

The minimum period of residence that I observed was 2 years, though others have reported less.

Now, besides malaria, the Dooars is notorious for amœbiasis. It is not common in the labour forces of the gardens, but it has a special predilection for a certain class of people such as Europeans, Bengalees, Punjabis, Marwaris, Chinese and up-country Hindus of the dhobi and sweeper class, and strangely it is in these people in whom blackwater fever is most prevalent.

Manson-Bahr writes that some workers recognize a pre-blackwater stage. One of the signs is 'an enlarged and tender liver'. Unfortunately, he does not mention if these workers noted whether or not the 'enlarged and tender liver' was still present after the blackwater stage had passed off and if emetine was required at some future date. Then some conclusion as to the cause of the 'enlarged and tender liver' could have been formed.

It may be that the amœbiasis in my series of blackwater cases is coincidence. This factor must be considered but in view of the work of Krishnan and Pai it is possible that amœbic hepatitis may, in some cases, cause liver damage of a type that will help in the production of the biochemical blood changes reported by them, especially when these cases suffer from a superimposed malarial infection. Quinine may, in these cases, cause further deterioration and act as an immediate excitant.

The exciting cause in all my cases of blackwater fever was quinine, which was taken either orally or by injection. In two cases where it was restarted the blackwater fever recurred and atebtrin was substituted. In the other cases, quinine was immediately stopped and either oral or parenteral atebtrin given. In no case did blackwater fever recur after atebtrin. The usual glucose and alkali treatment was also given, and some received *vitex peduncularis* in addition.

After convalescence, all patients were treated for amœbiasis with emetine and carbarson. Later, they were advised to take 5 grains of suppressive quinine daily, and in case of any malarial rise in temperature, they were further advised to stop the quinine and take atebtrin. There was no second attack of blackwater fever in any case but as they now took atebtrin, instead of quinine for any rise in temperature, no opinion can be given.

In the Dooars, there are a number of people, especially Bengalees, who suffer from chronic malaria and amœbic hepatitis, and it would be interesting to know if the blood changes in these people correspond to the changes reported by Krishnan and Pai in blackwater fever.

#### Conclusion

The importance of liver damage, partly due to amœbiasis, as a predisposing cause of blackwater fever, is stressed.

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### ORIENTAL SORE IN THE NIZAM'S DOMINIONS SOME EPIDEMIOLOGICAL FACTORS

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THE occurrence of oriental sore was first reported in H.E.H. the Nizam's Dominions by Young (1937) at Jalna as a condition simulating certain aspects of leprosy. Daver and Ahmed (1943) again reported the incidence of this conditions not only in Jalna but also in Pattan, a town in the Aurangabad district 72 miles from Jalna.

However late the disease may have been reported from these parts, the occurrence is by no means so recent as it seems. Many stories are in vogue in the affected areas regarding its importation, but the most plausible explanation is that it was introduced by the Moghal troops of Aurangzeb's days. The disease was rampant in the Moghal Empire's capital; hence it was called 'Delhi Boil', and it affected the Emperor Aurangzeb after whom it is also known as 'Aurangzeb Phora' at Delhi (Shah, 1941).

With a view to obtaining more definite information regarding this disease and the extent to which it prevailed in the affected parts, the present epidemiological survey was undertaken, and the junior author was specially entrusted with part of the work dealing with the vector (sand-flies). Unconfirmed reports of the occurrence of the disease in Aurangabad town led to the inclusion of the survey of this town during the present enquiry.

*Methods employed.*—A house-to-house search was made in the localities in the towns surveyed both for cases as well as for sand-flies. Details of the cases with age, sex, period of residence in the locality were recorded. Cases were first diagnosed clinically, but later in the majority of instances, diagnosis was confirmed by the demonstration of leishmania in smears taken from the sores. All sand-flies collected were first classified, and female sand-flies from houses where cases of oriental sore were found were dissected to detect the flagellate infection. Gravid females were specially chosen for dissection, as gravidity ensures that the fly has had at least one blood feed; since it is observed that

the development of the ovary is not possible unless it has taken the first meal of blood (McCombie-Young, Richmond and Brendish, 1926). Moreover, the development of the parasite is much more rapid in gravid females than in the unfertilized flies (Shortt, Barraud and Craighead, 1926). Search was also made for phlebotomus larvæ with a view to studying their bionomics, habits and habitat.

