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Abstract

In our country it has been believed that there is no histoplasmosis here in Japan. However, from the above clinical signs, radiological characteristics, laboratory tests, pathological and mycological examinations, and experimental findings, we believe this is the first case of histoplasmosis in Japan.

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A CASE OF HISTOPLASMOSIS
REPORT 1. CLINICAL, MYCOLOGICAL AND
PATHOLOGICAL OBSERVATIONS

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With a keen interest the authors have been studying on the various kind of fungus diseases such as candidiasis, cryptococcosis and aspergillosis etc. for the past several years. Last year (1956) the authors experienced an autopsy case with extremely characteristic findings, the like of which has never been reported in Japan. Thus far in our country it has been generally believed that there is no histoplasmosis here in Japan. However, we obtained many evidences to support the assumption of diagnosing this case as "a histoplasmosis combined with tuberculosis" as a result of the clinical, mycological and pathological examinations.

The authors present the whole perspective of this case in the following.

CASE REPORT

The patient was 17 years old school girl, and was admitted to our hospital on May 7, 1956, with the complaints of slight fever. Her father died of a pulmonary tuberculosis-like disease at the age of 44. She was born in Okayama city but moved to the present residence when she was 7 years old. Her dwelling was a small hut, which was in a valley of the Asahi river branch, and had been used as a storage cottage of fire woods for a long time before she moved. The patient had been treated with streptomycin, PAS and other medicaments for 5 years under the diagnosis of pulmonary tuberculosis. No improvement was noted after these treatments.

Physical examination at the admission revealed the following positive findings: The temperature was 37°C; the lungs appeared normal on percussion and auscultation; and diaphragmatic movements were good. Examination of the heart showed no abnormalities. The abdomen was soft and without palpable masses or tenderness. The liver was palpable 2 centimeters below the right costal margin and was described as smooth and non-tender. The spleen was not palpable.

The skin-tests of tuberculin and histoplasmin were both positive and the laboratory tests (see below) showed anemia, leucopenia and lymphocytosis, but serum calcium was in the normal level. On admission her X-ray revealed some peculiarities differing from the usual X-ray signs of pulmonary tuberculosis or sillicosis; i. e. there were many miliary calcified shadows scattered in all of her lung fields, especially densely in the lower fields, and some of them were gathering together and became even as large as an Indian bean (Fig. 2).

As compared with her X-ray film having been taken 4 years previously, the latest one showed that the infiltration had subsided and the calcification became more remarkable (Figs. 3, 4).

The patient was given streptomycin, trichomycin and symptomatic treatments for her emaciation. Despite these measures in our hospital, she died on September 13, 1956, of the failure of the heart caused by left spontaneous pneumothorax occurring in the first part of August (Fig. 5).

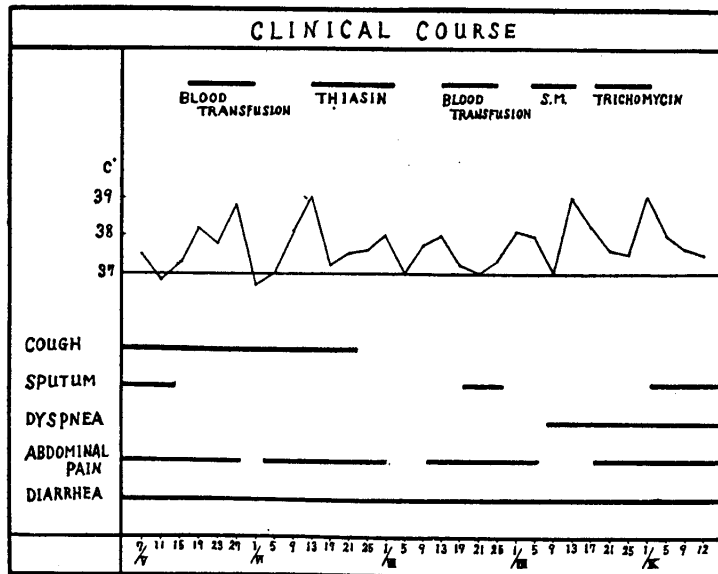


Fig. 1

Physical finding on blood and others are as follows ;

| | | |
|--|------------------------------|---------------------|
| Red blood cell count | 2.90 million per cmm. | |
| Hemoglobin | 7.8gm. per 100 cc. | |
| White blood cell count | 4.800 per cmm. | |
| Classification : | Polymorphonuclear leucocytes | 55.0 % |
| | Lymphocytes | 40.6 % |
| | Monocytes | 4.0 % |
| | Eosinophils | 0.4 % |
| Total serum protein | 4.7 g. | per 100 cc. |
| Serum calcium | 10.2 mg. | per 100 cc. |
| Cultures for acid-fast bacillus from sputum | | negative |
| Cultures for <i>Histoplasma capsulatum</i> from sputum | | negative |
| Tuberculin test | (\times 2000) | positive |
| Histoplasmin test | (\times 100) | positive |
| | | (5 \times 5 mm.) |

