

HISTOPLASMIN AND TUBERCULIN SENSITIVITY IN BURMA

Study of Tests on 3,558 Subjects

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“Histoplasmosis has apparently never been diagnosed on the continent of Asia . . . ”⁶ In Indonesia, however, a case has recently been reported in which *Histoplasma capsulatum* was isolated in culture; ³ four others, based on histological evidence, have been found in a search of the available literature.^{4, 5, 7, 9} In view of the nearness of Lower Burma to Indonesia (see fig. 1), the remarkable geographical resemblance between this area and known histoplasmosis-endemic centres elsewhere in the world, and the complete absence of published data on histoplasmin sensitivity among residents of south-east Asia, a pilot-type study was planned and carried out. The results of investigations of intradermal sensitivity to histoplasmin, together with other related evidence presented in this paper, make a strong case for the occurrence of as yet unrecognized histoplasmosis in Burma.

Through the interest of U Maung Gale, Director of Medical and Health Services of Burma, and the enthusiastic co-operation of U Ba Thein, Inspector-General of Prisons for Burma, and his entire staff, we were given access to Burma's three largest prisons for our work, which was carried out during April and May 1952. Our general procedure was to show a film on BCG vaccination, to have one of the prison physicians explain briefly to the prisoners, in Burmese, the significance of the histoplasmin test, and, finally, to demonstrate the tests on ourselves. Taking the tests was entirely voluntary; no one who took the tuberculin test, which was given first, refused the histoplasmin.

Material and Methods

Undiluted histoplasmin (Lilly, lot CT-189^a) was received via air mail and was kept under refrigeration when not in use. All work with histoplasmin (e.g., preparing dilutions of the antigen, skin testing, interpretation of reactions, etc.) was done by one of us (Tucker). One-tenth millilitre of diluted antigen was injected intracutaneously into the volar surface of the right forearm. Tests were read approximately 72 hours later, and the greatest diameter of induration was recorded. Induration of 5 mm or more in diameter was considered a positive reaction. New or dichromate-cleaned, autoclaved bottles were used for diluted antigen. New syringes and needles were employed, and stringent precautions were taken to prevent interchange of equipment.

Similarly, all work with tuberculin was done by one man (Kvisselgaard). Equipment was that used routinely by BCG field-teams in the area. Diluted purified protein derivative (PPD) was sent in 100-ml bottles by air from King's Institute, Madras, India; it was refrigerated in transit and when not in use. The tests were applied within ten days of the date of manufacture. One-tenth millilitre of antigen, containing 5 tuberculin units (equivalent to 0.0001 mg PPD) was injected intradermally into the volar surface of the left forearm. Tests were read after about 72 hours, according to the criterion mentioned above. Those who did not react were vaccinated with BCG.

Portable roentgenologic equipment was not available for use inside the prisons. However, roentgenograms of the chests of seven individuals who were histoplasmin-positive and tuberculin-negative were made in Rangoon at the Tuberculosis Clinic. Dr. B. Papanicolaou, former WHO Senior Adviser at the Clinic, remembered three cases of multiple foci of calcification among 8,661 miniature chest-films taken there during eight months of operation. It was possible to locate the clinical records and large films of two of these cases and to recall the patients for study and histoplasmin testing.

The first establishment in which tests were given, Rangoon Central Prison, had a population of which more than 90% claimed Lower Burma as residence; brief trips into central and northern Burma were disregarded. (In general, "Lower Burma" included the Arakan on the west, the delta surrounding Rangoon, and the Tenasserim section, which merges to the south with Malaya and Thailand.) Here, because results could not be anticipated, and because of previous experience with histoplasmin in a concentration of 1/100, that dilution was used on 1,258 individuals, including prisoners, prison personnel, and their families. Of these, 1,089 (86.6%) returned three days later for interpretation of the tests.

^a This histoplasmin, which was from the same lot as that previously used by Tucker in the Panama Canal Zone,¹⁰ was furnished by Dr. C. E. Roach, Medical Division, Eli Lilly and Company.

