sies was 33. The cases are described in the appendix.

Comments

In our series the finding of 4 primary carcinoma cases in such a short period is significant. At this stage we should not like to draw any statistical conclusions but only would point out that such cases are very often missed in our country, firstly, for the absence of any pathognomonic symptoms or physical signs leading to an ante-mortem diagnosis of the condition, secondly due to lack of 'biopsies of the liver' due to fear and lastly due to difficulty of obtaining bodies for routine postmortem examination.

Out of our 4 cases, 3 are in females and only one in the male. All are in Hindus, one from Madras, one form U.P. and the other two from this place. Three growths are 'Cholangiomas' (two in females and one in male) and one is a 'Hepatoma' (Female). Age group of 3 Cholangioma is 35-55 years. (Two between 35 and 40 and one above 50.) The age of the only Hepatoma case is 60 years.

The other fact that is brought to light in this series is the occurrence in the people of the lower strata of life taking a monotonous diet poor in proteins. Probably this defeciency resulted in the damage to the liver cells leading to the cancerous proliferations. (Glynn and Himsworth, 1944). The high incidence of this type of cancer is not purely racial in character. It is likely that it is attributable to the economic backwardness of the people affected. (Gillbert and Gilzar, 1944).

From the above it will appear that the statistical figures compiled during the days when better food and living conditions existed in India, may be completely upset if 'Liver Biopsies' are undertaken more freely in suspected cases and routine autopsies of all cases dying at least in the hospitals can be undertaken.

Summary

A review of published articles on the primary carcinoma of the liver with reference to India is made.

Four cases of primary carcinoma of the liver are described.

Plea for routine post-mortem examination of cases dying in hospitals and more frequent 'liver biopsies' in suspected cases is made.

We are indebted to Dr. S. C. Lahiri, Senior Visiting Physician and Dr. S. N. Chatterji, Prof. of Clinical Surgery for their cases. We are particularly indebted to Dr. A. B. Mukherji, Senior Visiting Physician, for the material which he obtained by undertaking a partial autopsy himself. We thank Dr. M. Pan our Principal, for allowing us to send these case reports for publication.

We also record our thanks to Dr. D. Basu, for the photo-micrographs and helpful comments and to Sri Jitendra Nath Bose for technical assistance.

REFERENCES

BASU, P. N., and VASUDEVAN (1929). *J. Path. and Bact.*, **32**, 342.

BERMAN, C. (1941) .. *S. African J. Med. Sci.*, **6**, 145.

GHARPURE, P. V. (1927). *Indian Med. Gaz.*, **62**, 315.
Idem (1928). *Ibid.*, **63**, 253.
Idem (1948). *Ibid.*, **83**, 5.

GILBERT, C., and GILLZAR, J. (1944). *Science*, **99**, 398.

GLYNN, L. E., and HIMSWORTH, H. P. (1944). *J. Path. and Bact.*, **56**, 297.

KHANOLKAR, V. R. (1945). *Indian J. Med. Res.*, **32**, 299.

STRONG, G. F., and PITTS, H. H. (1930). *Arch. Int. Med.*, **46**, 105.

TULL, J. C. (1932) .. *J. Path. and Bact.*, **35**, 557.

VISWANATH and GREWAL, K. S. (1935). *J. Ind. Med. Res.*, **23**, 149.

HISTOPLASMIN, COCCIDIOIDIN, BLASTOMYCIN, TUBERCULIN SENSITIVITY IN RELATION TO TROPICAL EOSINOPHILIA AND PULMONARY CALCIFICATIONS

By G. KAUR DHARIWAL, M.B., B.S. (Pb.), M.R.C.P. (Lond.), D.C.H. (Eng.)
and
N. K. CHAKRAVARTY, M.D. (Cal.)
(From the School of Tropical Medicine, Calcutta)