Pathological Findings

Patho-anatomical diagnosis : Histoplasmosis combined with tuberculosis. The main patho-anatomical findings were : 1) Histoplasmosis of the lung and hilar lymphnodes (calcification, fibrosis and catarrhal inflammation). 2) Pulmonary tuberculosis (productive, exudative and cavernous). 3) Spontaneous pneumothorax (left). 4) Ulcers of intestines and subserosal caseous nodules. 5) Diffuse fatty degeneration and miliary tuberculosis of the liver. 6) Miliary tuberculosis of the spleen. 7) Caseous tuberculosis and histoplasmosis of mesenteric lymphnodes. 8) Fibrous tuberculosis of the tonsil. 9) Ulcers and tuberculosis of the larynx. 10) Degeneration of kidney parenchyma.

Autopsy findings : Highly emaciated female with the skin anemic and dry. The right pleural cavity as a whole showed adhesion, while the left cavity is very wide due to the marked contraction of the left lung owing to the spontaneous pneumothorax. About 700 ml. of quite cloudy yellowish fluid was found in the peritoneal cavity. There was no adhesion of the intestine. Many light yellowish, white spots of a millet or a rice grain in size are scattered on the surface of intestinal serosa.

Lungs : The left lung weighs 180 g., showing the atelectasis and shrinkage. Numerous grayish white nodules as big as a millet or a broad bean of somewhat oval, clover-like, or irregular shape are scattered all over the cut surface of the lung. The majority of them show the distinct calcification, presenting on the whole chalky or bony appearance or an appearance of grains of sand. The calcification originates from the

periphery of the necrotic foci, forming several layers encircling the central part. These lesions are distinctly defined by thin capsule from the adjacent parenchyma. The right lung weighs 390 g. and the parenchyma is emphysematous in general. On the cut surface, besides numerous small nodules scattered all over as observed in the left lung, three cavities containing red brownish muddy substance are found in the apex and lower lobe, which are as large as the tip of the index finger and of an irregular shape. And several small calcified particles discharged are seen in the cavities. In the lower lobe, caseous lesions, partly calcified and of an egg or of the tip of the index finger in size can be recognized (Fig. 6).

Hilar and paratracheal lymphnodes: Swellings in sizes ranging from a soybean to the tip of index finger can be seen; and many grayish white nodules on the cut surface to be shown and a portion of them are calcified.

Spleen: The spleen is quite soft and follicles are indistinct, and many pulpy bulges can be seen on the cut surface. The tuberculous lesions are invisible to the naked eye.

Intestines: Many white nodules as big as a millet or a grain of rice are seen scattered over the mucous membranes of the duodenum and the jejunum. Many circular ulcers undermined and whose edges overhanging are recognized extensively from the ileum to the caecum (Fig. 7).

Liver: Yellowish tone is quite marked; and a slight greenish coloration can be seen around Glisson's sheath. Macroscopically the tuberculous nodules can not be noticed.

Larynx: Light ulcers from the left side of the epiglottis to the left false vocal cord, and severe ulcer formation on the true vocal cord can be recognized.

Tonsils: The tonsils are slightly swollen.

Mesenteric lymphnodes: Almost all lymphnodes are swollen to the size of a soybean or of the tip of index finger and the majority are hardened like bone. On the cut surface those that are still soft have become caseous while the hard ones are calcified.

No pronounced change can be observed in other organs.

Histopathological findings:

Lung: Non-calcified lesions and the wall of cavity are composed of nodular granulation tissues of varying sizes resembling tuberculous changes, consisted of epithelioid cells, LANGHANS's giant cells and lymphocytes which make a wall of these focuses; and many acid-fast bacilli are recognized (Fig. 8). Then, peculiar calcified lesions are occasionally

enveloped by a few epithelioid cells, LANGHANS's giant cells, or fibrous tissue ; the calcification is especially marked in the periphery of necrotic areas, forming several layers like the annual layer of tree (Fig. 9). In these necrotic areas numerous yeastlike fungi can be recognized by H. E. stain, periodic acid SCHIFF reaction, or GRAM's stain. The larger ones of these fungi measure 10—15 μ in diameter, while the small ones about 3 μ ; and the majority of them are vacuoles and some are greatly swollen or show indentations. On the other hand some organisms have a deep stained nucleus-like body within cytoplasm (Figs. 10, 11, 12, 13). Besides these fungi, a few acid-fast bacilli can also be seen. The former large fungi well coincide with the large form of *Histoplasma capsulatum* while the latter bacilli possibly with the *Mycobacterium tuberculosis*.

