There are no threads of fibrin and most cases tolerate treatment with silver and copper very well. There are no corneal complications.

(2) *Trachoma.*—Trachoma is often confused with the condition under consideration. Among the cases which Mulock Hower described in 1937 as acute trachoma, three at least belong to the condition described here. Our cases in Group 3 showed an extraordinary similarity to the conjunctival changes found in trachoma. Yet these cases did not have the cardinal sign of trachoma—pannus, even after many months. The slit-lamp can best settle this point of differential diagnosis (Wilson, 1932).

(3) *Conjunctivitis vernalis.*—A certain resemblance exists in that threads of fibrin are found in both conditions. In the disease under study, however, there are not the typical conjunctival vegetations nor changes at the limbus. In spring catarrh the characteristic conjunctival changes mainly affect the tarsal conjunctiva of

the upper lid while the fornices are free and the lower lid in pronounced cases presents only a milky hue. In contradistinction our conjunctivitis shows the most important changes in the conjunctiva of the lower lid (glistening "cellophane surface appearance" and papillary swelling). Moreover the corneal changes found near the limbus in spring catarrh have an altogether different character.

(4) *Koch-Weeks' conjunctivitis*.—In this condition there are likewise acute swelling of the conjunctiva, fibrinous secretion and enlargement of the pre-auricular gland, but the fibrin is present in gross amount and contains numerous polynuclear leucocytes and Koch-Weeks' bacilli which seem to disappear after short treatment. Finally the corneal changes in Koch-Weeks' conjunctivitis appear simultaneously with the conjunctival changes and are of quite different type. Sub-conjunctival haemorrhages appear less frequently in kerato-conjunctivitis than in Koch-Weeks' conjunctivitis.

(5) *Herpes corneae*.—This is practically never bilateral. There are no conjunctival changes, inclusion bodies are found fairly regularly, and experimental inoculation of the rabbit's cornea is followed frequently by encephalitis. This did not occur in our animal experiments.

(B) Experimental part

Small pieces of conjunctiva removed under sterile conditions from the lower lid and placed in a small amount of physiological saline were used as our material. These small pieces of tissue were pounded up with sterile sand and the larger particles removed by centrifuging. The supernatant fluid obtained after this process was considered to be a suspension of the infective agent and its sterility was proved by inoculation on blood agar and in serum broth. In the experiments described below this suspension was used without waiting for the bacteriological findings. Naturally only the results obtained with bacteriologically negative suspensions are included.

From a series of investigations it became clear that the pieces of conjunctival tissue in saline kept in the refrigerator, contained the infective agent in active form for as long as one month. On the other hand the suspensions remained infective for only 2-5 days.

In some cases the experimental materials used were scrapings from the conjunctival epithelium. This method soon appeared to be inefficient and was abandoned.

Animal experiments

Rabbits.—Inoculation was carried out as follows: The cornea, which slit-lamp examination had shown to be free from opacities, was scratched with a fine hypodermic needle and the suspension

described above was rubbed into the scratches. In addition, 0.1 c.c. of the suspension was injected sub-conjunctivally and when possible into the membrana nictitans. For the success of the experiment it did not matter whether or not the membrana nictitans or the conjunctiva were inoculated. Inflammatory changes in the conjunctiva were minimal and non-specific in that they did not resemble the changes found in the human conjunctiva. As a control, one eye only was treated with the suspension and the other inoculated simultaneously with physiological saline.

Summary of positive results from inoculation experiments

Of the 33 rabbits in which one eye was inoculated with the suspension, 30 eyes showed typical changes in the sub-epithelial layer of the cornea in the form of opacities similar in appearance to those found in most cases of human so-called keratitis epidemica and described as punctate keratitis. (The material used for these 33 inoculations came from 25 cases of Group 3 and 8 cases of Group 1 of the three negative results two belong to Group 3 and one to Group 1.) Some albino animals were used for inoculation and contrary to the experience of Wright (1930) no differences in susceptibility could be established between melanic and albino rabbits.

These fairly regular takes in the rabbit's cornea showed the following aspect and course: After an incubation period of 24-48 hours, sub-epithelial corneal infiltrates could be seen in the form of thin lines which in places showed bulbous thickenings. After several days hardly anything could be seen of the lines while the thickenings presented the appearance of small opacities as described above (see Figs. 1-3). Although one case lasted for almost 3½ months these opacities remained visible for an average of two months. Towards the end of this period the opacities faded in the following way: A central clear area appeared in the opacity giving it the appearance of a ring which finally faded away.

