Trachoma in the Sudan

A laboratory study

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The first isolation of the trachoma agent in the Sudan was made by Harper (1963), although the disease had been known to occur in the Sudan since antiquity. Harper investigated seven cases of trachoma and isolated only two strains of the agent. It was thus desirable that more laboratory investigations should be undertaken to confirm the previous work and to have more strains available for further study, perhaps for vaccine production.

Material and methods

SELECTION OF CASES

Some cases were seen in Khartoum Eye Hospital but most of the patients selected for this study were schoolchildren of 7 to 8 years of age in Abu Usher, a village which lies about 50 km south of Khartoum in the northern division of the Blue Nile Province. This village was selected because it is relatively near Khartoum and lies on a good motorway. It was preferred to villages nearer Khartoum because of the availability of treatment in the city. This village has about 7000 inhabitants, almost all farmers growing cotton in the Blue Nile (Gezira) cotton plantation area.

The upper tarsal conjunctivae were examined with a $\times 10$ monocular loupe and focal torch illumination. Those with active trachoma were selected for the investigation.

CONJUNCTIVAL SCRAPINGS

After instillation of 2 per cent amethocaine into the conjunctivae, scrapings were obtained from the upper palpebral conjunctivae with blunt pterygium knives sterilized by flaming. Each eye was scraped twice. The material from the first scraping was suspended in 1.0 ml sucrose-phosphate buffer solution containing 0.2 M sucrose in 0.02 M phosphate buffer, pH 7.2 (2 SP) (Gordon, Harper, Quan, Treharne, Dwyer, and Garland, 1969). This was immediately put into a large thermos flask containing wet ice. The second scraping was spread on to three clean glass microscope slides and air dried.

INOCULATION OF CHICK EMBRYOS

Before the investigation was carried out, fertile hens' eggs were obtained and incubated for 5–8 days, because there

was no -70°C deep-freeze, or other equivalent storage facility.

Bacteriological specimens were plated out by sterile loops on to blood agar plates which were then incubated aerobically and anaerobically at 37°C and cultures were also made into Robertson's cooked meat medium.

The rest of the sample was homogenized by being drawn into a disposable 2.0 ml syringe with a 19 gauge needle and pushed through a 23 gauge needle inside a cabinet with ultraviolet light. The volume was made up to 1.0 ml in 2 SP, containing 100 $\mu\text{g/ml}$ vancomycin and 10 000 $\mu\text{g/ml}$ streptomycin. All precautions necessary for the prevention of cross-contamination were taken (Harper, Dwyer, Garland, Jones, Treharne, Dunlop, Freedman, and Race, 1967). Inoculation of the chick embryos by the yolk sac method has been described previously; three chick embryos were used per specimen and 0.3 ml per embryo (Salim, 1969). The time between collection of specimens and inoculation was 4 to 6 hours. Eggs were incubated at 35°C in a humidified incubator. In the control group, eggs were inoculated with 2 SP alone.

The inoculated chick embryos were examined twice daily, embryos that died during the first 48 hours being discarded. No blind passages were made.

PASSAGE OF THE INFECTIVE AGENT

Yolk sacs were harvested from embryos that had died 3 days or more after inoculation, and smears were made on slides and stained using the method of Giménez (1964). Yolk sacs showing the typical elementary bodies were weighed and homogenized in Ten-Broek grinders as 20 per cent (w/v) in 2 SP solution containing streptomycin and vancomycin.

FREEZE-DRYING

When an isolate was established, a 20 per cent suspension of yolk sac was made in a solution made up of equal volumes of skimmed milk (10 per cent in water) and 15 per cent solution of glucose, giving final concentrations of 50 per cent skimmed milk and 7.5 per cent glucose. The yolk sacs were homogenized in Ten-Broek grinders in a cabinet with ultraviolet light. The material was distributed in 0.5 ml volumes in glass ampoules and freeze-drying was performed in a portable Edwards freeze-drying machine.* The freeze-dried material was stored at 4°C .