Spleen : Follicles are markedly atrophied and small tubercles are scattered here and there in the parenchyma. No acid-fast bacillus nor *Histoplasma capsulatum* can be found in these lesions.

Liver : Parenchymal cells show marked fatty degeneration, and occasional plugs of bile and many small tubercles can be recognized in the acinus. In these lesions only acid-fast bacilli are proven.

Intestines : Many undermined ulcers are remarkable, and the base of many of these ulcers reaches the tunica muscularis. The surface of ulcers are covered by necrotic substances ; and the granulative formation, chiefly consisted of epithelioid cells and surrounding the ulcers, is not so marked (Fig. 14). Although small tubercles can be recognized in and under mucous membrane as well as in the subserosa, they all present a weak tissue reaction. Acid-fast bacilli have been proven to exist in these nodules.

Mesenteric lymphnodes : Caseous lesions are discernible extensively in lymphnodes but not calcified, which are merely surrounded by very thin layer composed of LANGHANS's giant cells and epithelioid cells. Acid-fast bacilli are found positive to stains. In addition calcified lesions presented a picture similar to that in the lung ; and many yeast-like fungi resembling large form of *Histoplasma capsulatum* are detected.

Larynx : Many caseous lesions can be seen in the submucosa ; and the formation of deep seated ulcers are found at the apex of the vocal cord. From the edge to the base of these ulcers there are tuberculous nodules as mentioned previously. Acid-fast bacilli have been verified to exist around this lesion.

Tonsils : Many small nodules consisted of epithelioid cells are scattered and a few LANGHANS's giant cells are mixed as well.

No marked changes, however, can be seen in other viscera.

Mycological Findings

Tissue materials of several organs were submitted to the cultures. Materials from the lungs cultured on SABOURAUD's glucose agar at room temperature, yielded a few colonies markedly resembling themselves of the typical *Histoplasma capsulatum*. Cultures of material from spleen, lymphnodes and liver were negative.

In culture, the fungi were dimorphic, forming either a yeast-like or a mycelial form depending upon conditions of culture. On brain-heart infusion glucose blood agar at 37°C the organism grew as a dull white, wrinkled colony consisting of oval yeast-like bodies 1.0 μ to 5.0 μ in diameter (Figs. 15, 16). On SABOURAUD's glucose agar at 25°C the fungus grew slowly, producing a white aerial mycelium (Fig. 17). The mycelial form consisted of mycelium, many conidia and a few chlamydo spores (Fig. 18). These conidias were small and globular, 2.0 μ to 3.0 μ in diameter, and chlamydo spores were round, 7.0 μ to 8.0 μ in diameter. This mycelial form turned gradually to the yeast form on brain-heart infusion glucose blood agar, while the yeast form reverted to the mycelial form on SABOURAUD's glucose agar.

On the other hand, no culturable tubercule bacillus could be demonstrated from the tissue materials.

EXPERIMENTAL FINDINGS

For pathogenicity tests, four routes of inoculation, intraperitoneal, intracerebral, intravenous and intrarespiratory, were employed. Using mycelial form, as it was difficult to count the quantity exactly and to make it homogenous solution, therefore, the colony which was cultured on SABOURAUD's glucose agar for about two weeks at the room temperature was inoculated directly intraperitoneally in the mice with aseptic treatment. The animals were sacrificed after one week, and out of 5 cases, 2 cases were found with intraperitoneal abscess, and by the culture from this pus fungus was recovered in both cases. Then the yeast form inocula was prepared from the colonies grown on brain-heart infusion glucose blood agar for 2—4 weeks at 37 C. The growth was washed from the slants and diluted with sterile normal saline solution to yield a density which contained approximately 100—200 thousands spores per 1 mml. One half ml. of this inocula was injected intraperitoneally, and 0.02 ml. intracerebrally respectively. Sacrificing them after one week, subphrenical abscess, white patches on the liver, spleen and serosa of the intestines, and swelling of mesenteric and sub-

mandibular lymphnodes were macroscopically found in most cases. And the animals inoculated 12 hours after X-ray radiation of 300 r died one after another ; i. e. of 5 experimental animals two died after 1 week, two after 10 days, and the remaining one after 12 days. In these animals we could find the Gram positive, periodic acid Schiff stained spores in their livers, spleens and lymphnodes at autopsy. By the intravenous injection using 0.1-0.2ml. of yeast-form, few clinical findings were found, but there were revealed pathological changes similar to the histoplasmosis in all the cases 1—2 weeks later, and many yeast-like fungi, positive to GRAM'S and periodic acid SCHIFF stains, resembling to typical *Histoplasma capsulatum* were found in their livers, mesenterial lymphnodes and a little in the spleens (Figs. 19, 20).

