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Histoplasmosis, (U)

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DEPARTMENT OF THE ARMY
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HISTOPLASMOSIS

(Following is the translation of an article by Ludwik Debrowski, Dept. of Mycology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, the Polish Academy of Sciences, in the Polish-language journal Postawy Higieny i Medycyny Przyszalnej (Advances in Hygiene and Experimental Medicine), Wroclaw, Vol. 16, 1962, pp. 319-334.)

Necotic diseases, and especially organic mycoses, are beginning to attract, more frequently, the attention of the medical world. There are at least three important reasons which explain this fact. First, the classical infections caused by bacteria, spirilla, or Rickettsia, have been already sufficiently identified as to epidemiology as well as therapy. Secondly, in the era of common application of chemotherapeutics and antibiotics, arises the question of frequent complications of diseases which have bacterial etiology, as well as other diseases, through pathogenetic fungi. Eventually it became known that the role of fungi - of potential pathogenetic factors commonly found in nature - has been, until now, underestimated. A good example illustrating the latter is histoplasmosis. At first it was considered to be a rare disease. Today, it proves to be an epidemiological disease affecting millions of people.

Histoplasma capsulatum was discovered by Darling (20) in Panama, in 1906, in the course of a study of leishmaniosis. Darling first included Histoplasma capsulatum into protozoa, then he began to agree with the opinion of Dr. Rocha Lima (21) who, comparing Darling’s cases with the kala-azar disease and the inflammation of lymphatic vessels in isolated animals, ascertained, that Histoplasma capsulatum is similar to Cryptococcus Foagiuis, i.e. to the etiological factor, the above mentioned disease of salamps, and belongs to fungi. This was finally explained by examinations of Dr. Lebreux (22), as well as Fournier and Schenk (43) who, in the case of human histoplasmosis, have isolated Histoplasma capsulatum on an artificial medium and confirmed its belonging to imperfect fungi. From that moment cases of histoplasmosis were more frequently observed, thus confirming the identification through isolation of micro-organisms. Nevertheless, it was
presumed, that the infections, caused by Histoplasma Capsulatum, are unusually rare and do not have significant epidemiological meaning. A break-through came with the discoveries made by American scientists (6, 12, 35, 67, 73) and others, who found out that Histoplasma Capsulatum is responsible for many diseases of, until then, unknown etiology (36, 52).

Within the last decade the wide-spread presence of Histoplasma Capsulatum has been finally confirmed as well as the immensely frequent infection caused by this micro-organism existing, for example, in some states of the U.S.A. about 80% of the population (27, 37, 42, 57). It has been confirmed, in the majority of cases, that the infections do not show any symptoms in their course, or they are in sub-clinical form frequently leaving characteristic calcification within the lungs or in other internal organs which is similar to tuberculous calcifications.

Epidemiology and the Spread of Histoplasmosis

For a long time the appearance of fungus in nature was unknown. Only in 1949 Emmons (36) accomplished the isolation of Histoplasma Capsulatum from the soil in an endemic area. This discovery, which was later repeated many times by other scientists (2, 51, 56), determined, finally, the role of the soil as a natural environment for Histoplasma Capsulatum. Subsequently, many positive cultures of fungi were developed from the air (49), water (39), dust, chicken lodgings, rotten wood (42), old silos (40), from the areas of old, abandoned houses occupied by pigeons and bats (22, 33); as well as from many domestic and wild animals. (1, 3, 15, 29, 31).

Histoplasmosis has been described in many animal species, yet the immediate spread of infection from a diseased animal to a healthy one, or from an animal to a human being, has not been confirmed. It follows that such transmissions do not take place at all or they occur unusually seldom, and they don’t play a substantive role in epidemiology.

The infection develops as result of the fungus invasion into the organism through respiratory or alimentary organs. (57). Histoplasmosis has been also observed in humans; it resulted from penetration of the parasite through damaged skin or through mucous membranes. There is a great probability that the transmission of infection from an infected organism to a healthy one can occur through the medium of ticks (55).