In all the three towns surveyed, schoolboys and girls were specially examined with a view to finding the incidence of the disease among this particular group of the population.

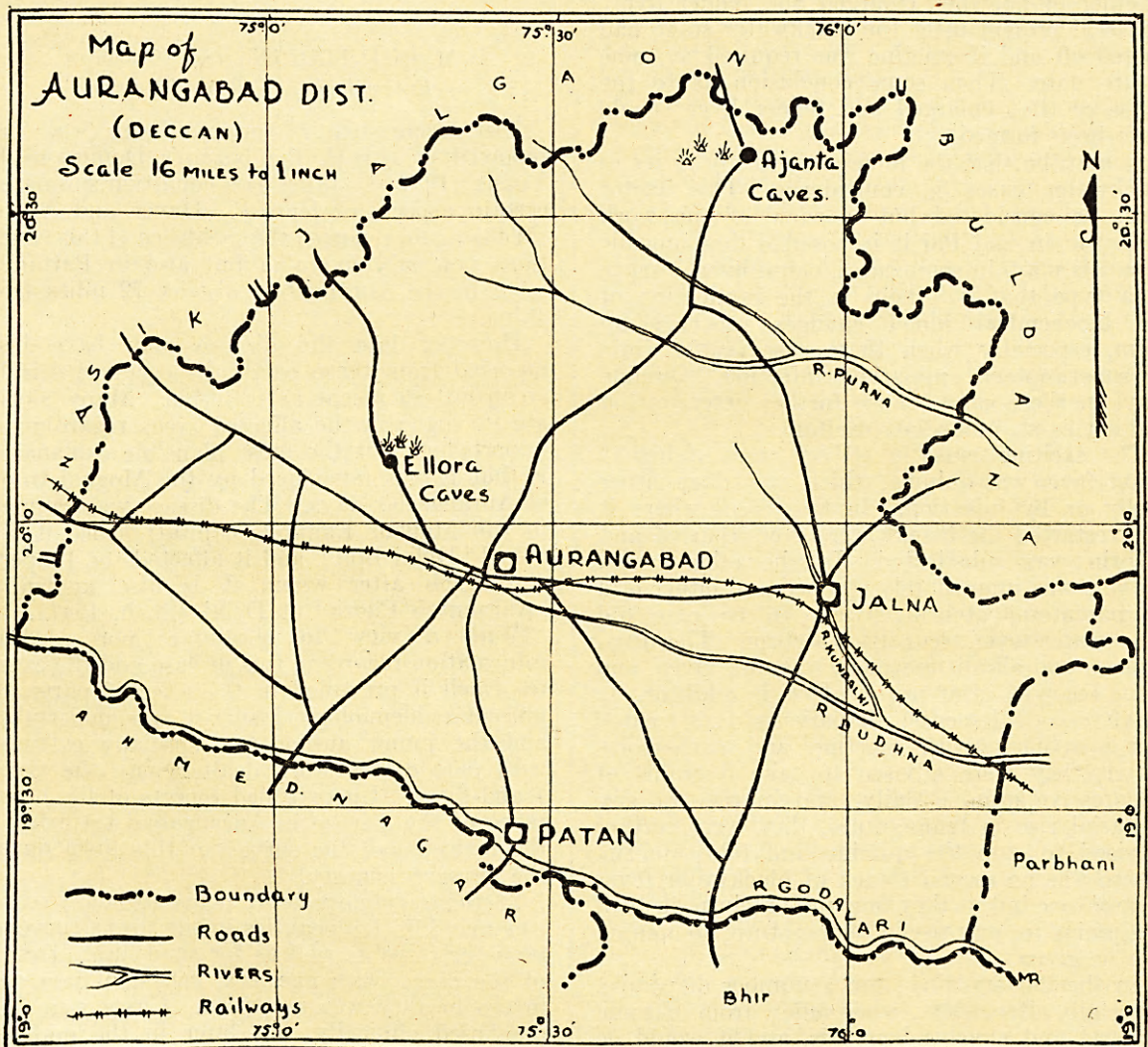
The three towns surveyed are indicated in the map, which shows their disposition in relation to one another and the existing communications.

Aurangabad is the headquarters of the district and the Division (Suba) of the same name.

The population of the town is 50,924. The Kham river, which is a tributary of the Godavari, flows through the town. The city proper is on its eastern bank; the cantonment and a suburb of the city are on its western bank. The mean height above sea level is 1,885 feet and the surface soil is loamy.