COMMENT

Histoplasmosis is a fungus disease caused by the generalized infection of the *Histoplasma capsulatum*. The knowledge concerning this disease has made a rapid progress in the recent ten years, especially in the United States of America ; and many cases of histoplasmosis have been reported in America since 1938. Formerly histoplasmosis had been thought to be a fatal disease but succeeding findings proved that it is not so, i. e. 95 per cent of cases of primary histoplasmosis are asymptomatic. Only less than one per cent or more likely less than 0.1 per cent of primary infections fall into the progressive and fatal type of this disease.¹

Skin tests by intracutaneous injection of 0.1 ml. of a 1 : 100 dilution of standardized histoplasmin become positive after a few weeks following infection. The reaction is read in 24 and 48 hours, and it must be shown at least 0.5 cm. in diameter of induration to be considered positive.¹ In Japan it has been generally believed that histoplasmosis has never been existing here. But already in 1953, ITO et al.² reported 2.9 % histoplasmin positive reactors who handled the sand imported from U. S. A. in Nagoya district. And TANAKA³ also reported 1.9% histoplasmin positive reactors of 838 employers of occupational armed forces in Nagoya city and 6 % positive reactors of 471 N-pottery factory workers in Nagoya. Especially, out of 80 persons who worked at the clay-cracking field, 16 persons (20%) were positive to histoplasmin sensitivity. Last year, we reported the data of Okayama Prefecture⁴ ; i. e. 96 of 1744 persons were positive (5.5 %). Among them the data of Mitsuishi district where clay industry is well developed were remarkably high (8.43%). HOSHIZIMA⁵ has reported the data of Tohoku district, resulting the positive reactors of 0.83—12.1

per cent. HOSHIZIMA⁵ is now investigating such cases all over the country. From these findings, a considerably high percentage of positive reactors, the like of which had never been thought in Japan, has been proven; and we must consider the possibility of the existence of latent infection. As regards the so-called tuberculoma, ZIMMERMAN⁶ mentions that of 35 pulmonary granuloma tubercle bacilli were detected by ZIEHL-NEELENSTAIN in six cases and *Histoplasma capsulatum* in 19 cases by special stains for fungi.

Histoplasmosis has been generally considered as an exogenic infection and the infection is primarily a pulmonary infection caused by inhalation of this fungus *H. capsulatum*. ISRAEL⁷ classified histoplasmosis into the following three groups; 1) Healed primary form, 2) chronic disseminated form, 3) acute disseminated form. Healed primary form recovers easily and STRAUB⁸ pointed out that this form often resembles to primary affected lesion of pulmonary tuberculosis on the X-ray. And this third form, the most fatal type is very rare and it occurs only when unfavorable conditions about somatic and environmental factors in individual are combined unbeneficially to the patient.

As to the somatic factor, ISRAEL et al⁷ stated that acute disseminated form can easily occur in emaciated persons with tuberculosis or malignant neoplasm. As to the environmental factor, PARSONS and ZARAFO-NETIS⁹ reported that the distribution of this disease are endemic at such valley districts as Tennessee and Missouri. This disease is often infected from firewoods or pigeon-droppings.^{10,11} The transmittance from man to man is not certain. Humidity is important for the growth of this fungus. MENGES¹² described that high humidity is necessary for the growth of this fungus in his experimental studies. There is a correlation between the prevalence of the fungus and the presence of a large river running through low country as mentioned by MOCHI¹³. The authors must point out the several factors on the environment of our patient. She had dwelt before death in the recess of a mountain along a small valley. This region is sparsely populated and frequently foggy. Her dwelling had been used for a long time as a storage of old firewoods before she went there to dwell. Although Emmons^{14,15,16} isolated *Histoplasma capsulatum* from soil, dog, mouse, cat, skunk and opossum in Virginia, in the neighborhood of this house, *Histoplasma capsulatum* could not be cultured from the soils.

Histoplasmosis is characterized clinically by anemia, leucopenia, cough, fever and hepatosplenomegaly in general, but these findings are non-specific to this disease. The roentgenogram of histoplasmosis shows

rather specific findings and the lesions have been divided by FURCOLOW¹⁷ into the following three types: the disseminated infiltrations, pneumonic infiltration and nodular foci. The large number, the uniformity of size and distribution, indicate this disease in 90 per cent of the cases. The roentgenogram of our case belongs to the disseminated form. Comparing with the roentgenogram of our case which was taken 4 years ago, the latest one showed that the calcification has become more remarkable.

In 1906, DARLING¹⁸ gave the name *Histoplasma capsulatum* to intracellular "protozoa" in the tissues obtained at autopsy of a patient in Panama. And he described the morphology of the organisms in detail. DE MONBREUM¹⁹ in 1934 succeeded in culturing from another case and proved that the organism was a fungus. Cultural characteristics were described by CONANT²⁰ in 1941. It was proven that this organism could occur in two forms: a yeast-like form found in infected tissues and a mycelial form which is presumed to be the form found in nature. As another characteristics of this fungus chlamyospore and conidia were found on mycelial form. We could also prove these characteristics on the fungus cultivated from our present case.