The infections caused by Histoplasma Capsulatum materialize in humans regardless of race, age or sex; however, they develop more frequently in children than in adults. The development of histoplasmosis in childhood has been observed, equally often, in both sexes; from 40 to 60 years of age mycosis appears more frequently in men than in women. It
has been also confirmed that the cases of histoplasmosis are slightly more numerous among the agricultural population than among the city dwellers.

Histoplasmosis has been observed in all continents. Quite frequently however, this disease is found in North and South America, especially in North America. Into endemic areas are included mid-west and north-eastern parts of U.S.A. The cases of histoplasmosis have been also observed in South and West Africa, Sudan, south-eastern Asia, in Malaya Archipelago, Philippines, Australia, and in the following European countries: England, Austria, Bulgaria, France, Spain and Portugal.

As an indicator of the frequency of infections caused by Histoplasma Capsulatum serves the allergic skin test performed with histoplasmin. This reaction, which was for the first time applied by Van Pernis and collaborators (54), made possible the examination of large groups of people. It demonstrated, in some countries, for example U.S.A., areas of endemic histoplasmosis. The tests with histoplasmin, carried out in other countries, were of a much smaller scope and, therefore, can not truly reflect the state of infection through Histoplasma Capsulatum.

In Poland the skin tests with histoplasmin were conducted in 1952 by a team from NHS Tuberculosis Research Office. Two groups of school children were examined. In the city of Krakow only one positive result was obtained out of 535 examined children. The infected child was a seven years old boy. In Siedlce all the tests performed on 396 children showed negative results. (27, 60).

In conducting tests with histoplasmin among large groups of people it was possible, beyond any doubt, to discover a connection between histoplasmin positivity and the presence of calcification in the lungs of individuals showing a negative tuberculin reaction. Thanks to numerous studies (12, 54, 65, 72, 73, 82, 86, 88, 89), in some states of U.S.A., for example, frequent cases of calcification centers in lungs have been established, formed because of the histoplasmic infection and not because of tuberculosis (26, 28). Thus, it became possible to solve the intriguing problem of the etiology of changes (lesions) in lungs, similar to tuberculosis, with negative inoculation of tuberculous bacilli and the lack of tuberculin allergy.

It is appropriate here to emphasize the great significance which the discoveries in the field of other organic mycosis - coccidioidomycosis - had for the study of histoplasmosis. Due to the fact that coccidioidomycosis is, in its many attributes, similar to histoplasmosis and that the studies of coccidioidomycosis have been made somewhat earlier, it was possible to avoid errors and unnecessary research in explaining the problems of histoplasmosis.
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Pathogenic Factor

Histoplasma capsulatum belongs to imperfect fungi, which appear in two phases: 1) the yeast-like tissue phase, called by American authors TP (Tissue Phase), and 2) the mycotic phase, also called HP (Mycelial phase). In an infected organism, micro-organisms appear exclusively in the mycotic phase, although cases have been described in which the presence of mycotic phase in infected tissues was confirmed. (42).

The mycotic form of Histoplasma capsulatum appears, under microscope, in the form of oval cells in diameter of 1 to 4 u, which are similar to yeast cells. It is possible to observe in the infected tissue, around the cells of Histoplasma capsulatum, translucent cones, sharply refracting light, which made it possible for Darling (20) to confirm the presence of a capsule and thus give the name of "capsulatum." Later examinations, using an electronic microscope, did not confirm existence of a capsule. (76).

Histoplasma capsulatum, in the mycotic phase, has the form of nematode. It is formed from clouds in diameter of 2.5 u, possessing ramifications and forming on extremities on the course of threads, so-called chlamydiaceous. A characteristic feature of the mycotic phase are so-called "tuberculous chlamydiaceous" (tuberculate chlamydiaceous). They are round, large, or oval spores, in diameter of 7-8 u, possessing on the surface membrane numerous tubercular stripes.