In five cases an attempt was made to introduce the condition from an infected cornea to a healthy one in order to obtain passage of the infective agent. The lesions of the diseased cornea were removed under slit-lamp; and the material used on a second rabbit's cornea in the manner described above. In only two cases was a typical picture obtained. Of the others one was doubtful, two were negative. Attempts to pass the condition from the two positive cases were unsuccessful. It is noteworthy that the incubation period which was 1-2 days originally, increased to 4-5 days in the passage.

Mice.—Young mice (3-4 weeks old) were inoculated intracerebrally (0.03 c.c.) and intra-peritoneally (0.5 c.c.) by the method

described by Sanders (1942). In addition inoculation was carried out with infected *membrana nictitans* of rabbits, a method which has given good results with herpes virus, while other methods of inoculation with the latter are hardly effective in mice (Steigman and McNair Scott, 1942).

All these methods were consistently negative in their results. Fifty mice were inoculated without a single one showing any evidence of disease. In order to find out whether the infectivity of the agent could be enhanced by passage, 15 mice were sacrificed five days after inoculation, the brain removed with sterile precautions and after being pounded up was used for intra-cerebral injections into other mice. Even after five passages there was not the slightest evidence of pathological changes in the animals.

Likewise inoculation experiments were negative in mice which had previously been treated with X-rays (500 R) in order to diminish resistance.

Experiments with vitamin-deficient animals (riboflavin, thiamine) were likewise negative.

Similar experiments on mouse brain, using tissue culture material gave negative results.

Control experiments.—As mentioned above for the purpose of control and for the purpose of removing any doubt regarding the specificity of the corneal changes, the second eye of the rabbit was always inoculated simultaneously with saline. These inoculations produced no changes apart from fine lines of infiltration which disappeared in 2-3 days without leaving any trace. With the same object inoculation experiments were performed using normal conjunctiva as the inoculated material. Constant changes were obtained in these experiments similar to those obtained when pathological material was used—lines with bulbous thickenings at the crossings, but they disappeared in several days or at most one week. This is in contrast to our positive inoculation results in which the lesions in the form of a macular keratitis remained for at least a month and usually 2½ months.

Immunity.—The following neutralisation experiments were carried out to determine whether or not immune bodies were present in the serum of the convalescent case and whether specific neutralisation was possible.

Infective material from human conjunctiva and from tissue culture, as will be described below, was diluted (1:1) with physiological saline or with convalescent serum. One eye of the experimental animal was then inoculated with the suspension diluted with physiological saline, and the other with the suspension diluted with serum. In all six cases the typical pathological changes described above appeared in the eye which was inoculated with the suspension diluted with saline while the other eye showed

no more than the non-specific changes described above in the control experiments, and which disappeared at most after one week.

In a number of cases further infection was attempted on corneae of rabbits which had previously been inoculated with positive results. This was done during the period of full development of the disease, and shortly after the disappearance of the lesions. As no diminution in the susceptibility of the cornea was found in this experiment (both in active cases and those shortly after disappearance of lesions), no appreciable immunity could be detected in the affected tissue.

Tissue culture

In view of the negative results obtained in mouse-experiments, a series of experiments with tissue culture was initiated according to the method of Sanders (1943).

Technique.—The brain was removed from 12-15 days old mice embryos, cut into small pieces, and planted into Carrel flasks. The medium used consisted of 0.5 c.c. chicken plasma, 0.5 c.c. tyrode solution, 0.5 c.c. normal human serum and 3 drops of saline chicken embryo extract. The cultures were incubated at 37° C. Four days after incubation the tissue cultures showing an abundant cell outgrowth were inoculated with 2-5 drops of a suspension of the infective agent (prepared in the way described above) and left standing at room temperature for 5-7 days. For the purpose of passage the inoculated tissue culture was cut into pieces and the infected fragments were put into another Carrel flask containing embryonic brain culture. At each passage a piece of the tissue was pounded up and the suspension inoculated into a rabbit's cornea.

Results.—The first two cultures showed bacterial contamination after a few days' incubation, despite the fact that the blood agar and serum broth cultures had remained negative. The cultures remained sterile only when the precaution was taken later of washing the human conjunctiva with 1/4000 oxycyanate of mercury before removal of the tissue.

