

INDUCED CORNEAL OPACITIES IN THE RAT*†

BY

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CORNEAL opacities were observed in the eyes of rats after subcutaneous administration of high levels of morphine sulphate. These lesions were first noted as an incidental observation and a series of studies was initiated to elucidate their nature and aetiology. These opacities apparently result from an irreversible deposition of calcium in the region of Bowman's membrane, a condition closely resembling band keratopathy in humans. In contrast to the transient, drug-induced, lenticular opacities described by Weinstock, Stewart, and Butterworth (1958, 1963), and Weinstock and Stewart (1961), these corneal opacities were irreversible. The lenticular opacities were ascribed to the deposition of drugs or metabolites of these drugs on the surface of the lens, while the corneal opacities observed in our studies resulted from a permanent histopathological change in the cornea.

Methods

General

All studies, except one, were done with male albino Charles River CD rats (weight from 100 to 130 g.). Male Long-Evans hooded rats (160–200 g.) were used in the one exception.

Morphine sulphate, administered by subcutaneous injection, was given as a solution in distilled water at progressively increasing dosage to counteract the rapid tolerance shown by the rats. A typical dosage regimen began with 20 mg. alkaloid/kg. on the first day, followed by 40 mg./kg. on the second day (two injections of 20 mg. each), 45 mg./kg. on the third day (one injection of 20 and one of 25 mg.), and so on for 3 to 12 days.

Corneal examinations were made by forcing the eye to protrude and illuminating the eye from the side. A representative number of eyes from the various studies was fixed in 10 per cent. formalin, embedded in paraffin, sectioned at 6 μ , and stained with haematoxylin and eosin. Other eyes were fresh-frozen, sectioned in a cryostat, and stained with alizarin red-S by a slightly modified method of Gurr (1956) for the demonstration of calcium.

I. Morphine Sulphate Sampling

Experiments 1–3.—The incidence of corneal opacities was determined in three studies using samples of morphine sulphate from various suppliers (Merck and Co. Inc., Mallinckrodt Chemical Works, and New York Quinine and Chemical Works Inc.). Morphine was administered to groups of twenty rats each in the dosages shown in the Table (opposite).

In the third study, on the eighth day, the rats were separated into three groups:

- (1) Those with no opacities;
- (2) Those with small opacities (1 mm. or less in largest dimension);
- (3) Those with large opacities (> 1.0 mm. in one dimension).

Morphine (30 to 100 mg./kg./day) was given to Groups 1 and 2 from the eighth to the twelfth day, while no further medication was given to the large opacity group. This was done to determine whether continued medication would produce opacities in those that remained negative and whether the small opacities would increase in size. Medication was withheld from those with large lesions to see if these would regress; some were studied for 10 months.

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TABLE
SUMMARY OF PROCEDURES AND INCIDENCE OF CORNEAL OPACITIES

Experiment No.	Morphine Dosage Regimen* (mg./kg./day)	Dosage Period (days)	Total Administered (mg./kg.)	No. of Rats Medicated	No. of Survivors	Rats with Corneal Opacities		Strain of Rat
						No.	Per cent.	
1	20-80	12	730	20	19	10	52.6	Charles River CD†
2	20-50 2 Samples	4	155	20	16	10	55.6	Charles River CD
		4	155	20	17	9	52.9	
3	20-55 2 Samples	5	210	20	20	9	45.0	Charles River CD
		5	210	20	17	14	82.4	
4	Morphine alone 20-50	3	110	20	18	10	55.6	Charles River CD
	Same + 10-20 Nalline	3	110 (morphine) + 50 (Nalline)	20	20	0	0	
5	20-50	4	160	20	19	2	10.5	Long-Evans ‡
	30-60	3	150	20	18	3	16.7	
6	20-60	4	170	20	17	9	52.9	Charles River CD
	Same + moistening with saline	4	170	20	19	2	10.5	
7	Eyelids sutured open to induce drying (No morphine)	3	—	15	15	10	66.7	Charles River CD

* Dosage progressively increased over period indicated † Albino ‡ Hooded

II. Influence of Nalorphine

Experiment 4.—Morphine was given to two groups of twenty rats each in doses of 20 to 50 mg./kg./day over a 3-day period. In one group, the morphine injections were followed immediately by subcutaneous injections of 10 mg./kg. Nalorphine hydrochloride.

III. Influence of Strain

Experiment 5.—Two groups of hooded rats, which have highly pigmented eyes, were given morphine in doses ranging from 20 to 60 mg./kg./day (Table).

IV. Influence of Evaporation

Experiments 6 and 7.—Drying of the corneal surface was commonly observed during the periods of maximum depression and exophthalmos, especially after the first two to three administrations. These two studies were designed to study the influence of drying on the development of corneal opacities.