Aurangabad was the capital of the Moghal Empire for some time during the reign of Aurangzeb. After the disruption of the Moghal Empire and the shifting of the capital to Hyderabad by the Asif Jahi dynasty, the town lost its fame and importance, and most of it now is but a heap of ruins. The older houses are generally multi-storied ones; the ground floor in most is used as cattle sheds. In poorer quarters even these ground floors, dark and damp, serve as living rooms.

Jalna is the headquarters of the taluq of the same name, with a population of 22,408. It is a commercial centre dealing mostly in cotton and grains and has



*Geographical situation and sanitary condition of towns surveyed.*—All the three towns are situated in the Aurangabad district in the north-west corner of the dominions, which has an average rainfall of 35.8 inches and a mean temperature of 98.6°. The district is famous for its cave temples at Ellora and Ajanta.

many ginning factories. It has a mean height above the sea level of 1,670 feet. The soil is black cotton soil and *morum* overlying basalt rock. It is situated on the bank of Kondalki, a minor tributary of the Godavari, which divides the town into two principal parts, north and south. The south is old town, highly congested with narrow winding lanes and in parts with close aggregation of buildings forming insanitary labyrinths which cannot be efficiently cleansed and in

which the air is almost always stagnant. Cattle sheds were frequently found in this part of the town quite adjacent to living rooms. It has a number of old ruins including the fort. It is here that most of the cases of oriental sore were detected.

In the northern part of the town, which includes the cantonment area, better sanitary conditions prevail, though slum areas are not infrequently found where cases of oriental sore were found in fair numbers.

Pattan, also known as Paithan, is the headquarters of Pattan taluq, with a population of 6,294. The town is situated on the left bank of the river Godavari, with 1,500 feet. It was the seat of the empire of the Andhra Kings, but is now reduced in importance. A few stone and brick houses remain from its prosperous days. The sanitation of the place is poor with many dark ill-ventilated houses; hence it is a stronghold of sand-flies. It is a place of pilgrimage due to the temple of Eknath, one of the staunch devotees of the deity of Pandharpur, about half a mile from the town. A *jatra* is held every year in the last week of March when about 40 to 50 thousand people congregate.

*Incidence of the disease.*—The following statement shows the incidence of oriental sore among the general population examined in the three towns surveyed :—

Town	Number of houses inspected	Number of persons examined	Number of cases found	Incidence rate per mille
Aurangabad	106	1,032	5	4.84
Jalna	485	3,242	90	27.76
Pattan	315	1,781	76	42.68
TOTAL	906	6,055	171	28.24

Age incidence of the cases detected in the three places is shown in the table below :—

Town	0-1 year	1-5 years	6-10 years	11-15 years	16-20 years	Above 20 years	Total
Aurangabad	1	2	2	..	..	..	5
Jalna	3	35	21	18	3	10	90
Pattan	4	39	13	11	2	7	76
TOTAL	8	76	36	29	5	17	171

It shows that 70 per cent of cases occur in children below the age of 10. This is again borne out by the high incidence rate among primary and middle school children examined. Recorded figures are tabulated below. The age of children examined did not exceed 12 years.

Town	Number of schools inspected	Number of boys examined	Number found infected	Incidence rate per thousand	Number of girls examined	Number found infected	Incidence rate per thousand
Aurangabad	11	1,511	43	28.45	178	10	56.18
Jalna	8	728	54	74.17	199	5	25.13
Pattan	3	292	34	116.44	..	..	..
TOTAL	22	2,531	131	51.76	377	15	39.78

The incidence of oriental sore among this selected group of population of children in the three towns was 28.45, 74.17 and 116.44 as against 4.84, 27.76 and 42.68 in the general population. This shows a higher prevalence of the disease among children than among adults. This is explained by the fact that those having ulcers in earlier life rarely suffer from a second in the later years; immunity produced appears to last a life time. In view of this fact, prophylactic inoculation with cultures of *Leishmania* has been practised in south-east Russia (Lawrow and Dubowskoj). Sores develop at the site of inoculation after an incubation period of 2 to 6 months. The immunity thus produced protected against further infection. We do not however see any advantage in such prophylaxis when the disease produced by artificial inoculation does not differ from that naturally contracted except that one could choose a convenient time to have the disease (and a convenient part of the body.—*Editor*).