FIG. 1. SOUTH-EAST ASIA : GENERAL GEOGRAPHICAL FEATURES OF BURMA AND ITS PROXIMITY TO INDONESIA



The cities indicated are cited in the text

Next we went to the Insein Central Prison and Annex, located in a suburb about 12 miles (19 km) north-east of the centre of Rangoon. Here, 1,288 subjects were tested with histoplasmin (1/1,000), of whom 1,194 (92.7%) returned as directed. These persons were likewise mainly (over 93%) from Lower Burma.

Finally, we visited the Mandalay Central Prison, in Upper Burma. This was a deliberate choice since its location provided a marked climatological and geographical contrast to the more southern region. Records showed that more than 94% of the prison population had lived most, or all, of their lives in Upper Burma. For purposes of comparison with data already obtained, a dilution of 1/100 was used on 142 men and a dilution

of 1/1,000 on all others. Here, 1,366 persons were tested, of whom 1,275 (93.5%) came back for reading.

Persons tested ranged in age from three months to 75 years, and age distribution was similar in the three groups. Female prisoners and their children, whom they were allowed to keep with them if six years of age or less, were tested inside the prisons. Female relatives of prison personnel were tested outside, in the prison compounds. In analysing the data obtained, no differences were found among prisoners, prison personnel, and their families in a given establishment; therefore, all have been considered together.

Results

Intradermal sensitivity

When analysis of all the results obtained in the first prison revealed that one out of four persons tested was histoplasmin-positive, two variables were introduced into the experiment in the hope that they might clarify the problem. First, a higher dilution of histoplasmin was used; and, secondly, a distinctly different set of geographical and climatological factors was introduced. The results are given in detail in tables I-IV according to these variables.

Tenfold reduction in concentration of antigen, from 1/100 to 1/1,000, reduced the number of histoplasmin reactors in the samples studied by approximately one-half; this was true in both geographical environments. There were significant differences as measured by χ^2 , with $P = < 0.01$ as the arbitrarily selected level of significance. It was also found that the proportion of 1+ reactions rose from 60.9% with a 1/100 dilution to 74.6% when a dilution of 1/1,000 was used. Similarly, in Mandalay, a 1/100 concentration induced 70.0% 1+ reactions, and a concentration of 1/1,000, 89.2%. Thus, intensity of reactions tended to vary directly with the concentration of histoplasmin used.

When the variable was geographical background of the majority of the individuals in the samples, it was found that those from Lower Burma had significantly higher prevalence of sensitivity than those in Upper Burma, as shown in tables I-IV. This was the case regardless of the concentration of antigen employed. Comparable differences in prevalence of sensitivity to tuberculin were seen in the same test subjects.

In the three sets of data in which both sexes were represented, females seemed to exhibit a lower prevalence of sensitivity to histoplasmin than males. This difference was found not to be a real one, however, but to be due to the lower age-distribution of the females tested, approximately one-third of whom were less than ten years of age. The same tendency was seen, erroneously, with regard to tuberculin sensitivity in our samples; large-scale tuberculin-testing throughout the Union of Burma has shown

TABLE I. PREVALENCE OF HISTOPLASMIN (1/100) AND TUBERCULIN SENSITIVITY IN LOWER BURMA

Ten-year age-groups	T—	H—	T+	H—	T+	H+	T—	H+	All	H+	All	T+	Total number tested
	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	
0-9	67	72.8	23	25.0	1	1.1	1	1.1	2	2.2	24	26.1	92
10-19	7	9.3	60	80.0	6	8.0	2	2.7	8	10.7	66	88.0	75
20-29	24	4.7	346	67.3	138	26.8	6	1.2	144	28.0	484	94.1	514
30-39	5	1.9	161	61.7	91	34.9	4	1.5	95	36.4	252	96.6	261
40-49			73	70.2	31	29.8			31	29.8	104	100.0	104
50-59	1	2.8	22	62.9	12	34.3			12	34.3	34	97.2	35
60-69			5	62.5	3	37.5			3	37.5	8	100.0	8
70-79													—
Total	104	9.5	690	63.4	282	25.9	13	1.2	295	27.1	972	89.3	1,089*

