EXPERIMENTAL CORNEAL ULCERS*

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DURING recent years we have been working in Edinburgh on experimental ulcers and infections of the cornea of various types. This work started at the beginning of 1940 with the investigation of experimental mustard gas lesions on the eye and their treatment. In the course of this work it was found that secondary infection played an important part in determining the severity of the lesions produced by mustard gas; this not only affected the immediate reaction (corneal oedema and conjunctivitis) observed during the first week following contamination, but also the liability of contaminated eyes to develop delayed "vascularised keratitis" (secondary oedema with vascularization) starting about a week to ten days after the application of the gas. It was found that local treatment with chemotherapeutic agents decreased the severity of the primary reaction (which lasts for about a week following contamination), and also the liability of the eyes to develop delayed "vascularized keratitis" in rabbits. These experiments suggested that chemotherapeutic agents might be of value in the treatment of mustard gas lesions of the human eye and the treatment with sodium sulphacetamide (albicid soluble) is, to some extent, based on this work.

It became obvious, however, that much further work was necessary on the treatment of ocular infections, and it was of especial interest to determine how infections produced by different types of organisms could be influenced by treatment with chemotherapeutic agents and also with antiseptics.

There is much previous work on the production of experimental corneal infections with various organisms and this will be referred to later. In the case of *B. pyocyaneus* methods for the production of ocular lesions suitable for experimental investigation have been accurately described, but no such methods appear to be available for the study of infections produced by organisms like the pneumococcus, staphylococcus aureus and streptococcus haemolyticus, and suitable techniques had to be devised. As an extension of this work we have also studied experimental tuberculous lesions of the cornea. As will be seen such lesions have been previously described, but we have further studied them in

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order to obtain lesions suitable for the experimental investigation of the action of various therapeutic substances.

Value of such Experiments

It is worth while considering what information can be obtained from experiments of this type. In the first place, they may be of value in connection with injuries of the eye produced by toxic gases and other noxious substances, in so far as they are affected by secondary infection, and it is indeed with this end in view that this work was started originally; but, and possibly more important still, information can be obtained which is of value in the investigation of the aetiology and treatment of other lesions of the eye, and especially of different types of corneal ulcers. In connection with treatment, properly controlled experiments would allow us to obtain information on a variety of important points:

1. The relative value of various antiseptic and chemotherapeutic agents on lesions produced by different organisms.
2. The relative value of various methods of administration; in particular local and general (*i.e.*, systemic) methods of administration can be compared.

In this connection the suitability of various ointment bases for local application of substances can be studied. Furthermore various methods have been described for increasing the penetration of agents applied locally to the cornea, and it would be possible to study whether such methods do indeed increase the therapeutic results. For example, it has been suggested by Ridley (1940) that wetting agents should be used together with substances applied locally in order to increase their penetration. Iontophoresis is another method which has been suggested by Boyd (1943) to increase the penetration of sulphonamides.

3. Such investigations can be of value, not only in determining the effect of various treatments in eye conditions, but can give valuable information in estimating the effect of such substances in the treatment of infections with various organisms in sites other than the eye.

Human Corneal Ulcers.—A study of the literature shows that, in the human subject, many types of organisms can be concerned in the production of ulcers of the cornea, either of the simple or of the hypopyon type (see Weiss, 1943; Dickson, 1942; Duke-Elder, 1938.) With few exceptions, however, of which the pneumococcus is by far the most important, different organisms do not produce a characteristic clinical picture. The hypopyon type of ulcer is usually due to the pneumococcus, but to some extent the aetiological agent varies with the geographical location and with the occupation of the patient. Thus for example, in France the

diplobacillus of Morax (*H. duplex*) and of Petit are not infrequently concerned. In Palestine the Koch-Weeks bacillus (*H. influenzae*) has been recovered. In the Glasgow district the staphylococcus aureus was recovered in 25 per cent. of the ulcers examined, according to a study reported by Dickson (1942).

A variety of organisms have also been recovered in simple ulcers and it is of interest to note that in China marginal ulcers are frequently caused by haemolytic streptococci.

Experimental Investigations

(a) *Pyocyaneus ulcers*.—These are uncommon in man but the lesion is usually very severe when due to this organism. The experimental production of pyocyaneus ulcers in rabbits was first described by Axenfeld in 1908. In our own investigations we have produced equal lesions in both eyes of rabbits by removing equal areas of epithelium from both corneae and infecting these areas with a culture of *B. pyocyaneus*. For experiments on local therapy one eye in any animal can then be treated with the appropriate agent while the other untreated eye is used as control. That equal areas of epithelium have been removed can be confirmed by staining with fluorescein.

The lesions produced vary in severity in different animals, but are mostly equal in the two eyes of any one animal (in the absence of treatment.)