Histoplasmosis.—Recent studies of the fungus *Histoplasma capsulatum* have indicated that human infection with the organism may manifest itself in a variety of ways. In the reported cases when the lungs were involved the most common

symptom seemed to be frequent "colds". The sputum is usually mucoid and occasionally blood-tinged. At times the severity of cough increases with sensation of tightness in the chest, wheeze, and fever. The patient usually considers these symptoms to represent an acute cold. Some patients have such symptoms more or less continuously for several years (Charr, 1953). A white blood cell count in the normal range of 5,000, to 10,000 is recorded in the majority of cases of histoplasmosis. The dfferential white blood cell count shows no consistent changes and is subject to wide variation (Parsons and Zarafonetis, 1945). A large number of people affected, however, fail to show any clinical symptoms of the disease, and the only evidence that an infection has been present at some time in the past is sensitivity to the skin-testing antigen, histoplasmin, and quite frequently, calcified areas in the lungs or hilar lymph nodes. Findings of this nature are most common in the States bordering the Mississippi River and its larger tributaries, particularly in the Kansas City area where about 80 per cent of the population are positive histoplasmin reactors (Beaden Koff, *et al.*, 1949; Christie and Peterson, 1945; Palmer, 1945). Besides such findings fatal cases of histoplasmosis have been reported from time to time. With respect to tuberculosis, it has been well established that the antecedent lesion is a soft type of infiltrate in a tuberculin positive individual from whom it is often possible to recover tubercle bacilli by careful examination. Rather similar soft lesions have been found in histoplasmin positive, tuberculin negative persons, and the fungus *histoplasma capsulatum* has been recovered in some of the cases. This type of evidence leaves little doubt that healing by calcification does take place in histoplasmosis as it does in tuberculosis.

The development of calcification in some of these precalcific lesions has now been observed (Furcolow, 1949), so that pathogenic process has been followed from the negative roentgenogram of the chest and negative histoplasmin reaction through the appearance of the parenchymal infiltrate (with concurrent change in the cutaneous sensitivity) to the final calcified focus in the lung associated with a positive reaction to the histoplasmin test. Sontag and Allen (1947) have recently described similar precalcific lesions in children who were sensitive to histoplasmin but not to tuberculin. Edwards Lewis and Palmer (1948) presented an analysis of the pulmonary infiltrates and mediastinal lymph mode enlargement among student nurses at the beginning of training and observed the relationship of these findings to tuberculin and histoplasmin sensitivity. They reported that there was a tendency for the infiltrates associated with tuberculin sensitivity (T +, H –) to occur in the upper portions of the lung fields, whereas those associated with histoplasmin sensitivity (T –, H +) were observed in all areas of the lung. This was essentially due to the wide-spread distribution of the nodular infiltrates which comprised a high proportion of the lesions observed among histoplasmin reactors.

Skiagrams of the chest may be entirely normal in some patients despite chronic cough and mucoid expectoration. There is no specific x-ray appearance for pulmonary histoplasmosis. In some there is exaggeration of the peribronchial tissue markings extending outward from the hilar regions, and in others there are areas of infiltration or consolidation. In some there are scattered nodules, either discrete or conglomerate in one or both lungs. In a few skiagrams the findings closely resemble chronic pulmonary tuberculosis with cavitation (Charr, 1953). Furcolow *et al* (1947) reported the roentgenoligic characteristic of persistent pulmonary infiltrates found among persons who were sensitive to histoplasmin but not to tuberculin. The parenchymal lesions observed were of the disseminated type and approximately two-thirds were nodular, sharply circumscribed foci. The remaining were diffuse patchy infiltrations with poorly defined borders which some times developed into nodular lesions. A large proportion of the infiltrates were associated with enlargement of the hilar lymph nodes. Some of the infiltrates disappeared completely, some apparently became fibrotic, but the majority gradually developed calcification (Furcolow, 1949). The lesion tended to calcify slowly and many infiltrates persisted without complete calcification during the 2 years of observation (Furcolow, *et al*, 1947). Sontag

and Allen (1947) also reported a considerable lag between the initial soft tissue lesion in the parenchyma and calcification. There was often a lag of two years or more between a soft tissue lesion and a hilus calcification.