P.R. = Prevalence ratio, or the percentage in a given category in a given age-group

* 910 males, 179 females

TABLE II. PREVALENCE OF HISTOPLASMIN (1/1,000) AND TUBERCULIN SENSITIVITY IN LOWER BURMA

Ten-year age-groups	T—	H—	T+	H—	T+	H+	T—	H+	All	H+	All	T+	Total number tested
	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	
0-9	58	92.1	4	6.3	1	1.6			1	1.6	5	8.0	63
10-19	27	23.3	77	66.4	10	8.6	2	1.7	12	10.3	87	75.0	116
20-29	21	3.5	489	81.8	86	14.4	2	0.3	88	14.7	575	96.2	598
30-39	6	1.9	254	81.2	53	16.9			53	16.9	307	98.1	313
40-49	2	2.4	64	76.2	18	21.4			18	21.4	82	97.6	84
50-59			14	100.0							14	100.0	14
60-69			5	83.4	1	16.6			1	16.6	6	100.0	6
70-79													—
Total	114	9.6	907	75.9	169	14.2	4	0.3	173	14.5	1,076	90.1	1,194*

P.R. = Prevalence ratio, or the percentage in a given category in a given age-group

* 1,084 males, 110 females

TABLE III. PREVALENCE OF HISTOPLASMIN (1/100) AND TUBERCULIN SENSITIVITY IN UPPER BURMA

Ten-year age-groups	T—	H—	T+	H—	T+	H+	T—	H+	All	H+	All	T+	Total number tested
	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	
0-9													—
10-19	2	18.2	7	63.6	2	18.2			2	18.2	9	81.8	11
20-29	13	14.8	66	75.0	8	9.1	1	1.1	9	10.2	74	84.1	88
30-39	1	3.5	28	96.5							28	96.5	29
40-49			11	91.6	1	8.4			1	8.4	12	100.0	12
50-59			2	100.0							2	100.0	2
60-69													—
70-79													—
Total	16	11.3	114	80.3	11	7.7	1	0.7	12	8.4	125	88.0	142*

P.R. = Prevalence ratio, or the percentage in a given category in a given age-group

* All males

TABLE IV. PREVALENCE OF HISTOPLASMIN (1/1,000) AND TUBERCULIN SENSITIVITY IN UPPER BURMA

Ten-year age-groups	T—	H—	T+	H—	T+	H+	T—	H+	All	H+	All	T+	Total number tested
	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	no.	P.R.	
0-9	51	100.0											51
10-19	42	38.9	64	59.2	2	1.9			2	1.9	66	61.1	108
20-29	130	22.6	422	73.4	20	3.5	3	0.5	23	4.0	442	76.9	575
30-39	40	15.0	212	79.7	12	4.5	2	0.8	14	5.3	224	84.2	266
40-49	12	12.4	79	81.4	6	6.2			6	6.2	85	87.6	97
50-59	3	9.1	29	87.9	1	3.0			1	3.0	30	90.9	33
60-69			2	100.0							2	100.0	2
70-79			1	100.0							1	100.0	1
Total	278	24.6	809	71.4	41	3.6	5	0.4	46	4.0	850	75.0	1,133*

P.R. = Prevalence ratio, or the percentage in a given category in a given age-group

* 1,019 males, 114 females

that prevalence of sensitivity is essentially the same for both sexes in all areas studied (Kvisselgaard—unpublished data). For these reasons no separation by sexes has been made in the tabulations.