Twelve hours after inoculation the lesion has developed into an infected abrasion and 12 hours later definite corneal ulceration is present. Two to three days after inoculation the corneal ulceration is severe and fairly extensive and in some cases this may develop into a ring abscess with or without hypopyon. At the beginning of the second week the ulcer or abscess involves most of the cornea. Ultimately the lesion may (a) perforate and be followed by shrinking of the eye, or (b) develop into a grossly vascularised scar, usually with staphylomatous changes, or (c) occasionally the scarring may be limited to only about half the cornea.

Treatment.—Joy (1940) has shown that the course of such lesions in rabbits can be favourably influenced by the oral administration of sodium sulphapyridine. Shortly after this it was shown that the local application of a sulphonamide could also favourably influence the course of such lesions (Robson and Scott, 1941, 1942). When a 30 per cent. solution of sodium sulphacetamide was applied within 1 hour of inoculation ulceration was completely prevented in more than half the treated eyes, but when treatment was delayed for 12 hours its value was greatly reduced though there was still a definite effect.

Boyd (1943) found that iontophoresis increases the penetration of sodium sulphadiazine into the cornea and aqueous, as compared with simple diffusion. V. Sallmann (1942) investigated the value of this technique in the treatment of experimental pyocyaneus ulcers in the rabbit. He obtained the best results by a combination of sodium sulphadiazine iontophoresis and sodium sulphadiazine administration by the mouth and under these conditions beneficial effects could still be obtained when the treatment was started 24-30 hours after inoculation.

In contradistinction to these results, Klein and Sorsby (1943) found that sulphonamides did not control pyocyaneus ulcers. They probably used a strain of *B. pyocyaneus* which was resistant to sulphonamides. It is doubtful whether too much importance should be attached to such negative results, since it is easy enough to obtain negative results in chemotherapeutic experiments even when dealing with such a favourable organism as the haemolytic streptococcus.

Pneumococcal ulcers.—These are most important from the point of view of lesions in the human eye and therefore of great value in therapeutic experiments.

Ginsberg and Kaufmann (1913) described the production of infiltrates following the injection of a culture of pneumococcus into the cornea. They used these lesions in the investigation of treatment with various quinine derivatives and obtained some beneficial effects as judged by the action of these substances on the bacterial content of the infiltrates. Robson and Scott (1943 *a*) investigated the action of various strains of pneumococcus on the eyes of rabbits. Most strains injected into the cornea produce infiltrates but no ulceration; but the injection of a type 19 pneumococcus was followed by the development of ulcers associated with hypopyon and severe iritis with plastic exudation. The infection was obviously of a virulent character since about one third of the animals died from pneumococcal septicaemia, usually within the first 3 days following inoculation.

Method.—The young culture (6-15 hours) of the pneumococcus in plain broth is injected in both corneae of each rabbit to make small blebs. There is considerable variation in the severity of the lesion in different animals and especially in experiments performed on different days, but the lesions produced in both eyes of any one animal are usually very similar in character.

Twenty-four hours after inoculation there is slight infiltration at the site of inoculation and this is surrounded by a zone of oedema. The central part of the area stains with fluorescein and in the more severe cases there may already be the beginning of ulceration.

At 48 hours a definite ulcer is developed and this is surrounded

by a zone of oedema. There is subepithelial infiltration beyond the margin of the desquamated area and a fairly severe iritis can already be observed.

By the third day after inoculation a deep spreading ulcer about 4 mm. in diameter has developed and there is hypopyon and a severe plastic iritis. Subsequently the ulceration spreads both in depth and extent (see Fig. 1) and is accompanied by a massive

FIG. 1.

Microphotograph of pneumococcal corneal ulcer on 14th day after inoculation (animal 1329); to show spread of ulcerative process under margin. ($\times 35$).

cellular infiltration of the substance of the cornea. The hypopyon at the same time increases in size.

During the second week there is a natural tendency to healing in these lesions and ultimately a dense vascularised scar is formed. Occasionally, however, the lesion is progressive and ultimately results in perforation of the cornea.

Treatment.—The local effect of a number of substances has been investigated. These were applied in solution at hourly intervals in order to maintain a high concentration in the cornea. The best results were obtained with penicillin, though quite striking effects were also seen with sodium sulphacetamide.

When the treatment with penicillin was started 1 hour after inoculation, ulceration was completely prevented in 7 out of 8 eyes,

and only a small ulcer developed in the 8th animal. The control eyes all showed severe lesions. When treatment was delayed for longer periods the beneficial effect was greatly reduced, but some effect was still observed when the treatment was started 24 hours after inoculation.

The results with sodium sulphacetamide were not as good but quite marked effects were obtained when the treatment was started 1 hour after inoculation; when it was delayed for 12-24 hours the effects were slight.