On artificial media both phases show a different type of growth. The mycotic phase grows in constant media in the form of round, smooth, glossy white colonies of medium size; the mycotic phase has the appearance of dry, flat, irregular, squiggled colonies, which are white at the beginning and after a dozen or so days they turn yellow-brown.

The cultures of Histoplasma capsulatum are developed in quite a great range of temperatures: 20° to 37°. To procure the growth of the mycotic phase the 37° temperature is indispensable. The reaction of the medium is constant acid, fluctuating from pH 5 to pH 7. The growth takes place in oxygen conditions, though it is helpful to increase the access of CO₂ (15-20% CO₂). As the most frequently used constant media belong Sabouraud's media containing 2-4% of glucose, and media containing grain starch. The latter serve in establishing the presence of tuberculous chlamydiaceous.

It can be seen from the studies of Pinca (76) that media containing agar do not always constitute a suitable substrate for growth. Pinca found out that fatty acids, which are in agar, are inhibitors of Histoplasma capsulatum. The activity of these acids can be abolished with the help of compounds containing SH groups in the reduced form (ex.: cysteine, glutathione). Certain blood, and especially red blood corpuscles, show a clear influence by stimulating the growth of Histoplasma capsulatum.
Histoplasma Capsulatum multiplies through transversal partition or through budding, creating blastospores or chlamydospores. There are tuberculous chlamydospores as well as smaller ones, in diameter 2.5 to 4 μ, having thin and long walls. Both kinds of chlamydospores can develop directly from schizos or they come into being on conidiophores.

Passing from the mycotic phase into the yeasty phase, and vice versa, depends on the conditions of environment. The returnation into mycotic phase occurs in natural conditions. To preserve the micro-organism in the yeasty phase or to develop it from the mycotic into yeast phase, is a difficult task requiring complicated media and, sometimes, the only effective mean is the passage through a living organism. A good medium for passing from the mycotic phase into the yeast phase is the medium of composition given by Litwin (55). It contains liver and spleen extracts, human blood, glucose, agar, and antibiotics.

The antigenic structure of Histoplasma Capsulatum is relatively little known. The majority of studies were concentrated on the problem of antigenic affinity with other pathogenic, diphasic fungi like: coccidiodes immitis, Blastomyces dermatitidis, Candida albicans, etc. In making examinations, the fixation reaction, precipitation, passive hemagglutination and allergic reaction, were used. Agglutinative reaction can not be applied on account of spontaneous agglutination of Histoplasma Capsulatum cells.

Histoplasma Capsulatum has, in contrast to the majority of pathogenic fungi, relatively strong antigenic characteristics. The method of obtaining immune sera was gradually improving. At first, experimental animals were immunised during a period lasting several months; now, good sera can be obtained by applying large doses of antigen in short intervals of time, i.e. 3-5 weeks (76).

The studies of antigenic affinity of deep mycoses etiological factors showed that Histoplasma Capsulatum contains some antigenic fractions in common with Coccidiodes immitis, Blastomyces dermatitidis and Candida albicans. The description of natural antigenic affinity between above mentioned micro-organisms explained many questions connected with diagnostics of pathogenic, diphasic fungi.

Histoplasmosis in Humans

The course of histoplasmic infection in man shows a great variety. It depends on many factors such as predisposition, age, the state of natural immunity, the intensity of infection, etc. As result of the infection developing most frequently per os, and only sporadically through damaged skin, a primary complex is formed future fate of which can be various. In the majority of cases the infection, subjectively and objectively, is asymptomatic. Only in a certain group of people suffering from such form of the disease, the changes remain in the form of a single one or several centers of
Classification within the area of lungs or spleen. The only certain cause of infection that passed in mild, asymptomatic forms, is the state of produced allergy to histoplasmin.