Pathologically remarkable findings are many calcified nodules forming in both lungs. Non-calcified lesions resemble microscopically to tubercles as in miliary tuberculosis, but on the whole it is strange that the borders of them are demarcated distinctly from the surrounding parenchyma, in which the inflammatory figure, usually found around a tuberculous nodule, is almost absent. The nodules show caseations in their centers and many of them concurrently remarkable calcifications, forming several layers from the periphery.

It is significant that many yeast-like fungi are demonstrated in these calcified lesions; the smaller ones measure 3—5 μ in diameter, while larger ones 15—20 μ in diameter, and both are positive to the periodic acid SCHIFF reaction and GRAM's stain. We can find the same calcified lesions in hilar and mesenteric lymphnodes.

On the other hand, we can find an acid-fast bacillus resembling tubercule bacillus in the caseous lesions, a small cavity-wall of the right lung and the undermined intestinal ulcers.

Histologically we proved a fungus and an acid-fast bacillus in the same tissue. Then we must determine which of the two, the fungus or the acid-fast bacillus, is more responsible to these characteristic changes.

In the present case we found histologically the acid-fast bacillus in nodules, caseous lesions and small cavity of the lungs, undermined ulcers

of larynx and small intestine, and caseous lesions of lymphnodes. But strange to say, in spite of the repeated cultures from sputum ante mortem and the carefull cultures from the materials of the tissues post mortem, tubercule bacilli were all negative. According to ZIMMERMAN,⁶ however, it is said that the cultures for *Mycobacterium tuberculosis* (and/or fungi) are negative in more than three fourths of the cases of so-called tuberculoma. Moreover, he claims that the differential histopathologic stains are even more valuable than culture studies. Therefore, the negative result of cultivation of tubercle bacillus from our present case seems to fall in line with his opinion. Nevertheless, the characteristic phenomenon such as the peculiar calcification in numerous lesions can not be explained merely as tuberculosis. Hence we may assume that an intimate relation exists between the fungi and such calcification. As mentioned by ANDERSON²¹, and Conant et al¹., in the case of a patient showing such a remarkable calcification in the lungs as in our case histoplasmosis must be suspected first of all.

HANSMANN²², SCHULZ²³ and ANDERSON²¹ described that *Histoplasma capsulatum* is often found to be phagocyted in the cells of the reticuloendothelial system. In the present case, however, the phagocytic picture in reticulum cells of these organs as described in many literatures could not be observd. Therefore, at the beginning we could not determine histologically whether the diagnosis of this case was really histoplasmosis or not.

However, later we arrived at a conclusion that this case should be adjudged as a histoplasmosis on the basis of exhaustive examinations and bibliographic consideration.

The statistical report of the SCHULZ²³ shows the frequency of such lesions in organs to be : 63 per cent in lungs, 59 per cent in liver, 59 per cent in lymphnodes, 58 per cent in spleen and 31 per cent in bone marrow. And ISRAEL et al⁷. described that most lesions of chronic disseminated histoplasmosis was often found in the lungs, larynx and lymphnodes, and that sometimes it was found to be even localised in the lungs or genitalia.

Moreover, the authors have succeeded in culturing a fungus from the tissues of the lungs of our patient, which resembled much to *Histoplasma capsulatum*. Subsequently, by inoculating this fungus intracerebrally, intravenously, intraperitoneally or by inhaling respiratorily in mice, we detected the phagocyted figures resembling the findings of the small form of *Histoplasma capsulatum* in the cells of the reticuloendothelial system.

The yeast-like fungus appeared in the necrobiotic foci and the fungus

of the large form in our autopsy case is morphologically the same, and yet this fungus resembles much to the large form of *Histoplasma capsulatum*.

CAMPBELL and SASLAW²⁴ stated in experimental studies that *Histoplasma capsulatum* in the yeast-phase when suspended in 5% gastric mucin and administered intraperitoneally into white mice caused a high percentage of fatal infections and that equivalent challenge doses of the organism suspended in saline rarely caused death. HOWELL and KIPKIE^{25, 26, 27} also reported that mice are relatively resistant to intravenous injection of the yeast-phase of *Histoplasma capsulatum* and that intraperitoneal injection of organisms suspended in 5% mucin increased the death rate. In a series of our experiments using the agent suspension with saline solution we found that this fungus has pathogenicity to mice and the intracerebral injection was far superior to any other routes employed and that the mortality rate of mice increased by X-ray radiation of 300r before the fungus inoculation to the mice. Experiment using mucin is still being conducted. Other findings of our experiments with animals seem to bear many similarities to the experimental studies of *Histoplasma capsulatum* by KIPKIE and HOWELL^{25, 26, 27} and we will make detailed report on them in near future. By culturing the necrotic tissues of the infected mice on SABOURUD's glucose agar and brain-heart infusion glucose blood agar, we succeeded in proving mycelial or yeast form on each medium. The variety of the sizes from 3 μ to 15 μ can be seen in this patient's tissues and inoculated animal's tissues, and this variety has already been confirmed as *Histoplasma capsulatum* by MOORE et al²⁸, MOORE and LAYTON²⁹ reported that the large form of this fungus is usually found in the old necrotic foci, the calcified lesions and the granulative tissues. These findings coincide with our findings.