Coccidioidomycosis.—Dickson and Gifford (1938) have reported that the benign infection called valley fever represents initial benign stage of coccidioidomycosis and the granuloma or disseminated form is a more serious late stage of systemic disease. This concept is further supported by Cox and Smith (1939) and Aronson *et al* (1942) by the demonstration of role of the fungus *coccidioides immitis* in the production of pulmonary disease in certain localised regions of California. The initial benign stage of coccidioidomycosis or valley fever is often spoken of at first as a bad cold or "flu". Usually the patient complains of feeling ill, with headache and often with general body aches and pains. Some complain especially of aches and pains about the chest as in pleurisy, and some of indefinite gastro-intestinal disturbance. Frequently, there is a history of mild sore throat which is some times attributed to tonsillitis. Fever may begin at the time of onset of symptoms or four or five days later and the temperature is not higher than 100 to 101°F. There is usually bronchitis some time with unproductive cough, but varying amounts of sputum are common. At times the sputum is produced in considerable amount and may be streaked with blood. After a few days' illness erythema nodosum usually appears on the skin, primarily on the shins. The leucocyte count usually ranges from normal to about 15,000 and the differential count often shows eosinophilia. The highest count which the authors have found is 13 per cent.

The skiagram of the chest shows evidence of enlargement of the hilar glands, branching shadows from the hilar regions of the lungs and more or less scattered areas of shadows indicating involvement of the parenchyma of the lung, in either the upper or lower lobes. Further contribution to the clinical manifestations of coccidioidomycosis is made by Bass (1950) and Bass *et al* (1948). They have found the residual lesions of all types, i.e., nodular densities, cavities, mottled infiltrations, fibrosis, pleural effusion and calcification. These residual pulmonary lesions characteristically show little or no change over a period of observation of from two or five years. The resemblance of the residual pulmonary lesions to those in tuberculosis is striking. All the patients reacted to coccidioidin in 1:100 dilution. However, an occasional case reacted positively to both tuberculin and coccidioidin tests. In areas of endemic coccidioidomycosis Cox and Smith (1939) and Aronson *et al.* (1942) reported that pulmonary calcifications were associated with this infection.

Blastomycosis.—Blastomycosis is another fungus infection of the lungs which can be misdiagnosed as tuberculosis until dissemination of the infection with the formation of subcutaneous abscesses occurs. Primary infection in the lungs does not show any characteristic X-ray findings (Stokes, *et al*, 1950). A number of intradermal tests for blastomycosis have been tried (Daris, 1911; Lowg, 1926; Peck, *et al*, 1940; Stober, 1914). It is reported that at times the intradermal test may fail to become positive and also the previously positive result may become negative in the terminal stages (Smith, 1949).

Material and methods of study

The present report is based on the study of 64 cases of tropical eosinophilia. There were 17 outdoor cases and 47 indoor patients admitted under Dr. R. N. Chaudhuri. In each case a total and differential leucocyte count was done. A skiagram of the chest was taken before performing the cutaneous tests. The patients are being followed-up.

Each patient was given skin tests with tuberculin, histoplasmin, coccidiodin, and blastomycin, administered intracutaneously in 0.1 c.c. doses, each in particular areas of the fore-arms. Separate set of syringes and needles was used for each antigen. The tuberculin used was Koch's old tuberculin 1:1000 and 1:100; the histoplasmin and blastomycin were 1:1000 dilutions; the coccidiodin in a dilution of 1:100. The patient was instructed to report back for skin test interpretations in either 48 or 72 hours whichever was convenient. At that time the diameters of both the erythema and the induration of the reactions were measured. A skin test was considered positive if the induration measured 5 mm or more in diameter, regardless

of the erythema. A doubtful reaction was defined as one showing 1-4 mm of induration. These doubtful reactions were counted as negative in the analysis that follows.

Age and sex distribution.—The patients ranged in age from 7 to 58 years. Their age and sex are given in table 1.

