

the economically better off and socially more advanced women, soap is used and the use of turmeric and mustard or gingili oil is confined to certain ceremonial occasions.

To ascertain whether turmeric and vegetable oils have any repellent effect, the following tests were made. The first author collected in the Jeypore Hills over a dozen anopheline mosquitoes, mostly *A. fluviatilis*, in a cage and put inside it one of his hands previously besmeared with turmeric and mustard oil and then washed with plain water. The mosquitoes refused to feed on it whereas immediately afterwards they rapidly fed on his other hand not so besmeared. He repeated the experiment next night with the same result. About the same time, the second author carried out a similar experiment using gingili oil instead of mustard oil with *A. subpictus* and *A. annularis* at Naupada (North Madras Coast) with precisely similar results.

Since the above observations were made, we came across, by the courtesy of Major R. Senior White, the statement made by Bispham (1944) which bears on our findings. He says that, in certain tropical countries, local habits of the population either limit the spread of malaria or increase the spread. In Fezzan, for instance, women are not often infected because they anoint their bodies, as a cosmetic habit, with an oil which is deterrent to mosquitoes. On the other hand, small children in most hot countries wear no clothing until they are six or even more years of age and are easily bitten by the infected mosquito.

#### Summary

(a) Turmeric and vegetable oils appear to exercise a repellent effect against anopheline mosquitoes.

(b) The relative freedom from malaria of women in the Jeypore Hills and in the bilingual areas of the North Madras Coast may be attributable to their habit of besmearing themselves with these two substances before their daily bath.

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### TROPICAL ULCER

#### A STUDY OF ONE HUNDRED CASES COLLECTED IN THE CITY OF MADRAS

By N. SESHADRINATHAN, M.B., B.S., D.T.M.  
 King Institute of Preventive Medicine, Guindy

AT the Stanley Hospital, Madras, a study of tropical ulcers (*Ulcus tropicum*) was first started on 27th November, 1943, at the request of the superintendent. A few cases were investigated, but subsequently they became scarce at the hospital. Later it was observed

that a large number of cases of tropical ulcer were attending the dispensary conducted by the Ramakrishna Mission at Mylapore. With the kind assistance of the authorities of the Mission and the medical officer of the dispensary, the ulcers were investigated. A total of one hundred cases formed the series under investigation.

The ulcers were recognized as 'ulcus tropicum' by their indolent nature, shelving base, often foul-smelling profuse discharge, and the associated pain and tenderness. The diagnosis was confirmed by the presence of Vincent's bacillus either alone, or with spirochaetes in the discharge.

The following observations were made:—

The size of ulcers varied from  $\frac{1}{4}$  inch to  $2\frac{1}{2}$  inches. Of 84 ulcers, 73 did not exceed one inch in diameter. Most of the ulcers were circular or oval. Of 95 ulcers, 62 were on the foot, 32 on the leg, and one on the elbow.

Pain was almost invariably present which rendered the patient often sleepless. The ulcers were very tender. Twelve out of 84 cases examined showed enlarged lymph glands. In these, there was associated inflammation of the cellular tissue surrounding the ulcer.

#### Age incidence

Below 7 years	7-15 years	15-30 years	30-50 years	Above 50 years	Total examined
Nil	28	39	14	9	90

The lowest age at which the ulcer was noted was in a boy of seven years. The largest number occurred among adults engaged in active work.

*Sex.*—In a total of 98 patients in whom the sex was recorded, there were 88 males and 10 females.

*Occupation and history of injury (see table).*

In the group 'others' are included 19 school children in addition to persons following occupations such as toddy tapping, goldsmith, barber, etc.

*Comment.*—The highest number was among the weavers. Only 4 out of the 30 weavers gave a history of injury as a provocative cause for the ulcer. This is interesting, since, nearly 50 per cent of other cases have given a history of injury. The weavers also admitted that these ulcers were very commonly prevalent among them.

*Treatment.*—No controlled experiments were conducted. The impression formed is that mere antiseptic treatment such as the application of gauze soaked in 1 in 40 lysol lotion or eusol lotion was not effective. Ointments such as boric acid and zinc oxide were equally useless. The discharge freely oozed out of the ulcer through the dressings, the margin gradually extending under the discharge.