The similarity of the large form of *Histoplasma capsulatum* to *Blastomyces* has often been pointed out as an important factor in the differential diagnosis of these two diseases by MOORE²⁸, TUTTLE LICHTWARDT and ALTSHULER³⁰, but from the cultural findings and peculiar pulmonary calcifications in our case, we can exclude *Blastomyces* for the time being.

In Japan, it was thought that this disease has never been in existence. But our finding shows that histoplasmosis really exists in Japan, and moreover, various fungus diseases including histoplasmosis must be sought after throughout the country.

SUMMARY

In our country it has been believed that there is no histoplasmosis here in Japan. However, from the above clinical signs, radiological characteristics, laboratory tests, pathological and mycological examinations, and experimental findings, we believe this is the first case of histoplasmosis in Japan.

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LEGEND OF PLATES

Plate 1.

Fig. 2. X-ray taken November 7, 1952: Infiltrative lesions were scattered in all lung fields.

Fig. 3. X-ray film taken April 9, 1954: The calcification became more remarkable.

Fig. 4. X-ray film taken Juli 2, 1956: The infiltration had subsided and the calcification became more and more remarkable.

Fig. 5. X-ray film taken September 4, 1956, shows the left spontaneous pneumothorax.

Plate 2.

Fig. 6. The cross section of the lungs showing many calcified lesions (white nodule) and cavities and confluent nodular masses in right lower lobe.

Fig. 7. The sections of opened ileum (upper) and cecum (lower). Numerous undermined ulcers are observed.

Fig. 8. Section of the lung showing many acid-fast bacilli in the granulation tissue. Ziehl's acid-fast stain. $\times 1000$.

Fig. 9. Section of the lung showing peculiar calcified lesion, capsulated by a thin fibrous wall. The calcification originates from the periphery of the necrotic focus. H. E. stain. $\times 100$.

Fig. 10. Section of the calcified nodule in the lung showing many large form of *H. capsulatum* scattered in the necrotic masses. Goodpasture's method of Gram's stain. $\times 1000$.

Plate 3.

Fig. 11. Same focus as in Fig. 10. Large form of organisms are phagocyted in giant cell in surrounding tissue (center). One shows an indentation (right lower). Goodpasture's method of Gram's stain. $\times 1000$.

Fig. 12. The large form of organism in the periphery of calcified focus of the lung. Goodpasture's method of Gram's stain. $\times 1000$.

Fig. 13. Section of the lung showing budding organisms in the granulation tissue, which surrounds calcified masses. Periodic acid Schiff stain. $\times 1000$.

Fig. 14. Section of ileum showing deep undermined ulcer. Its base reaching the tunica muscularis. H. E. stain. $\times 40$.

Fig. 15. *H. capsulatum*. Yeast form on brain-heart infusion glucose blood agar.

Plate 4.

Fig. 16. *H. capsulatum*. Yeast form on brain-heart infusion glucose blood agar, 21 days, at 37°C. $\times 100$.

Fig. 17. *H. capsulatum*. Mycelial form on Sabourau's glucose agar.

Fig. 18. *H. capsulatum*. Mycelial form on Sabourau's glucose agar, 21 days, at room temperature. $\times 400$.

Fig. 19. Section of liver obtained from the No. 2 mouse, killed after one week from the intravenous inoculation. Micrograph showing a fungus-laden giant cell in the sinusoid. Periodic acid Schiff stain. $\times 1000$.

Fig. 20. Section of mesenterial lymphnode, the same mouse in Fig. 19, showing many organisms similar to the large form found in the autopsy case (cf. Fig. 10). Fungi are scattered in the necrotic masses. Periodic acid Schiff stain. $\times 1000$.