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* Obtainable from Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex

MICROSCOPIC EXAMINATION OF CONJUNCTIVAL SMEARS

Smears on microscope slides were fixed with methyl alcohol for 10 min and left to dry at room temperature. One smear on a slide was stained with iodine (Sowa, Sowa, Collier, and Blyth, 1965) and the other with Giemsa stain.

Results

The results of the clinical and laboratory investigations are shown in Table I. It can be seen that 46 patients suspected of having trachoma were investigated, and from these 25 strains of the trachoma agent were isolated in chick-embryos.

Table I Result of laboratory examination of 46 patients diagnosed clinically as cases of trachoma

Case no.	Sex	Age	Stage of trachoma	Inclusions in smears		Yolk sac culture	Bacterial culture	Strain no.
				Iodine	Giemsa			
K1	M	23	III	—	—	—	Sterile	—
K2	F	27	III	—	—	—	Sterile	—
K3	M	42	III	—	—	—	Sterile	—
K4	M	24	III	—	—	—	Sterile	—
K5	M	35	III	—	—	—	Sterile	—
K6	M	42	II	—	—	—	Sterile	—
K7	M	70	III	—	—	—	Diphtheroid	—
K8	F	45	II	—	—	—	Sterile	—
K9	F	45	III	—	—	—	Sterile	—
K10	F	20	II	—	—	—	<i>Staph. albus</i>	—
K11	M	31	II	—	—	—	Sterile	—
K12	M	34	III	—	—	—	Diphtheroid	—
K13	M	40	II	—	—	—	Diphtheroid	—
K14	F	22	I	—	—	—	Sterile	K1
K15	F	18	II	—	—	—	Sterile	K2
BN1	M	7	II	+	+++	+++	Sterile	BN1
BN2	M	7	II	+	+++	+++	Diphtheroid	BN2
BN3	M	16	II	—	—	—	<i>E. coli</i>	—
BN4	M	14	II	+	++	+++	<i>Staph. albus</i>	BN4
BN5	M	14	II	—	—	—	Sterile	—
BN6	M	7	II	—	++	—	Sterile	—
BN7	M	7	II	+	++	+++	Sterile	BN7
BN8	M	7	II	—	—	—	Sterile	—
BN9	M	7	II	+	++	+++	Sterile	BN9
BN10	M	7	II	+	++	+++	Diphtheroid	BN10
BN11	M	7	II	—	—	—	Diphtheroid	—
BN12	M	8½	II	—	++	+	<i>Staph. aureus</i>	BN12
BN13	M	8	II	—	++	+	Sterile	BN13
BN14	M	8	II	—	++	+	Sterile	BN14
BN15	M	8	II	—	++	+	<i>Staph. albus</i>	BN15
BN16	M	8	II	+	++	+	<i>E. coli</i>	BN16
BN17	M	7½	II	—	—	+	Sterile	BN17
BN18	M	8	II	—	—	+	Sterile	BN18
BN19	M	8	II	+	++	+	<i>Staph. aureus</i>	BN19
BN20	M	8	I	—	++	+	Sterile	BN20
BN21	M	8	II	—	—	—	Sterile	—
BN22	M	8	II	+	—	+	<i>E. coli</i>	BN22
BN23	M	8½	I	+	+	+	Diphtheroid	BN23
BN24	M	8	II	—	+	+	Diphtheroid	BN24
BN25	M	8	II	—	+	+	Sterile	BN25
BN26	M	8	II	—	+	+	Sterile	BN26
BN27	M	8	I	+	++	+	Sterile	BN27
BN28	M	8	I	+	+	—	Sterile	—
BN29	M	8	I	—	++	+	Diphtheroid	BN29
BN30	M	8	II	+	+	—	Diphtheroid	—
BN31	M	8½	II	—	—	+	Sterile	BN31