Using three affected conjunctivae as material, tissue cultures were infected and these were maintained to the 7th passage. All subcultures of these three series were inoculated on rabbits' corneae with positive results. As there is a dilution of 1:10 with each subculturing, and therefore in seven passages a dilution of 1:10,000,000, it can be readily seen how much enhanced the agent becomes in tissue culture. These experiments show that here we most probably deal not only with maintenance of the infective agent but that a real multiplication of the latter had taken place. The original suspension of the agent, on the other hand, was infective only up to a dilution of 1:20—and higher dilutions gave negative results.

Inoculation experiments on mice with tissue culture material, were completely negative and thus a neurotropic tendency could not be demonstrated. The infective agent was tested for dermatropic properties by inoculation into the skin of a number of patients during the initial, fully developed and chronic stages of the disease. In no single instance was a local reaction obtained.

Filtration experiments.—Pieces of tissue were taken from 12 Carrel flasks under sterile precautions, pounded up, suspended in about 15 c.c. of sterile tyrode, and lightly centrifuged. The supernatant fluid was passed through a Seitz filter. The filtrate was specifically infective for the rabbit's cornea in direct experiment and after transmission in tissue culture to the 4th passage.

These tissue culture experiments strongly suggest that the infective agent in the disease under consideration is a filtrable virus. Many examinations of the scrapings of the conjunctiva were made by us in a vain search for inclusion bodies. Major van Rooyen to whom we are greatly indebted was kind enough to examine at least 50 specimens which failed to show any inclusions.

Histology

Histology of human conjunctiva.—Histological examination of cases at the commencement and at the height of the disease gave fairly characteristic appearances. In all cases the conjunctival tissue examined came from the lower fornix. The tissues were fixed in Zenker's solution and stained with haematoxylin-eosin and diluted Giemsa solution. Sections show an early flattening of the cylindrical cells of the epithelium (Fig. 6). In the sub-epithelial tissue marked capillary dilatation and oedema are the outstanding features. The epithelium is thinned at first especially over places where the subepithelial tissue shows considerable infiltration and oedema, most probably as a result of mechanical pressure. Later on thickening of the epithelium accompanied by many mitotic figures occurs. During the first few days the diffuse subepithelial infiltration at many places appears separated from the epithelium by oedema (Fig. 4). Soon, however, the infiltration is everywhere in contact with the epithelium and presents a constant picture consisting almost entirely of two types, common lymphocytes and large histiocytic cells of epithelioid character (Figs. 5 and 7); the latter show numerous mitotic figures (Fig. 7). In an early case single eosinophils can be seen also in the subepithelial infiltrate and in later ones a varying number of polymorphonuclear leucocytes may be found scattered between the epithelium and the sub-epithelial tissue which may be considered as evidence of secondary infection (Figs. 8 and 9). In the later stages there is also a moderate perivascular infiltration.

As is common in chronic conjunctivitis one frequently notices epithelial changes with the formation of goblet cells, several of which conglomerate to form large detritus-containing crypts some of which open to the surface (Fig. 8). In very chronic cases showing strong papillary hypertrophy, there is a histological picture reminiscent of that of trachoma. Rows of epithelial cells seem to pass inwards, while the essential feature is a marked proliferation of the papillae towards the surface of the conjunctiva. Often with these changes the superficial epithelial cells are not only flattened but also disorganised, and in places reduced to a single layer.

Histology of a rabbit's cornea.—The cornea was examined histologically of a rabbit which had been successfully inoculated five days previously. The portion of the cornea containing the typical lesions (macular keratitis) presented the following changes:—

In circumscribed areas, beneath the epithelium a marked increase in numbers of elongated cellular elements is to be seen, originating either from the corneal corpuscles or from wandering cells. These cells are prominent, their cytoplasm is distinctly basophil and the nuclei are enlarged. Here and there they form short, more or less compact bundles (Figs. 10 and 11).

The epithelium above these spots has mostly lost its regular architecture. The cells of the basal layer lose their palisade-like arrangement and the nuclei change their position from perpendicular (Fig. 13) to the corneal surface to horizontal (Figs. 11 and 12). The epithelial layer is clearly thinned; in some places the mobilised cells seem to intrude into the epithelial cover and at such a bulge the epithelium is reduced to 2-3 layers (Figs. 10 and 14). It should be noted that the corneal surface over the subepithelial lesions described is slightly irregular and covered with some detritus, resulting from the incompletely healed mechanical injury produced at the time of inoculation (Figs. 11 and 12). (No relation can be established to Bowman's membrane as this membrane is non-existent in rabbits.)

Comment

There are no doubts concerning the specificity or the infectivity of the disease under consideration. The mode of infection is not known although predisposing circumstances for infection can be recognised such as trauma and climatic conditions.