TABLE 1

Age and sex distribution

Age	Male	Female	Total.
0— 5	..	..	..
6—10	3	2	5
11—15	3	..	3
16—20	11	..	11
21—25	10	..	10
Above 25	28	7	35
Total	55	9	64

The majority of the cases were from the poor class with the exception of a few who came from upper or middle class families. Most of them belonged to Calcutta, and only a few came from other provinces. Their occupation is shown in table 2.

TABLE 2

Occupation of patients

Occupation	*Number*	*Occupation*	*Number*
Students	15	Engineer	1
Shop-keepers	7	Advocate	1
Clerks	7	Higher administrative service	1
Peons	7	Painter	1
Housewives	7	Tailor	1
Drivers	5	Cook	1
Labourers	3	Shoe-maker	1
Washermen	2	Unemployed	1
Sweepers	2	Unknown	1
Total	55		9=64

Blood count.—Most of the patients had an absolute eosinophil count between 2,000 to 10,000 per c.mm. The highest white cell count per c. mm. was 52,000 with 88 per cent eosinophils (45,760 absolute eosinophil count per c. mm.)

X-ray findings.—In the majority of cases there was accentuation of broncho-vascular shadows with hilar reaction. In some skiagrams fine or coarse mottling was present. Sixteen cases showed calcification. In none of the cases investigated in this series were tubercle bacilli found in the sputum examined by direct smears.

Results

Of the 64 cases tested with histoplasmin, 3 showed positive reaction. These three patients also reacted to tuberculin. Fifty seven subjects were tested for blastomycosis and 38 for coccidioidomycosis and all gave negative result. Six out of 24 patients did not react to tuberculin.

TABLE 3

Antigen reactions

	Histo-plasmin.	*Tuber-culin.*	*Cocci-dioidin.*	*Blasto-mycin.*
Total No. tested	64	24	38	57
Positive ..	*3	18	0	0
Negative ..	61	6	38	57

*They also showed positive tuberculin reaction.

Efforts were made to isolate histoplasma from the three patients showing a positive reaction. Cultures were made from the sputum and sternal bone marrow. A growth of monilia was obtained from the sputum of one of them. Further biochemical tests showed this fungus to be pathogenic. A skin test was done to find out sensitivity of the patient to monilia. Antigen was prepared by adding one loopfull of fresh culture of monilia to 3 c. c. of sterile normal saline and 0.1 c. c. of this antigen was injected intradermally in the fore-arm of that case. The result obtained was strongly positive. The second case showing positive histoplasmin reaction was found to be suffering from advanced ringworm of the groins and thighs for over two years. The third case who reacted to histoplasmin had sternal bone marrow cultured but was found to be negative for *H. capsulatum*. This case had coarse mottling over the lower zone of the right lung field with calcification of the hilar lymph nodes on the same side. The patient was given aureomycin one capsule 4 times a day for ten days on two occasions. The second skiagram of the chest taken 55 days after the second course of treatment showed disappearence of the coarse mottling and the absolute eosinophil count came down from 12096 to 1230 per c. mm.

The individuals who reacted to one antigen, to combinations of the antigens, and the non-reactors were studied to determine their relationship to pulmonary calcification. The total number of cases showing pulmonary calcification was sixteen and all were tuberculin positive. The patients showing calcification in their lung parenchyma or hilar lymph nodes were all above 19 years of age. Two were females and the rest all males. Out of the three patients showing positive reaction to histoplasmin and tuberculin only one revealed calcification in the hilar lymph nodes.

There was no specific pattern of pulmonary calcification either in the lung parenchyma or hilar lymph nodes in the tuberculin positive cases. The common finding was one or two and in three cases 4 calcified areas of 4-5 mm. in diameter in the lung parenchyma. In two cases a calcified area was present in the hilar lymph nodes. The "Halo" primary focus described as a specific lesion with respect to the diagnosis of histoplasmosis was not found in the present series as also by previous workers (Absher and Cline, 1949; Silverman, 1950; Wahi, 1952).