Gauze soaked in warm 5 per cent copper sulphate solution was found to be very effective, reducing the discharge in about 3 to 7 days. Thereafter healing took place rapidly.

forms of Vincent's bacilli were non-motile while the long forms were more slender, and motile. In addition to the spirochaetes and Vincent's bacilli, *B. proteus* and some other very actively

	Weavers	Coolie	Domestic servant	Cart driver	Carpenter	Others	Total examined
Total in each group ..	30	7	7	2	2	43	91
With history of injury ..	4	3	4	2	2	20	35
Without history of injury ..	26	4	3	0	0	23	56

*Epidemiology*

The possible factors that lead to the incidence of 'ulcus tropicum' appear to be :—

1. *Trauma*.—If the weaver group is excluded from the series, it will be apparent that nearly 50 per cent (31 out of 61) give a history of injury initiating the sore.

2. *Bites by insects*.—A high proportion of the ulcers were among the weavers (30 out of 91). In this 30, only 4 gave a history of injury initiating the ulcer, while 26 did not. Another feature observed is that among the weaver group, with the exception of 2, all were adults above 15 years. All these people were males. These men evidently had been engaged in their vocation of weaving in their houses. The occupation necessitates them keeping their legs in pits for long hours. These pits are long pits in which the handlooms are lodged. The pits are kept moist, warm and lined with cowdung. Insect larvæ and adult insects may be sheltered in the pits. Whether insects have anything to do with initiating the ulcer has to be investigated.

3. *Nutrition*.—It was a common observation made during the study of this series that the class of patients coming with these ulcers consume little or no milk.

*Bacteriological observations*

Microscopic examination was done at first by staining the smears from ulcers by Gram's stain, but later on freshly diluted carbol fuchsin stain was substituted.

Total specimens examined	Fusiform bacilli alone present	Fusiform bacilli and spirochaetes present	Long forms of fusiform bacilli present
100	48	44	14

Streptococci and staphylococci alone were found in 8 cases which did not resemble the typical tropical ulcer in all respects. The short forms of Vincent's bacilli were tapering at the ends and slightly curved. They were seen in large numbers.

Examination of wet preparations by dark ground illumination showed that the short

motile bacilli (*Ps. pyocyanea*?) and some cocci were seen.

The long forms of Vincent's bacilli were fewer in number and were seen at a deeper level. Any current in the preparation could not disturb these organisms very much. The motility was very characteristic, namely, a lateral oscillatory movement of the progressing end, and a bending and straightening of the whole length of the organism. The bacilli could often be seen progressing slowly across the field. Fox (1921) has described motile Vincent's bacilli in wet preparations. He describes them as 'having a peculiar wriggling movement suggestive of mosquito larvæ.' Smith (1933) describes motile fusiform bacilli in wet smear preparations from tropical ulcer cases.

*Cultural examinations*.—The medium used was that of Smith (1930) described as modified Wenyon's medium. Instead of human blood, rabbit blood was used. The fusiform bacilli grew along with *B. proteus* and other organisms. It took about three days to one week for the fusiform bacilli to multiply. The spirochaetes were alive for the first few days, but failed to multiply.

Total cultures examined	Fusiform bacilli grown
39	12

In seven cases in which the long forms of fusiform bacilli were seen from the direct smear, positive cultures were obtained. The organisms showed the same kind of motility. They were slender, adhering to masses of cellular debris especially decaying leucocytes. Sheaves of the bacilli could sometimes be seen twisted round one another. Often they were seen arranged in a radiating manner around a central mass of cellular debris. In one case, however, these long forms showed no motility, and had a granular appearance. The appearance was identical both in direct smear as well as in culture.

Pure cultures of the fusiform bacilli were obtained in a few instances on solid media (one

per cent agar containing 25 per cent rabbit blood) incubated at 37°C. in an atmosphere of 90 per cent hydrogen and 10 per cent carbon dioxide. Sub-cultures could not be maintained; further attempts are being made.