In asymptomatic forms the changes and symptoms of the disease are of unusually great variety and, therefore, create much difficulty in the formation of individual clinical complexes. On the basis of Wilson's study, (35), who attempted to establish the clinical forms on the bases founded in other mycosis - coccidiodomycosis - following complexes can be singled out:

1. primary histoplasmosis of the skin
2. primary histoplasmosis of the lungs
3. disseminated histoplasmosis.

The first form, the primary histoplasmosis of the skin, appears sporadically and is characterized by forming of ulceration on the spot of infection as well as by the inflammation of surrounding vessels and lymphatic nodes. A similar case, described by Gantle and Cowley (19), ended favorably; in the body of the diseased no other histoplastic changes were noted. The individual, in the course of further observation, did not show recurrence of the disease.

The primary histoplasmosis of the lungs can develop in bronchial or blood vessel tracts. On the spot where the germ settles a reaction is formed which resembles the picture of tuberculous deposit inundation. The inflammatory process, produced in palmonic membrane, can have various exits. It can lead to complete resorption, inundation, disintegration with the creation of a cavity, fibrosis, calcification, or formation of histoplasmin. The inflammatory changes can be numerous, and then, in unfavourable cases, it leads to fibrosis of the entire segments of lungs, emphysema and bronchial catarrh. Histoplastic process attacks, sometimes, pleura. Then comes to inflammation of pleura with formation of emenate (4). The mucous membrane of upper respiratory channels also succumbs, quite frequently, to pathologic process, whether as result of primary or secondary nestling of the bacillus. The cases of histoplasmosis of the mouth cavity, throat, and larynx have been described; the changes in these cases had the ulcerative character, sometimes imitating neoplastic tissue (5, 19, 62). To subjective symptoms, which appear in this complex, belong typical complaints about diseases of respiratory organs. The primary histoplasmosis of the lungs encompasses not only symptomatic forms, but also frequent asymptomatic cases.

The disseminated histoplasmosis can occur, like the preceding complex, in symptomatic or asymptomatic forms, though asymptomatic course is rather infrequent. As result of the generalization of infection a characteristic picture is being formed: the reticular-endotheliaceous system is occupied which is manifested by hyperplasia of the spleen, liver or lymphatic glands. This form is accompanied by symptoms of
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... changes in peripheral blood, retina, lymphoma, fever, dis-
... ... nition in the form of vomiting, diarrhea, bleeding
... ... nfection, formed because of ulceration and perforation.

... result of dissemination of infection many internal organs are injured.

... result are changes within the liver and in suprarenal body, causing

... their insufficiency.

The course of the disseminated histoplasmosis is usually fatal.

... cases end in death within few weeks, lingering cases can last even
... ... 13 to 20 years. The generalized form materializes in individuals
... ... demonstrating certain immunological defects in regard to Histoplasma

... Capulatum infections, which does not let them to develop normal

... defensive mechanisms.

... Interesting is frequent coexistence or joining of histoplasmosis
... with disease in which the disturbance in reticuloendothelial system
... are continued; for example, in various forms of leukaemia, in Hodgkins
... disease, or in Lymphosarcoma. This phenomenon can be explained doubly:

... it is possible, that as the result of primary damage to hematopoietic

... system and R.E.S the resistance power of the organism are insufficient

... to liquidate histoplasmosis infection or, conversely, as result of the

... infection develops a reaction of these tissues which reminds of lympha-

... blastomatous states. (35).

The Histopathological Picture of Histoplasmosis

... The majority of scientists agree that the histopathological

... picture of histoplasmosis is similar to tuberculosis. In the form of

... disseminated histoplasmosis, or pulmonary histoplasmosis there appear,

... within lymphatic glands, producing changes showing tendency for dis-

... ... integration. In the lungs, which are almost always attacked, milky

... tubercles are formed and the lymphatic glands are swelling. Within the

... liver and spleen, which are enlarged, hyperemic, appear tubercles and

... necrotic area. Bone-marrow and suprarenal glands are also frequently

... involved in the disease process.