Clinical findings

Dr. Papanicolaou was of the opinion that all of the seven histoplasmin-positive, tuberculin-negative individuals from the Rangoon Prison who obtained roentgenograms of the chest had bilateral hilar deposits of calcium. One 26-year-old warder had, in addition, two parenchymal foci about 5 mm in diameter in the right lung field. In none was there evidence of active pulmonary disease.

Of the three patients with teleroentgenograms showing multiple foci of calcification which Dr. Papanicolaou recalled, two were brought in for interview and further study. Pertinent features of these cases follow :

(1) M. P. A., 43-year-old Burman

This patient had spent all his life in the neighbourhood of Rangoon. He gave a history of an acute, severe, respiratory illness when he was 20 years of age but had since felt entirely well. His teleroentgenogram, reproduced in fig. 2, showed multiple, round or oval foci of calcification scattered throughout both lung fields, most of them being located towards the bases. His tuberculin reaction was strongly positive (4+), and histoplasmin (1/1,000) produced an induration of 8-mm diameter after 72 hours.

(2) T. R., 40-year-old Indian male

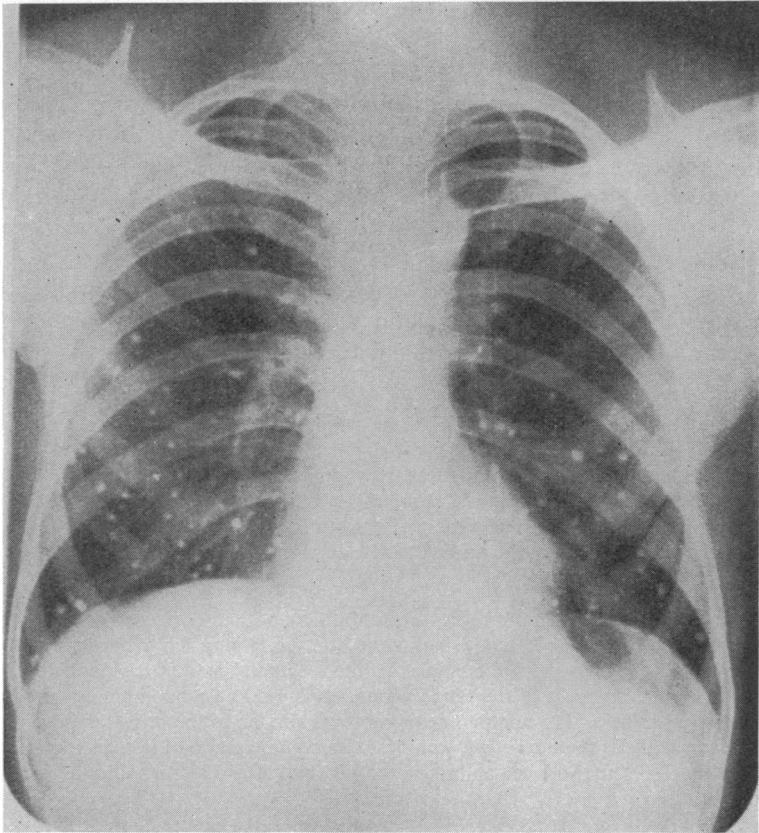
This man had been born and reared near Madras and had come to Burma five years before. Like the patient cited above, he worked on the Hlaing River docks in Rangoon proper and had come to the Tuberculosis Clinic originally as a participant in a tuberculosis survey of employees of the Port Commission. He had no complaints and denied previous serious illness. His teleroentgenogram was similar to the one illustrated (fig. 2). The foci were fully as numerous but were less radiopaque and had less distinct margins. Histoplasmin (1/1,000) produced an induration of 7 mm after 72 hours, and the tuberculin test was positive.

Discussion

Although it is not yet possible to draw a complete picture of the relationship between prevalence of sensitivity to histoplasmin and such geographical factors as humidity, latitude, altitude, annual rainfall, and temperature, at least in the majority of published studies⁶ high prevalence has been found in association with rainy, low-altitude regions of the temperate or tropical zones. In fact, it was the remarkable resemblance between all these features in Lower Burma and those in regions of the American tropics where histoplasmosis has recently been found^{8, 11} which suggested that investigations in Burma might yield provocative results.