In Experiment 6, two groups of albino rats were given morphine in doses from 20 to 60 mg./kg./day over a 4-day period. In one group, the eyes were moistened with warm saline during the period of depression, inactivity, and exophthalmos. The eyes in the other group were not treated.

In Experiment 7, fifteen rats were anaesthetized with ether and the eyelids of one eye were sutured so that they could not be closed. The sutures were left in place for 3 days and then removed. The eyes were closely observed for drying and for the development of opacities.

Results

Incidence and General Appearance of the Lesions.—A summary of the results is given in the Table. In albino rats the incidence of morphine-induced corneal opacities ranged from 45 to 82 per cent. In two groups of hooded rats the incidence was lower (11 and 17 per

cent.), but the opacities were similar. There was no apparent relationship between incidence and source of morphine sulphate, as similar incidences were observed when samples from three different suppliers were used.

The majority of the lesions developed within 4 to 5 days, and continued administration of morphine after 5 days had very little effect on increasing the incidence or size of lesions. The opacities, which became grossly visible after two to three medications, were first characterized by an area of slight cloudiness or by fine grey stippling. In 4 to 5 days, distinct opaque areas were seen which in most cases had well-defined borders.

The initial dose of morphine was large enough to produce a period of marked respiratory depression, inactivity, and exophthalmos of approximately 3 hours' duration. During this time, drying of the corneal surface was observed in most rats. Following subsequent medications the depth and interval of depression rapidly decreased in spite of the increased dosage.

No corneal opacities developed when the various doses of morphine were followed by subcutaneous doses of Nalorphine hydrochloride at 10 mg./kg. This dose of Nalorphine produced a marked reduction in the depression and exophthalmos observed when morphine was given alone.

Moistening of the eyes with warm saline during the period of deep depression and exophthalmos markedly reduced the incidence of corneal opacities. In the group in which the eyes were repeatedly moistened after each dose of morphine, lesions occurred in only two of nineteen rats, an incidence of 10.5 per cent. compared with 53 per cent. in those receiving only morphine. These two lesions were minimal and consisted of small areas of cloudiness which could be seen only by shining a light obliquely through the cornea. Lesions in the control group were readily visible by ordinary room lighting and without manipulation of the eyes.

Suturing of the eyelids of one eye so that they could not be closed resulted in corneal opacities in ten of the fifteen eyes so treated. No changes were observed in the opposite (normal) eyes. Drying of the corneal surface of eyes in which the eyelids were sutured occurred while the rats were anaesthetized and for a period of approximately 2 hours after recovery. After this period, lacrimal secretion seemed to be sufficient to keep the eyes moist even though the eyelids could not be closed.

Microscopical Appearance of the Lesions.—The histological changes observed in these corneal opacities were basically similar in all cases. A normal cornea is shown in Fig. 1.

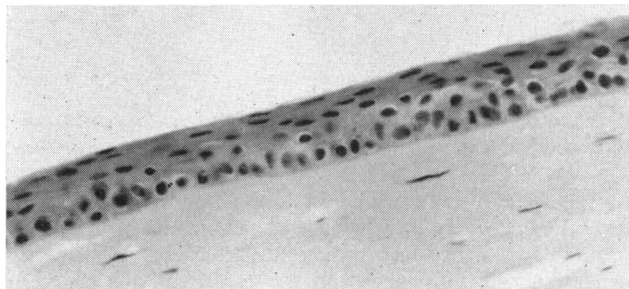


FIG. 1.—Normal cornea of albino rat. $\times 360$.

Note:

All photomicrographs are of rat cornea, sectioned at 6μ , and stained with haematoxylin and eosin.

The earliest change (24–36 hours) seen was an area of compressed corneal epithelium with degenerative changes in the basal layer (Fig. 2, opposite). At 5 days there was focal mineralization of Bowman's membrane and the epithelium was thickened (Fig. 3, opposite). The reaction of this mineralization with haematoxylin and alizarin red-S stains indicated that the deposition was calcium. Mineralization was followed by vascularization and infiltration with lymphocytes and fibroblasts in the corneal stroma adjacent to the calcium deposit (Fig. 4, opposite). These developments corresponded grossly to a progressive increase in

FIG. 2.—Early degeneration of corneal epithelium 24 hrs after first medication. $\times 360$.