Discussion

The primary purpose of this paper is to present data on a series of patients suffering from tropical eosinophilia for the purpose of studying the role of fungi in this disease and pulmonary calcifications. Out of the 64 patients tested not even a single case reacted to histoplasmin alone. Three cases were positive to both tuberculin and histoplasmin. One of them had monilia grown from the sputum and the other had dermatomycosis. None of these two had any pulmonary calcification. From these observations one might say that histoplasmin in these two cases probably gave non-specific reactions; that it may well serve as an index of sensitization to various fungi. It has been stated by various workers (Christie and Peterson, 1945; 1946; 1946*a*; Emmons, *et al.*, 1945; Palmer, 1945; Wahi, 1952) that such a situation might exist; that the histoplasmin sensitivity may be indicative of sensitization by some agent having an antigenic complex closely related to that of histoplasmin. The negative reactions suggest that pulmonary fungus infection is not common here. Investigations in Uttar Pradesh, on the role of fungi in pulmonary disease (Wahi, 1952) have led to very similar conclusions. However, one case of disseminated histoplasmosis has been reported recently (Sen, 1953).

Conclusion

Though a small number of patients have been studied it may be concluded on the basis of the above findings that infection with histoplasma, blastomyces and coccidioides has no aetiological relation to tropical eosinophilia.

The follow-up skiagrams of the chest in tropical eosinophilia have not shown healing by calcification.

Tuberculin sensitivity was present in all cases showing pulmonary calcification.

Summary

1. Literature on the symptoms, clinical findings, and skiagrams of the chest in pulmonary mycosis is reviewed.
2. A study of 64 cases, including 7 children with regard to skiagrams of their chests, eosinophil count and the cutaneous sensitivity to histoplasmin, coccidioidin, blastomycin and tuberculin, is presented. No positive reactors were found out of the 57 cases tested for blastomycosis and 38 for coccidioidomycosis. Three cases out of 64 reacted to histoplasmin and one of them showed pulmonary calcification. Eighteen patients including the 3 histoplasmin positive cases were sensitive to tuberculin and six gave negative reaction out of the 24 cases tested. Sixteen of the tuberculin positive group showed pulmonary calcifications.
3. No relation has been found between the fungus infections mentioned above and tropical eosinophilia.

Acknowledgment

The writers are indebted to Dr. R. N. Chaudhuri for advice and helpful criticism in the preparation of this paper. They are also grateful to Dr. P. N. Wahi for supplying the antigens, to Dr. P. K. Sen for providing the tuberculin and to Dr. A. Chakravarty for the fungus culture.

REFERENCES

ABSHER, W. K., and CLINE, F. (1949). *Amer. Rev. Tuberc.*, **59**, 643.

ARONSON, J. D., SAYLOR, R. M., and PARR, E. I. (1942). *Arch. Path.*, **34**, 31.

BASS, H. E. (1950) .. 1041. *J. Amer. Med. Assoc.*, **143**,

BASS, H. E., SCHOMER, A., and BERKE, R. (1948). *Arch. Intern. Med.*, **82**, 519.

BEADENKOFF, W. G., LOOSLI, C. G., LACK, H., RICE, L. A., and SLATTERY, R. V. (1949). *Pub. Health Rep.*, **64**, 17.

CHARR, R. (1953) .. *Amer. Rev. Tuberc.*, **67**, 370

CHRISTIE, A., and PETERSON, J. C. (1945). *Amer. J. Pub. Health*, **35**, 1131.

Idem (1946). *J. Pediat.*, **29**, 417.

Idem (1946*a*). *J. Amer. Med. Assoc.*, **131**, 658.

COX, A. J., and SMITH, C. E. (1939). *Arch. Path.*, **27**, 717.

DAVIS, B. F. (1911) .. *J. Infect. Dis.*, **8**, 190.

DICKSON, E. C., and GIFFORD, M. A. (1938). *Arch. Intern. Med.*, **62**, 853.

EDWARDS, L. B., LEWIS, I., and PALMER, C. E. (1948). *Pub. Health Rep.*, **63**, 1569.

EMMONS, C. W., OLSEN, B. J., and ELDRIDGE, W. W. (1945). *Ibid.*, **60**, 1383.

FURCOLOW, M. L. (1949). *Ibid.*, **64**, 1363.