A whole series of facts suggest that the inciting agent is a filter-passing virus. Examinations of direct smears and of cultures failed to reveal any bacteria.

Experimentally secretion from the conjunctiva did not produce the disease, while conjunctival tissue direct or after tissue culture gave positive results. In the absence of bacteria the material remained infective; this can be explained only by active propagation of the infective agent within the living cell.

The neutralisation experiments were indicative of the presence of immune bodies in human convalescent serum. The clinical fact of non-recurrence of the disease seems to indicate that in man it is followed by immunity. On the other hand it is noteworthy that (1) the presence of lesions on the rabbit's cornea and (2) the subsidence of these lesions seemed to confer no immunity to the cornea in the rabbit. This might be explained by the fact that in the rabbit, unlike in man, the disease is confined to the cornea, the conjunctiva is not affected and presumably no general immune reaction with production of antigens occurs. This point, however, requires further investigation.

Pathological findings in the keratitis under discussion are scarce and inconclusive. The changes found histologically in a rabbit's cornea are interesting. It is of course not permissible to draw conclusions on the specificity of these changes from this one case. Should, however, the changes prove to be due to the specific agent, this finding would acquire importance in that it is illustrative of the earliest changes occurring in this form of human keratitis. The reversibility of the latter process, *i.e.*, the gradual disappearance of the macular lesions in the course of recovery, might then find an explanation in the assumption that the elements of infiltration, the mobilised cells, which do not form scar tissue regress gradually without leaving any trace.

Changes in the virulence of the disease in the course of epidemics are reported from time to time. The protean appearance of the corneal lesions in particular induced Wright (1930) to suggest the name keratitis diversiformis for the disease. In Palestine, too, some change in the appearance of the corneal lesions could be observed. While in the beginning epithelial alterations such as minute vesicles and subsequent epithelial defects taking fluorescein stain were in the majority, in the past few years mostly sub-epithelial macular lesions or even deeper-seated ones were seen. This may possibly be due to multiple passages in the course of time.

In this connection the immense spread of the disease since the end of the thirties may be recalled. The movements of populations before and to a far greater extent during the war naturally suggest themselves as factors in the spread of the disease.

Summary

- (1) Three hundred and eighty-one cases of kerato-conjunctivitis were investigated in the Middle East, chiefly from Palestine.
- (2) The majority of these cases occurred during epidemics.
- (3) In the Middle East the beginning of the rainy season appears to be the period of maximum incidence of the disease.

(4) In Palestine the disease although now occurring inland, first manifested itself in the coastal plain. This is in accordance with findings in other parts of the globe.

(5) In some cases trauma appeared to play a definite rôle in determining the onset of the condition.

(6) The affection of lids, conjunctiva, regional glands and cornea formed a well defined clinical picture differentiable for the most part on clinical grounds from swimming bath conjunctivitis, spring catarrh, trachoma, Koch-Weeks conjunctivitis and herpes corneae.

(7) In 60.4 per cent. of cases the cornea was involved.

(8) The relevant cases free from corneal lesions were diagnosed from the clinical picture, the absence of bacterial smears and the occurrence during epidemics.

(9) The mildest therapies proved the best. Douchings with cool water, instillation of adrenaline and, if necessary, cocaine and atropine drops were the most satisfactory remedies. Energetic treatment prolonged the disease and caused gross changes in the conjunctiva.

(10) Histological examination of human conjunctiva showed flattening of the epithelium, marked capillary dilatation and oedema in the subepithelial tissue and subepithelial infiltration with lymphocytes and large mononuclears, multiple mitoses and, in chronic cases, marked hypertrophy of the papillae.

(11) Histological examination of a rabbit's cornea in one case of inoculation keratitis showed notable changes.

(12) Experiments with rabbits confirmed the specific infective nature of the causal agent.

(13) This agent was not infective for mice by different methods of inoculation.

(14) Tissue culture and filtration experiments showed that the infective agent is a filtrable virus.

(15) Clinical immunity was apparently present in all of our cases; this was not found in experiments on the rabbit's cornea. Immune bodies were shown to be present in the blood of convalescent human cases.

Conclusion

Epidemic kerato-conjunctivitis is a specific disease of the conjunctiva and cornea caused in all probability by a filter-passing virus which can produce a similar condition in the cornea of the rabbit.

It has its own well defined epidemiological and clinical features.

Treatment must not be energetic. Douching with cool water and instillation of adrenaline and, if necessary, cocaine and atropine drops are the most helpful remedies.

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