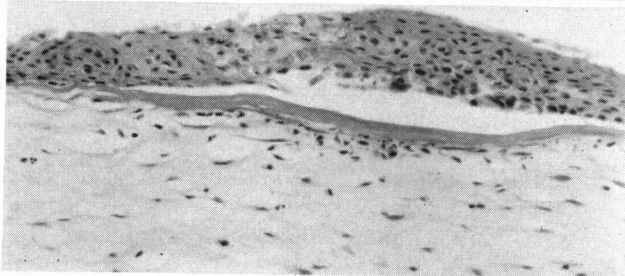
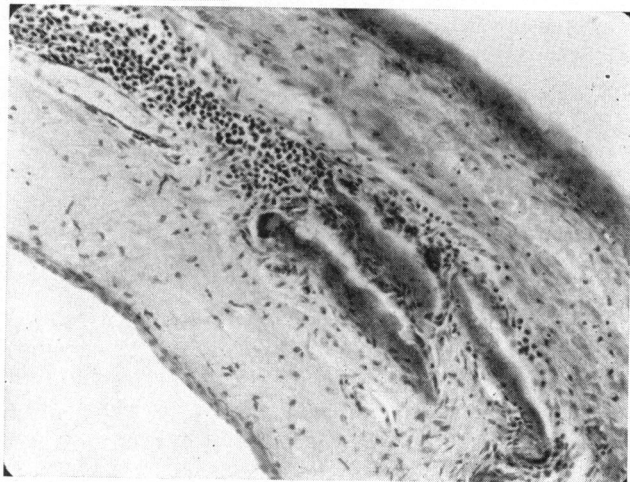


FIG. 3.—Calcification in region of Bowman's membrane at 5 days. $\times 192$.

FIG. 4.—At 4 weeks the deposit lies within the stroma. There is vascularization and a copious inflammatory exudate. $\times 144$.



density of the opacity. Even after 10 months calcium deposits were still found lying in the stroma (Fig. 5).

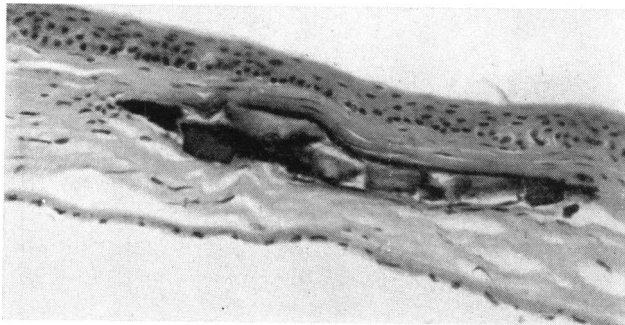


FIG. 5.—At 10 months the deposit lies within the stroma with minimal inflammatory activity. $\times 144$.

At this time, the corneal epithelium over the deposit was essentially normal and there were very few inflammatory cells surrounding the deposit.

Discussion

Evaporation from the corneal surface appeared to be an important aetiological factor in the development of the corneal opacities observed in these studies. After large doses of morphine, periods of deep sedation, exophthalmos, and analgesia occurred which permitted evaporation from the surface of the eye to take place. Suturing of the eyelids so that the lids could not be closed also permitted excessive evaporation from the corneal surface. In both of these circumstances typical corneal opacities developed.

Radnót (1948) and Zeiter (1962) discussed the possible role of evaporation in the development of band keratopathy in man, and suggested that evaporation in the area where the eyelids open produces an oversaturation of calcium which then precipitates. The results of the studies reported here are compatible with this explanation. The production of corneal calcification was not dependent upon the administration of morphine sulphate with which it was first observed. In fact, it could be produced simply by suturing the eyelids so that they could not be closed, and occurred in the pigmented eyes of hooded rats as well as in the non-pigmented eyes of albino rats. The majority of the opacities appeared during the first few days of medication with morphine, the period in which the depressant effects of morphine were the greatest and most drying took place. Nalorphine inhibited production of the opacities because it prevented exophthalmos and reduced the intensity of the depressant effects, so that this excessive drying did not occur. Moistening the eyes with saline during the period of deep depression and exophthalmos almost entirely prevented the occurrence of opacities—the two minute lesions that were observed may have been overlooked in the examination before the study began, or the moistening procedure may not have been adequate.

Forbus (1952), discussing dystrophic calcification in general, listed three factors essential to the local deposition of calcium:

- “(1) The presence of dead or devitalized tissues;
- (2) A state of relative alkalinity;
- (3) A continuous supply of fluids.”

These conditions are met in the corneal lesions which have been described. Devitalized corneal epithelium was observed before the deposition of calcium. Analysis of the aqueous humour of the rabbit (Pirie and van Heyningen, 1956) indicated that the pH of the humour is higher than that of the plasma, thus providing a state of alkalinity. The continuous supply of fluids is provided by the constant flow of aqueous humour which bathes the inner surface of the cornea.

These results indicate that the occurrence of corneal opacities in animal studies involving highly depressant effects may be merely the result of corneal drying. In any event, careful consideration should be given to this possibility in assessing the role that the drug in question may have had in their causation.

Summary

The occurrence of corneal opacities in rats after administration of high levels of morphine is described. Histologically these opacities are seen to be due to the deposition of calcium in the region of Bowman's membrane.

Since opacities of the same type were subsequently produced by suturing the eyelids so that they could not be closed, evaporation from the corneal surface seems to be an important aetiological factor.

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