M = Male

F = Female

+ to +++ arbitrary gradient from light to heavy infection

BN = Blue Nile

K = Khartoum

Table II summarizes the investigations comparing the detection of infection by inclusion body findings and yolk sac cultures. It can be seen that when smears from the conjunctivae were examined, out of 46 cases diagnosed clinically as trachoma, only 28·3 per cent were confirmed by iodine staining and 47·8 per cent by Giemsa staining. On the other hand, 54·3 per cent were confirmed by isolating the causative agent. It can also be seen that about 39·1 per cent of samples obtained from the conjunctivae were contaminated with some bacteria, most of which were commensals—for example, diphtheroids and *Staphylococcus albus*. *Staphylococcus aureus* was isolated from two cases complicated by muco-purulent conjunctivitis and *E. coli* from two cases of simple conjunctivitis and one from another case showing no acute inflammation.

Table II Summary of some of the data presented in Table I

No. of cases tested	Stage of trachoma			Inclusions in smears		No. of strains isolated	Bacterial contamination
	I	II	III	Iodine	Giemsa		
46	6	32	8	13/46 (28·3)	22/46 (47·8)	25/46 (54·3)	18/46 (39·1)

Percentages are given in parentheses

Five to six months later the freeze-dried material was tested in chick embryos and was found to be still active.

On repeated passage, it was noticed that eggs began to lose their susceptibility to the trachoma agent and had it not been for freeze-drying of early passages the isolates would have been lost.

Discussion

These laboratory studies carried out on patients diagnosed clinically as cases of trachoma have confirmed the pioneer work of Dr I. A. Harper on trachoma in the Sudan. The results of isolation of the agent, 54·3 per cent in chick embryos, was remarkable and this may partly be due to:

- (i) The selection of severe cases, mostly Stage II
- (ii) The use of wet ice in a thermos flask for transporting the specimens, thereby avoiding freezing
- (iii) The early inoculation of chick embryos: only 4 to 6 hours elapsed between collection of the specimens and inoculation
- (iv) The combined use of high doses of streptomycin and vancomycin for combating bacterial contamination.

Isolation of the causative agent was found to be more sensitive than the detection of inclusion bodies in conjunctival smears, as a method of laboratory diagnosis. This is in line with previous findings (Sowa and Collier, 1960; Darougar, Dwyer, Treharne, Harper, Garland, and Jones, 1970). Giemsa staining was more sensitive than iodine staining.

We were fortunate in having most of our specimens free of bacterial contamination as the specimens were collected in the winter (February) when the incidence of bacterial conjunctivitis is usually low. However, this contamination was effectively controlled by the antibiotics applied.

The loss of virulence for the chick embryo in the case of some isolates was of interest. Whether this was due to a change on the part of the organism or the chick embryos was not fully investigated. It was observed by Jawetz (1962) that chick embryos lost susceptibility to trachoma agent during warm weather. Most of our passages were carried out during the months of March, April, and May which coincide with our summer.

For most laboratories in endemic areas where sophisticated storage facilities—for example, -70°C electric-driven deep-freezers, solid CO_2 , or liquid nitrogen—are not available, a simple portable freeze-drying machine is very useful. The glucose-skimmed milk solution used for freeze-drying proved to be fairly reliable. Some of the agent was lost but what survived was enough for further passage.

Workers in endemic areas should be encouraged to attempt isolation of the trachoma agent in ordinary laboratories provided they ensure that inoculation of chick embryos 5 to 8 days old is done immediately, or within 6 hours, and that a portable freeze-drying machine is available.

Summary

Out of 46 patients in the Sudan who had been diagnosed clinically as having trachoma 25 strains of the trachoma agent were isolated.

Bacterial contamination was easily controlled by streptomycin and vancomycin.

On repeated passaging, chick embryos lost their susceptibility to the trachoma agent during the summer.

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