FURCOLOW, M. L., MANTZ, H. L., and LEWIS, I. (1947). *Ibid.*, **62**, 1711.

LONG, E. R. (1926) .. *J. Amer. Med. Assoc.*, **87**, 1441.

PALMER, C. E. (1945) .. *Pub. Health Rep.*, **60**, 513.

PARSONS, R. J., and ZARAFONETIS, C. J. D. (1945). *Arch. Intern. Med.*, **75**, 1.

PECK, R. L., MARTIN, D. S., and HAUSER, C. R. (1940). *J. Immunol.*, **38**, 449.

SEN, S. (1953) .. *Bull. School Trop. Med.*, **1**, 12.

SILVERMAN, F. N. (1950). *Amer. J. Roentgenol. and and Radium Therapy*, **64**, 747.

SMITH, D. J. (1949) .. *J. Amer. Med. Assoc.*, **141**. 1223.

SONTAG, L. W., and ALLEN, J. E. (1947). *J. Pediat.*, **30**, 657.

STOBER, A. M. (1914) .. *Arch. Intern. Med.*, **13**, 509.

STOKES, J. H., BEERMAN, H., and INGRAHAM, N. R. (1950). *Amer. J. Med. Sci.*, **220**, 435

WAHI, P. N. (1952) .. *J. Res.*, **1**, 205.

BIBLIOGRAPHY

DAVIS, H. V., and NEFF, F. C. (1946). *Amer. J. Dis. Child.*, **71**, 171.

FEREBEE, S. H., and FURCOLOW, M. L. (1947). *Pub. Health Rep.*, **62**, 631.

GODDARD, J. C., EDWARDS, L. B., and PALMER, C. E. (1949). *Ibid.*, **64**, 820.

GROOVER, M. F., CLEVE, E. A., BORNSTEIN, S., RICE, A. G., GALLOWAY, A. F., and MACALUSO, C. P. (1947). *Arch. Intern. Med.*, **80**, 496.

HIGH, R. H., ZWERLING, H. B., and FURCOLOW, M. L. (1947). *Pub. Health Rep.*, **62**, 20.

LUMSDEN, L. L., and DEARING, W. P. (1940). *Amer. J. Pub. Health*, **30**, 219.

OLSEN, B. J., WRIGHT, W. H., and LOLAN, M. O. (1941). *Pub. Health Rep.*, **56**, 2105.

PALMER, C. E. (1946) .. *Ibid.*, **61**, 475.

SMITH, C. E., BEARD, R. R., ROSENBERGER, H. G., and WHITING, E. G. (1946). *J. Amer. Med. Assoc.*, **132**, 833.

SMITH, C. E., SAITO, M. T., BEARD, R. R., ROSENBERGER, H. G., and WHITING, E. G. (1949). *Amer. J. Pub. Health*, **39**, 722.

SEROLOGICAL CLASSIFICATION OF C. DIPHTHERIÆ IN CALCUTTA

By B. P. TRIBEDI, M.B. (Cal.), D.Bact. (Lond.), F.N.I.

and

D. BARUA, M.B. (Cal.)

Department of Pathology and Bacteriology, Medical College, Calcutta

CLASSIFICATION of *C. diphtheriæ* into gravis, mitis and intermedius groups according to their cultural and other biological character is well known (Anderson *et al.* 1931). A perusal of literature shows that serological classification of *C. diphtheriæ* though known since 1896 has been placed on a sound footing only recently by Hewitt (1947a) in England and Ferris (1950) in Australia after a thorough and comprehensive survey under the recognised cultural groups.

Soman and Neil (1949), Das and Ghosal (1951) and Tribedi, Sarkar and Barua (1952) reported that unlike Western countries, *C. diphtheriæ mitis* is the more prevalent organism in India. The latter two works also drew attention to the more virulent nature of local *C. diphtheriæ mitis* organisms on clinical and post-mortem evidenecs.

We took up this problem to determine whether the local strains are homogenous or can be serologically classifical into different types and if so, how far the types corroborate or differ from the findings in other countries.