Plate 1



Fig. 2

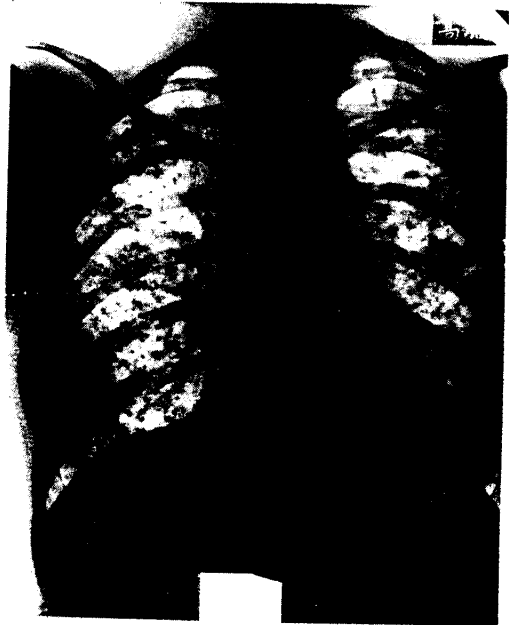


Fig. 3

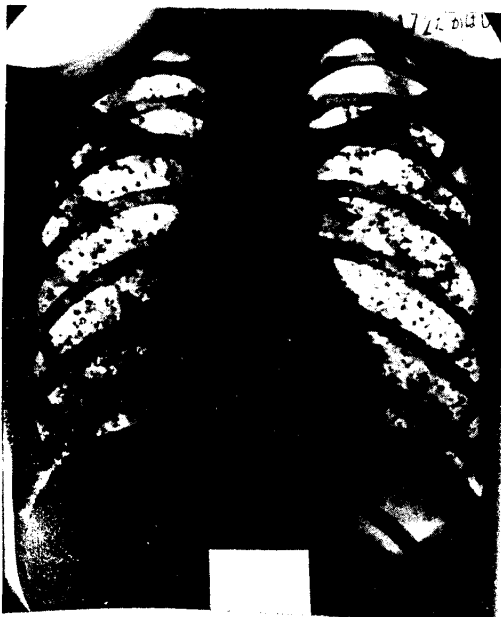


Fig. 4

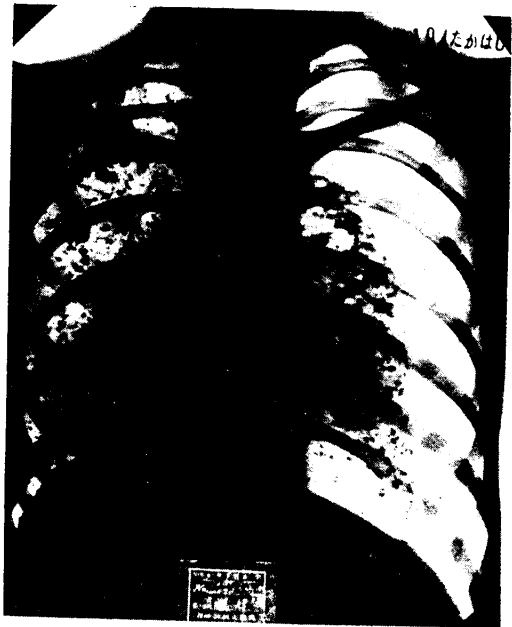


Fig. 5

Plate 2



Fig. 6

6



Fig. 7

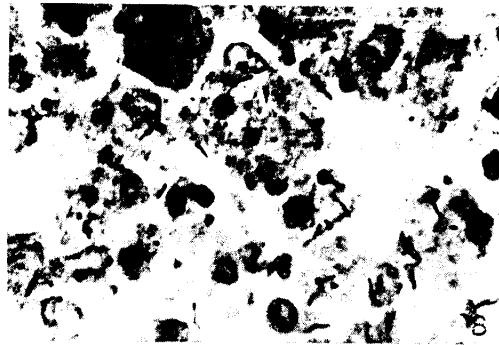


Fig. 8

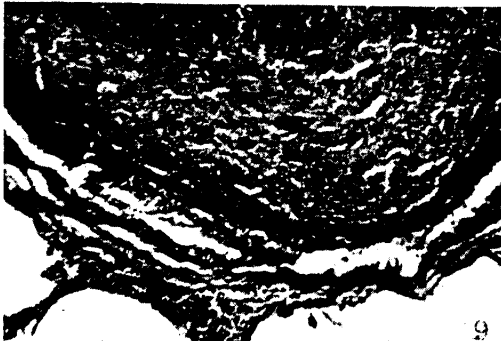


Fig. 9

9

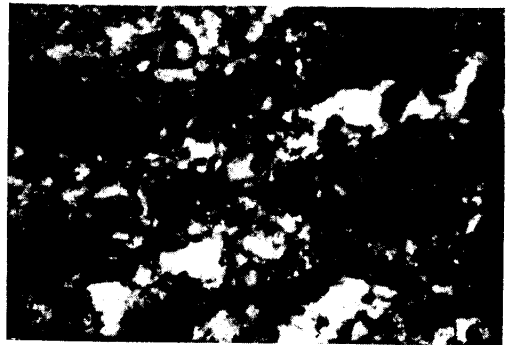


Fig. 10

Plate 3