A number of experiments were also performed with sodium sulphapyridine which appeared to be of some value in preventing the development of the lesion; unfortunately this solution is alkaline ($\text{pH} > 10$) and its repeated instillation in an inflamed eye produces quite marked irritation. It seems probable that alkaline solutions will not prove very suitable for local treatment of ocular infections since not only do they tend to produce an irritant action, but they also interfere with the natural lysozyme of the tears which produces its optimum antibacterial effect at the reaction of the body (Ridley, 1940).

There has been much discussion recently about the value of acridine derivatives, and especially of proflavine, in the prevention and treatment of sepsis (see Browning, 1943). A number of experiments were undertaken to determine whether proflavine would be of any value in ocular infections. (Robson and A. A. B. Scott, unpublished data). It was found that, when treatment of pneumococcal lesion was started immediately after inoculation, a 0.1 per cent. solution of proflavine sulphate produced no beneficial effect whatever. With a 0.2 per cent. solution there was a slight beneficial effect, but this concentration of the drug produces irritation of the cornea and conjunctiva and would thus be unsuitable for clinical use. Proflavine was also tried in experimental staphylococcus aureus and haemolytic streptococcal infection (to be described below), but was found to be of comparatively little value.

Staphylococcus aureus lesions.—These organisms are uncommon in human corneal ulcers, though they were recovered from 25 per cent. of the ulcers (84 patients) investigated in Glasgow (Dickson, 1942). They are very common in lesions in rabbits due to irritant substances, e.g. in mustard gas lesions.

A number of attempts have been made to produce experimental lesions suitable for the investigation of therapeutic agents. Brown and Pugh (1936) succeeded in producing a fairly satisfactory ulcer in rabbits by the injection into the cornea of a strain of staphylococcus aureus found after many trials.

Robson and Scott (1943 b) tested a number of coagulase positive strains obtained from human lesions and chose the most virulent

of these for their further experiments. They found that the injection of an undiluted culture of this strain produced a very acute, almost fulminating, lesion unsuitable for therapeutic experiments, but found that by diluting it to a suspension containing 1500 organisms per c.c. satisfactory lesions could be produced.

Again equal blebs were produced by intracorneal injection into both eyes of rabbits, and although there were variations in different animals and especially in different groups of animals done at different times, the lesions which developed in both eyes of any one animal were amazingly similar.

In untreated eyes ulcers of varying severity developed and these were usually associated with hypopyon and iritis. In no case did the injection of the diluted culture fail to produce a corneal ulcer.

Twenty-four hours after inoculation the area stained with fluorescein and there was subepithelial infiltration at the margin.

At 48 hours the ulcer had already developed and a hypopyon was usually present. The lesion then increased in severity producing ulcers of various types and sizes. In some cases there was ultimately extensive necrosis of the cornea with or without perforation.

Treatment.—As was to be expected the local application of penicillin produced very striking results. The solutions available at that time were of low potency but even so they markedly inhibited the development of the lesion. The application of sodium sulphacetamide solutions, though less effective also gave definite results: 10 per cent. and 30 per cent. solutions appeared to be equally effective, but a decrease in the concentration of the sulphonamide to 2.5 per cent. made the treatment entirely ineffective.

Since sulphathiazole is amongst the most effective of the sulphonamides against staphylococcus aureus, this was applied as a powder to the experimental lesions, but was found to be ineffective. Sulphathiazole does not, however, readily penetrate into tissues and a soluble preparation, sulphathiazole sodium formaldehyde sulfoxylate was tried. This too proved to be ineffective.

The effectiveness of penicillin and of sodium sulphacetamide was greatly decreased when the interval between inoculation and the first treatment was lengthened and at 24 hours after inoculation neither of these chemotherapeutic substances was of any value. These results again emphasize the importance of early treatment.

In a number of experiments cultures were taken from the conjunctival sac in untreated eyes. Staphylococcus aureus could be recovered for a number of days after inoculation. Treatment with penicillin eliminated the organism from the conjunctival sac, but the application of sodium sulphacetamide did not have this effect. Hence the effect of an agent on the flora of the conjunctival sac

is not necessarily an absolute index of its effectiveness in the treatment of corneal lesions.

Haemolytic streptococcus.—This organism is uncommon in corneal lesions in the human subject though it is occasionally recovered. The experimental investigation of such lesions can, however, give valuable information about the effectiveness of various remedies in the treatment of infections with this organism.

Various investigators have produced experimental infections of the eye by the inoculation of the aqueous and vitreous (Rambo, 1938; Vence, 1939; Klein and Sorsby, 1943), but did not produce primary corneal ulcers.

Robson and A. A. B. Scott (1943, unpublished observations) injected a number of strains of haemolytic streptococcus into the cornea. In the majority of cases progressive lesions leading to ulceration were not produced. Two strains were, however, ultimately obtained which did produce corneal ulcers. One was recovered from the throat in a patient suffering from scarlet fever and this organism was particularly virulent and effective in the production of corneal ulcers; the other was recovered from an infected wound.