Burma lies between latitude 10°N. and 28°N., thus being mainly in the tropics. It has an area of about 262,700 square miles (750,000 km²) and an estimated population of roughly 18,500,000. It is bounded by natural barriers, the whole mountain system consisting of north-south offshoots

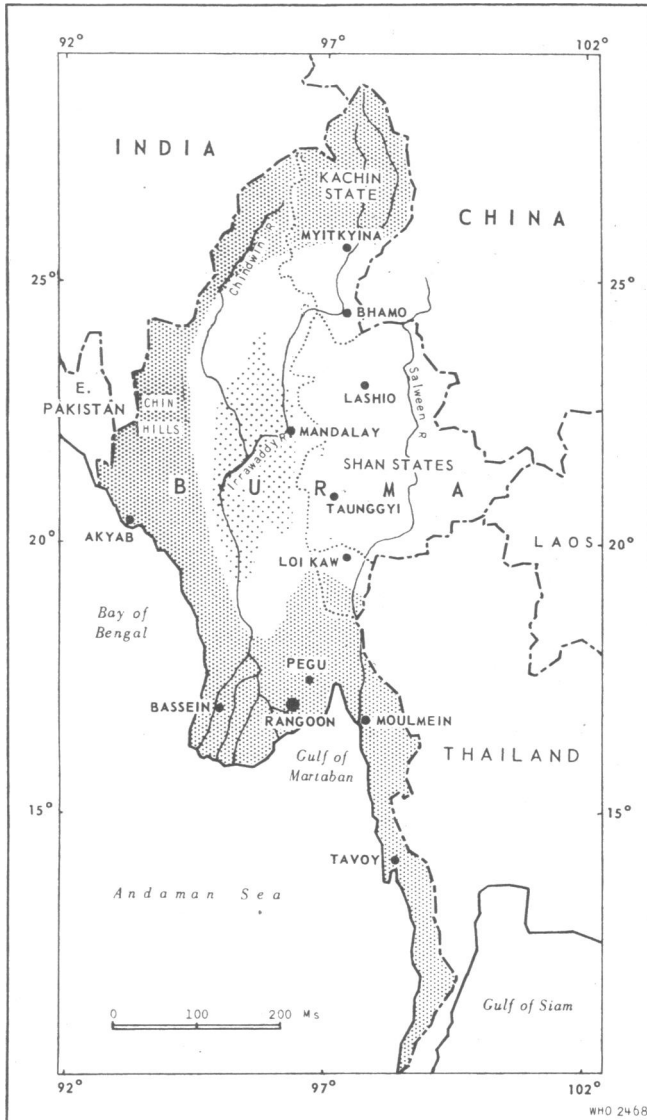
FIG. 2. TELEROENTGENOGRAM OF CHEST SHOWING ROUND OR OVAL HEAVILY CALCIFIED FOCI SCATTERED THROUGHOUT THE LUNG FIELDS






Never out of Burma, this patient reacted to histoplasmin (1/1,000) and to tuberculin and gave a history of severe respiratory illness 23 years before.

of the Tibetan mountains in the shape of a horse-shoe. The country is made up of a series of river valleys, the most important being that of the Irrawaddy, which arises in Tibet and flows south past Mandalay finally to break up into branches in the delta region before entering the Andaman Sea. Rainfall varies greatly in different parts of the country, as is shown in fig. 3. The monsoon season, from mid-May to mid-October, brings about 200 inches (500 cm) of rainfall a year to the coastal regions of Arakan and Tenasserim and to the mountain ranges of the extreme north. The Irrawaddy delta receives about 100 inches (250 cm) of rainfall annually, and the hills in the west and east, about 80 inches (200 cm). The Arakan Yomas, however, cut off the monsoons from the central part of the country, and only 20-45 inches (50-115 cm) of rain fall there. A temperature of over 100°F (38°C)