*Sex incidence.*—Among a total of 171 cases examined in the general population, 91 or 53.2 per cent were among males and the rest 80 or 46.8 per cent were among females. This slight difference can be easily accounted for by the general tendency among the local females not to come forward for examination. The incidence among both sexes appears to be almost the same.

*Situation of the ulcers.*—While examining the cases, a careful record was kept regarding the sites affected. A summary of the results is given in the table on page 88.

The exposed parts, such as the extremities and face, were thus more frequently affected than the covered parts, such as the trunk. Cases with

multiple sores were not infrequent, particularly at Pattan and among children, while single sores were the general rule among the adults in all the three towns examined.

*Species of sand-flies caught.*—In all the three towns surveyed, 940 adult sand-flies of different

## Sites affected in cases of oriental sore examined

Site affected		Total number of cases found
<b>EXTREMITIES—</b>		
Upper	.. ..	50
Lower	.. ..	38
<b>TOTAL</b>	.. ..	88
<b>FACE—</b>		
Cheeks	.. ..	40
Lips	.. ..	11
Eye-lids	.. ..	1
Ear	.. ..	9
Nose	.. ..	6
<b>TOTAL</b>	.. ..	67
<b>TRUNK—</b>		
Chest	.. ..	6
Abdomen	.. ..	2
Back	.. ..	11
Neck	.. ..	2
<b>TOTAL</b>	.. ..	21

species were caught and of this number 483 were females and the rest males. The table below gives the details of the various species caught:—

Town	<i>P. papatasii</i>			<i>P. argentipes</i>			<i>P. antennatus</i>			<i>P. babu</i>			<i>P. bailyi</i>			<i>P. sergenti</i>		
	M.	F.	Total	M.	F.	Total	M.	F.	Total	M.	F.	Total	M.	F.	Total	M.	F.	Total
Aurangabad	48	36	84	39	25	64	6	7	13	6	9	15	8	9	17	1	..	1
Jalna	133	101	234	40	85	125	5	9	14	4	5	9	2	6	8	..	..	..
Pattan	80	90	170	59	69	128	21	24	45	5	8	13	..	..	..	..	..	..
<b>TOTAL</b>	261	227	488	138	179	317	32	40	72	15	22	37	10	15	25	1	..	1

*Phlebotomus papatasii*.—This was by far the commonest species met with during the survey. Of the 227 females caught, 200 were dissected and in two of them (one from Jalna town and the other from Pattan) flagellate infection was detected in the midgut.

*Phlebotomus argentipes*.—This is the second commonest species of sand-flies found in the towns surveyed. One hundred and thirty-eight males and 179 females were caught. Of the 160 female flies dissected, none was found infected.

*P. sergenti*.—The solitary male specimen of *P. sergenti* was found at Pattan and was preserved after identification. Further search for sand-flies of this species both at Pattan and other places proved futile.

*P. antennatus*, *P. babu* and *P. bailyi*.—In the minutus group the largest number found (72) belonged to the species *P. antennatus*, while the other two species, viz, *P. babu* and *P. bailyi*, were represented by the small numbers of 37 and 25.

*Breeding grounds of sand-flies*.—In order to determine the local nature of the breeding

grounds of sand-flies, an intensive search was made for phlebotomus larvæ. Out of a variety of samples of earth examined from all possible breeding places, two larvæ were discovered in a blend of litter and soil from a cowshed in Jalna. As the larvæ were distorted very much during the process of search, no specific diagnosis could be made. Adult catches from the same area showed only *Phlebotomus papatasii*.

*Control measures recommended*.—Advantage in control measures should be taken of the breeding habits of the vector. They breed in soil in which there is nitrogenous matter, as supplied by household refuse or domestic animal droppings; in cracks and crevices protected by the overhanging eaves of houses, under trees and bushes, or other heaps of refuse. Proper soil sanitation, the keeping of cattle sheds away from living quarters and, where this is not possible, the keeping of cattle sheds clean by removing the litter to manure pits away from the houses and burning the litter, if it is suspected of actually breeding sand-flies; these are important practical measures.

*Spray killing of the adult flies*.—Brief experiments carried out in the laboratory have shown pyroicide mixture, in concentration as used for

killing of mosquitoes, to be equally effective for sand-flies. It is recommended that spraying should be done twice a week as these flies become infective as early as the fifth day after ingesting leishmania bodies (Shortt, Sinton and Swaminath, 1935). Spraying should be vigorous in the affected localities. The fly, unlike the mosquito, has a very limited range of flight, and is therefore nearly always found not far from its breeding grounds; advantage of this fact can be taken in limiting spraying to infected localities or their immediate neighbourhood, special attention being paid to houses where cases have been detected.