#### Summary

A study of a hundred cases of 'ulcus tropicum' collected in the city of Madras exhibited the following features:—

1. The fusiform bacillus was present in every case of 'ulcus tropicum.'
2. A motile form of Vincent's bacillus is present in some of these ulcers.
3. The lower limbs form the site of election for the ulcers in almost all cases.
4. Though injury is responsible for starting the ulcers, there might yet be another factor in their causation, *viz.*, the insect factor.

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### APPLICATION OF THE D.E.C. MEDIUM TO THE ISOLATION OF TYPHOID BACILLI FROM SEWAGE IN CALCUTTA\*

By G. PANJA, M.B., D.Bact. (Lond.), F.N.I.

Officiating Professor of Bacteriology and Pathology and

S. K. GHOSH, B.Sc.  
 Assistant Chemist

(From the Department of Bacteriology and Pathology, School of Tropical Medicine, Calcutta)

As the D.E.C. medium of Panja and Ghosh (1943) is markedly inhibitory to coliform organisms, it was thought desirable to try it for isolation of *Bact. typhosum* from the sewage of Calcutta. This work was first undertaken here by Stewart and Ghoshal (1932) using the Wilson and Blair medium, and *Bact. typhosum* was isolated from the sewage. There are however certain disadvantages with the latter medium: it is more difficult to prepare; it has to be used on the same day that it is prepared; at least two or three plates have to be used for one sample of sewage; the suspected colonies are to be examined after 48 hours of incubation instead of 24 hours; recognition of the colonies is difficult; and the medium is often too soft for satisfactory work. We therefore wished to see whether our medium would remove the above disadvantages, and whether a higher percentage of isolation would be obtained.

\*The paper was read before the Indian Science Congress Association held at Nagpur in January 1945.

The Wilson and Blair medium was prepared according to the latest formula given by Wilson and Blair (1931). First of all, it was tested with pure cultures of typhoid bacilli. After 24 hours' incubation, most of the colonies were greenish in colour, but a few were small and black with a metallic sheen. There was no further change after 48 to 72 hours' incubation. It may be mentioned here that on the D.E.C. medium, the colonies were about 2 mm. in diameter, smooth, slightly opaque and undifferentiated.

A loopful of young broth culture of the typhoid bacillus was mixed with about 12 c.cm. of sewage and plated direct on the Wilson and Blair medium. Eleven suspected black colonies were tested but none of them was of the typhoid bacillus. The mixture was plated again after 24 hours, and out of 14 colonies tested, 4 proved to be of typhoid bacilli. Similarly plating was done on the D.E.C. medium direct after mixing and again after 24 hours. In the first instance, 10 colonies were tested and all were of typhoid bacilli and in the second instance 3 colonies out of 4 tested were of typhoid bacilli. This simple preliminary experiment suggested the possibility that the new medium might be superior to the Wilson and Blair medium in the isolation of typhoid bacilli from sewage.

One hundred samples of sewage were collected from different pits in northern Calcutta and kept in the ice-chest for 15 to 30 minutes before plating. By practice, it was found that the sewage had to be diluted 1 in 3 before plating; otherwise colonies were so numerous that the isolation of typhoid bacilli was not possible. Large plates 6 inches in diameter were selected, one containing D.E.C. medium and two containing Wilson and Blair medium, for each sample of sewage. Plates were marked into 4 quadrants, and 4 drops of diluted sewage were put in one quadrant of the D.E.C. medium and 6 drops in the Wilson and Blair medium. Thus the total number of drops on two plates of Wilson and Blair medium was 12, *i.e.* three times more chance of success was given to this medium. Suspected colonies were fished out and stroked on small areas on MacConkey's plates. It was easier to select lactose-nonfermenting clear colonies from D.E.C. plates. This preliminary sub-culture on MacConkey's medium eliminated many colonies as lactose-fermenters and thus a lot of extra work was saved. The result of selection of colonies from the two media is given in the following table:—

	D.E.C. medium	Wilson and Blair medium
Total number of colonies fished out.	1,012	2,229
Lactose non-fermenters ..	858	1,146
Percentage .. .. .	85	51
Identified as <i>Bact. typhosum</i>	7	1