FIG. 3. RAINFALL IN BURMA



-  More than 80 inches (200 cm) annual rainfall
-  40-80 inches (100-200 cm)
-  Less than 40 inches (100 cm) (central "dry" zone)

in the shade is not uncommon in the "dry zone"; and, immediately before the monsoons, the temperature in the delta region may be equally high. In the coolest months (December and January), the temperature in southern Burma is in the neighbourhood of 60°F (16°C) minimum and falls even lower in the "dry zone". The delta and the coastlands are extremely humid throughout the year.

With minor changes, this brief description of Lower Burma could apply to those regions of the Western Hemisphere where histoplasmosis has been found and prevalence of histoplasmin sensitivity has been shown to be high. In south-east Asia it could likewise apply to Indonesia. In Java, for example, which seems to be the common factor in the history of residence in the five reported cases of histoplasmosis in Indonesia,^b rainfall, temperature, and humidity are high, altitude is low (northern Java is a coastal plain), and the island lies even closer than Burma to the equator (latitude 6°-9°S.). This combination of features seems to be favourable to the continued development of the fungus once *Histoplasma* has been introduced. In such geographical areas the histoplasmin test may serve as a useful epidemiological tool since high prevalence of sensitivity indicates that the fungus may be present.^c Thus far, no work with histoplasmin has been reported from Indonesia.

However, two studies have been carried out on the continent of Asia in geographical areas which are dissimilar to those described in the preceding paragraphs (WHO Tuberculosis Research Office—unpublished material). In Kelur, south-west of Madras, India, no positive reactions were noted in tests with histoplasmin (H-40, 1/500) of 341 schoolchildren and of 261 leprosy patients of all ages. This area is hot and so dry—20-40 inches (50-100 cm) of rainfall annually—that irrigation is extensively employed. Again, in Darjeeling, West Bengal, 1,159 native Indians, between 1 and 20 years of age and in approximately equal numbers of both sexes, were tested with the same lot and concentration of histoplasmin; only nine had reactions of 5 mm or more in diameter. Darjeeling is located high in the eastern Himalayas above Calcutta. Rainfall is high, and humidity and temperature are low, as is the case in the extreme north of Burma. It is perhaps to be regretted that this effort was not expended on work in the Ganges delta, in the vicinity of Calcutta or in Chittagong, East Pakistan.

The apparent relationship between low-lying river valleys and endemic areas of histoplasmosis has been pointed out by many workers; and some have suggested² that the rivers play an important role, the fungus being disseminated by floods carrying a soil-fungus mixture which is later airborne. In spite of apparent exceptions to this opinion (both Rangoon and Mandalay

^b Although six cases have been cited in the references listed,^{3, 4, 5, 7, 9} the third case mentioned by Bras et al.⁹ was later reported in detail by Hausman & Hiemstra⁴ (personal communication from Dr. Sutomo Tjokronegro, Professor of Pathology, Universitas Indonesia, Jakarta).

^c A detailed consideration of the possible explanation of the apparent absence of the fungus in areas showing high histoplasmin-sensitivity prevalence is given by Tucker.¹⁰

are located on the Irrawaddy River), Mochi & Edwards⁶ have stated that, in the search for new endemic centres of histoplasmosis, priority might be given to low-altitude regions along large rivers. It seems to us that other factors, such as temperature, humidity, altitude, and annual rainfall should not be underrated, as consideration of fig. 3 emphasizes. The provocative regions in south-east Asia to which we would give priority for future work would be, in addition to Indonesia, the Ganges delta region of India and East Pakistan, the vicinity of the Menam Chao Phaya about Bangkok, Thailand, and the great Mekong delta, near Saigon, in Cochinchina.