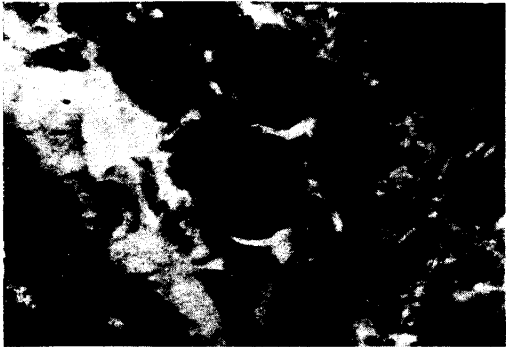


Fig. 11

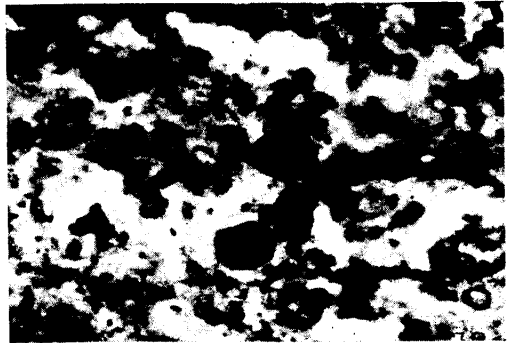


Fig. 12

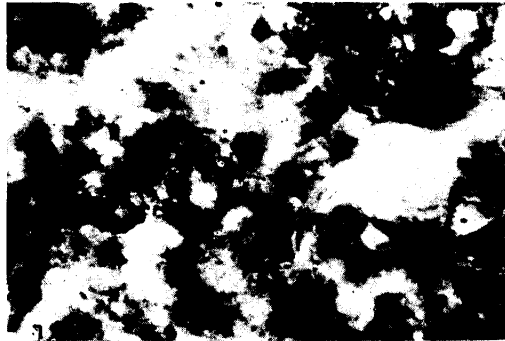


Fig. 13



Fig. 14

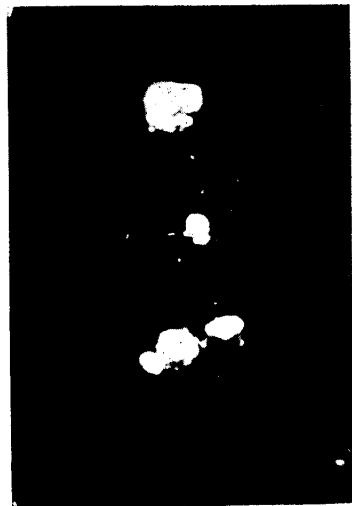


Fig. 15

Plate 4

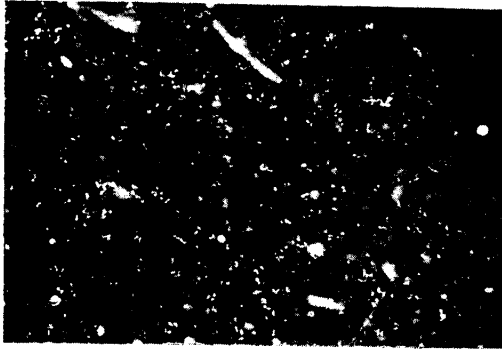


Fig. 16

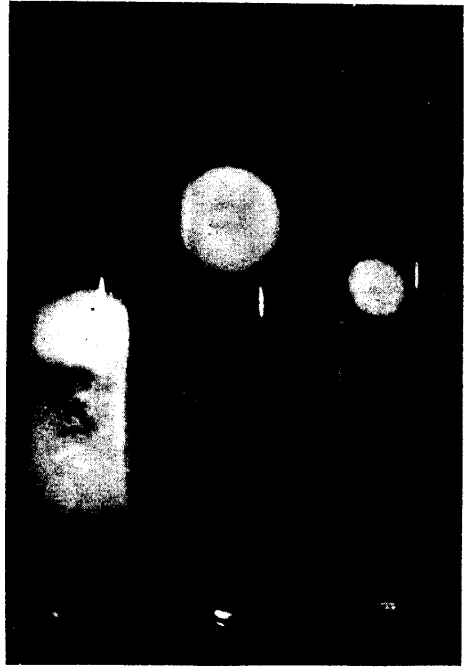


Fig. 17



Fig. 18



Fig. 19

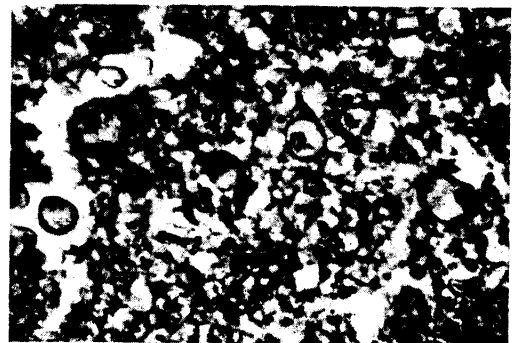


Fig. 20