Stray dogs with suspicious-looking sores should be destroyed. Sinton has shown that the leishmania occurring on the face of dogs in India are transmissible to man.

The use at night of fine-mesh mosquito netting—40/45 mesh (as against 25/26 mesh used for protection against mosquitoes) is necessary to exclude these tiny insects. An electric fan can also repel the flies. A relative protection from the disease is provided by occupying a bed-room

in the upper stories, as fewer sand-flies are found at higher levels.

Treatment centres to eliminate the source of infection in the affected areas should be started.

*Summary and conclusions.*—An oriental sore and sand-fly survey of three towns Aurangabad, Jalna and Pattan in the northern part of H.E.H. the Nizam's Dominions was carried out.

The incidence of oriental sore ranged from 42.6 per mille in Pattan to 4.8 in Aurangabad.

There does not appear to be much difference as far as the sex incidence is considered, but the disease is most common among children under 10 years of age.

Six species of sand-flies were found in this area. The most commonly found species were *Phlebotomus papatasi* and *Phlebotomus argentipes*. The other species which were found in small numbers were *P. babu*, *P. bailyi* and *P. antennatus*. A solitary specimen of *P. sergenti* was found in Aurangabad.

Out of 414 female sand-flies dissected, two were found to be infected. Both these specimens (one from Pattan and the other from Jalna) belonged to the species *P. papatasi*.

Control measures are recommended.

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### VOGES-PROSKAUER TEST WITH LEIFSON'S REAGENT

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Most bacteriologists held that the old Voges-Proskauer's test (1898) was not a satisfactory test

for the differentiation of the coli-ærogenes group of organisms. During carbohydrate fermentation by the ærogenes group of bacilli, acetoin (acetylmethyl carbinol) is formed, and this is detected by caustic potash and peptone. Acetoin is oxidized to diacetyl, and this reacts with caustic potash and peptone and gives a faint pink colour in the old Voges-Proskauer's test. O'Meara (1931) introduced a more sensitive test by adding creatin which helped to detect acetoin in a dilution of 1 in 20,000 with great ease, and 1 in 50,000 dilution was just capable of recognition. Leifson (1932), by using copper sulphate as a catalyst, introduced a more sensitive test. His reagent was very simple, less expensive, and could be preserved for a long time in a glass-stoppered phial. To 1 c.cm. of culture, 1 c.cm. of this reagent was added and mixed. The reaction was markedly positive, showing bright red or eosin-like colour at the surface in 10 to 20 minutes at room temperature. The maximum time required was 2 to 4 hours. Only two days' old culture in glucose phosphate broth at 37°C. was required. The formula of his reagent is as follows:—

CuSO <sub>4</sub> , 5 H <sub>2</sub> O	1 gm.
Conc. am. hydroxide	40 c.cm.
(sp. gr. 0.90).	
NaOH 10 per cent	960 c.cm. to be added last.

The reagent should be kept in stoppered bottles. Barritt (1936) introducing  $\alpha$ -naphthol discovered a more sensitive and specific test, and was able to detect acetoin in a dilution of 1 in 1,000,000 in 2 to 5 minutes. But he used 3 days' old culture at 37°C. Seetharama Iyer and Raghavachari (1939) used Barritt's test in bacteriological analysis of water and found it to be specific and highly sensitive. They did not compare the utility of the test with that of Leifson. They advocated carrying out both methyl-red test and Voges-Proskauer's test in succession in the same culture tube. Seshadri-nathan and Venkataswami (1943) carried out Barritt's test on coliform organisms of the urinary tract and confirmed the test as satisfactory, but they also did not compare the test with Leifson's.

We could not procure  $\alpha$ -naphthol and creatin, and hence we had to use Leifson's reagent alone in comparison with the old Voges-Proskauer's test, and found the test very reliable. The few coliform organisms that we tested with Leifson's reagent and by Barritt's method showed that the tests tallied with each other. The positive reaction is indicated by the development of a bright red or pink colour in 10 to 20 minutes. The maximum time required is 2 hours. A bluish violet colour or absence of any colour indicates a negative reaction. No reading should be taken on the next day after addition of the reagent. The test should not be done on 3 days' old culture. Our results are given in the following table.