Our study suggests that histoplasmosis may exist in Burma. Although "pulmonary calcifications are apparently nonspecific reactions to a variety of organisms",¹ the occurrence of multiple, rounded, sharply defined, uniform foci, scattered bilaterally throughout the lung fields, is an unusual picture in pulmonary conditions other than histoplasmosis. The roentgenographic evidence presented was suggestive rather than conclusive, as were the results of our intradermal testing. Nevertheless, our findings in Burma, when compared with similar data from parts of the world where histoplasmosis is known to occur, suggest that an active search by local clinicians and pathologists may result in the isolation of *Histoplasma capsulatum* in Lower Burma.

SUMMARY

Although *Histoplasma capsulatum* has, in one instance, been isolated in Java, histoplasmosis has apparently never been diagnosed on the continent of Asia. Since environmental conditions in Lower Burma closely resemble those in many areas where this disease is endemic, a pilot-type study of sensitivity to histoplasmin was carried out in conjunction with tuberculin testing in three prisons in Burma.

Histoplasmin-sensitivity tests using Lilly antigen (CT-189, 1/100 dilution) revealed a prevalence of 27.1% among 1,089 prisoners, prison personnel, and their families at a large Rangoon prison. Using a 1/1,000 dilution of histoplasmin in tests at another prison in Lower Burma, the authors found that 14.5% of the 1,194 persons tested had indurations of 5 mm

RÉSUMÉ

L'histoplasmose semble n'avoir jamais été diagnostiquée dans le continent asiatique, bien que *Histoplasma capsulatum* ait été isolé, dans un cas, à Java. La Basse-Birmanie, dont les conditions géographiques et climatiques ressemblent beaucoup à celles que l'on observe dans plusieurs des zones où la maladie est endémique, fut choisie pour une étude-type sur la sensibilité à l'histoplasmine. Les essais furent effectués dans trois prisons de Birmanie, parallèlement à des épreuves tuberculiques.

Les épreuves de sensibilité à l'histoplasmine effectuées avec l'antigène Lilly (CT-189, dilution 1/100) dans une grande prison de Rangoon sur 1.089 personnes (prisonniers, membres du personnel et leurs familles) ont donné 27,1% de résultats positifs. Des épreuves analogues furent effectuées dans une autre prison de Basse-Birmanie avec une dilution d'histoplasmine

or more in diameter, which was considered a positive reaction.

In Upper Burma, a hot, dry area, 8.4% of 142 prisoners tested with a 1/100 dilution of the antigen, and only 4.0% of 1,133 persons tested with a 1/1,000 dilution, reacted positively, thus demonstrating, with both concentrations of histoplasmin, a significantly lower prevalence of histoplasmin sensitivity in residents of the "dry zone".

The same subjects were tested concurrently with 0.0001 mg of purified protein derivative (PPD). Roentgenograms of two histoplasmin- and tuberculin-positive patients showed the typical picture of "multiple calcific pulmonary foci" found in histoplasmosis.

It was concluded that the sum of circumstantial evidence was strongly in favour of the occurrence of *Histoplasma* in Lower Burma.

de 1/1.000; 14,5% des 1.194 personnes soumises à l'épreuve présentèrent des indurations d'un diamètre de 5 mm ou plus, considérées comme des réactions positives.

En Haute-Birmanie, région chaude et sèche, 8,4% des 142 prisonniers ayant reçu la dilution 1/100 et 4,0% seulement des personnes auxquelles fut injectée la dilution 1/1.000 réagirent positivement. Ces résultats, obtenus avec deux dilutions différentes, montrent que la proportion de personnes réagissant positivement à l'histoplasmine est notablement plus faible chez les habitants de la « zone sèche ».

Les mêmes sujets reçurent en même temps 0,0001 mg de dérivé protéinique purifié (PPD). La radiographie montra chez deux sujets positifs à l'histoplasmine et à la tuberculine une image typique de « foyers pulmonaires multiples de calcification ».

Les auteurs concluent de l'ensemble des observations faites, à la présence de *Histoplasma* en Basse-Birmanie.

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