Method.—Undiluted cultures were injected into the cornea and produced lesions of the same type as those obtained with staphylococcus aureus, though a hypopyon did not form as frequently as with the latter organism. In order to make certain that the haemolytic streptococcus, and not some contaminant, was actually responsible for these lesions cultures were taken from the depth of the abscesses prior to ulceration. The infecting organism, *i.e.*, the haemolytic streptococcus, was in all cases recovered in pure culture.

Treatment.—A fairly concentrated solution of penicillin (220 units per c.c.) was available for these experiments and when the treatment was started 1 hour after inoculation the development of the lesion was practically completely inhibited. When the treatment was delayed for 12-24 hours the beneficial effect was only slight.

Unexpected results were obtained with sulphonamides (sulphanilamide and sodium sulphacetamide). They proved entirely ineffective when the treatment was delayed for 1 hour even though the strains tested proved not to be sulphonamide-resistant. When, however, the treatment was started immediately after inoculation quite appreciable beneficial effects were obtained both with sulphanilamide and sodium sulphacetamide.

Tuberculous lesions.—The production of experimental tuberculous lesions of the cornea was apparently first described by Langhans in 1867, *i.e.*, 15 years before Koch discovered the bacillus of

tuberculosis and established its causative relation to various forms of tuberculosis. The data of Langhans are not, at present, available and have been obtained from the paper by Haensell (1879). Both these investigators produced lesions in rabbits by injecting human tuberculous material into the cornea. After a latent period infiltration, vascularisation and caseation were produced. In some animals tuberculous lesions ultimately spread throughout the body (lungs, liver and other organs). Panas and Vassaux (1885) performed similar experiments and describe the growth of infiltrates by the formation of secondary tubercles around it, " mais, fait important, ce tubercule, arrivé au summum de son évolution, se reproduit, en émettant autour de lui de jeunes colonies qui ne tarderont pas à se rattacher à la source primitive, à s'ulcérer elles aussi, et concourir à l'agrandissement de l'ulcération ".

Igersheimer (1922) and Churgina (1930, 1931) produced tuberculous lesions of the cornea by the injection of cultures of the organism. They also investigated the effect of one localised tuberculous lesion against a second inoculation of the organism and found that some protection was given.

We have investigated the production of tuberculous lesions of the eye suitable for the study of the action of chemotherapeutic substances (Robson and A. A. B. Scott, unpublished observations). For this purpose it is important that equal lesions should be produced in both eyes of any one animal and that the minimum inoculum which will produce a suitable lesion in all animals be used. This will of course give the optimum conditions for the action of chemotherapeutic agents.

We have used two strains, namely :

- (a) bovine strain from the National Type Collection.
- (b) human strain isolated from a tuberculous lesion of the human eye.

Suspensions of the organisms were injected into the cornea and in every experiment in which 100 or more organisms were injected tuberculous lesions have been produced. The actual amount of fluid injected into the cornea is about 0.01 c.c. and the minimum amount of bovine culture with which a lesion has been produced is 0.00001 ug. This contains about 5 organisms.

The course of the lesion with the bovine and human strains was rather different.

Lesion with bovine strain.—This produces a lesion of the ulcerative, caseative type. After a latent period of a week or more, depending on the dose inoculated and during which period only the lesion produced by the needle track is seen, macroscopic infiltration begins to develop at the site of inoculation. This sometimes seems to be made up of a number of small nodules. The

infiltrate gradually increases in size and minute nodules appear around it. Vascularisation starts at about two weeks or more after inoculation. The minute outlying nodules increase in size and coalesce with the growing main infiltrate.

At about three weeks after inoculation the infiltrate breaks down producing caseating ulceration. Simultaneously vascularisation spreads from the periphery towards the centre but the central caseating area which can be of considerable size in the more severe cases, does not become vascularised. Nodules are continually forming under the vascularised area and may spread into the conjunctiva and sclera. Occasionally the central ulcer may perforate.

General dissemination of the tuberculous process may occur, secondaries being found in the cervical lymph glands, lungs, kidneys, etc.

Lesion with human strain.—This produces a more benign type of lesion with a typical macroscopic appearance. There is less tendency to caseation and ulceration and in the great majority of cases the lesions tend to regress after three or more months. We do not know of course whether the differences between these two strains will apply to all bovine and human strains or whether they are merely due to differences in their virulence.

FIG. 2.

Showing the development of tuberculous lesions in the right and left eyes of groups of rabbits.

We have studied quantitatively the development of the lesions of both eyes of a number of animals and found that they progress at a remarkably equal rate. This is illustrated in Fig. 2.

The investigation of the treatment of these lesions is still at a very early stage. So far only lesions produced by massive injections have been treated, and the results have been negative, but further investigations are in progress.

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