... According to Wilson (35), one can observe in the changed tissues

... the appearance of Histoplasma Capulatum within the cells of the R.E.S

... system, in the form of round or oval formations, surrounded by transglysant

... zones indicate an areola. Here and there are visible germating cells.

... The concentrations of parasites within tubercules are surrounded by

... pseudo-vascularous tissue, composed of lymphocytes, plasmatic cells,

... fibroblasts, macrophage, epithelialoid cells, and macro-cells. The

... combining of such centers can cause the formation of large necrotic

... areas. Sometimes the productive changes are subjected to fibrosis.

... Einford (3), while conducting a particular histopathological
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Histology, confirmed the existence of three basic forms of changes in the lungs. They were histiocytic-mycotic changes, in which Histoplasma capsulatum in the yeast phase develops within the protoplasm of histiocytes. The constant appearance of new histiocytes leads to enlargement of tissue content and the organ. Nuclei of caseous necrosis are formed which are surrounded by a mantle of spindle-shaped cells. Other elements, such as plasmatic cells and lymphocytes, do not play a major role here.

The appearance of epithelioid granuloma, developing within lymphatic glands, in which in addition to epithelioid cells appear also Langhans cells, belongs to other changes. The lesions are very similar to tuberculous lesions, especially because the nuclei of caseous necrosis have been confirmed in them. The non-caseous nuclei are well-confined and resemble Böck's sarcoids.

The inflammatory changes (lesions) in interstitial tissue of the lungs constitute the third form which, in addition to characteristics of the two above mentioned forms, are also characterized by formation in interalveolar areas of exudate containing many inflamed cells and gradually succumbing to organization and fibrosis.

Of great interest was the discovery made by Puckett (73), who in lesions defined as tuberculous, was able to discover Histoplasma capsulatum. Davis and collaborators (22) as well as Forsee and coll. (34) confirmed later this discovery through the histopathological examination and the positive isolation of fungus and proposed for those changes (lesions) the name: histoplasmosis.

Consequently, it can be concluded from these studies as well as others, that histoplasmosis is a cellular, mycosis--cytomycesis, attacking all tissues, with the exception of osseous and cartilaginous tissues.

SUMMARY

The treatment of organic mycoses, including histoplasmosis, is still a difficult task. Chemotherapeutics and antibiotics, commonly applied today proved to be ineffective in histoplasmosis and even on the contrary, some of them stimulated the growth of the parasite. Campbell and Caspari (3), for example, confirmed in vitro the stimulating influence of streptomycin on the growth of the mycotic phase of Histoplasma capsulatum. This effect was not observed in infected mice (10).

Recently, much hope is placed on amphotericin B, an antibiotic isolated by Gold and collaborators (33) as well as Vandegutta and coll. (33) in 1955, from the genus Streptomyces.
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Ampicillin B was used, with great success, in combination with sulfadiazine in treatment of experimentally infected hamsters. According to Littau and collaborators(5), substantially smaller quantities of lesions in the lungs were observed in the course of such therapy as well as the prolongation of the survival of experimental animals. A similar report was given by Leu and coll. (58), applying ampicillin B to infected white mice.

The first tests of ampicillin B application in human histoplasmosis indicate that this antibiotic will produce good results. Leu and coll. (53), applying ampicillin B orally in doses of 2-5 grams daily during 2 - 3 months, observed substantial alleviation of clinical symptoms, and in some patients, remarkable improvement. Results of the therapy were much better in cases of fresh infections than in old infections in which substantial organic lesions had developed.

Good results were likewise obtained by Ellis and coll. (20) and Zimmerman (57), applying ethyl ester of vanillic acid in skin histoplasmosis and in mucous membranes of the larynx, as well as Christie (11) in case of disseminated histoplasmosis.

In addition to the etiopathogenetic therapy which to date in many cases does not give the desired effects, a symptomatic therapy is applied as are surgical operations of resection of the transformed parts of pulmonary tissue.(47, 59, 71).

Diagnosis of Histoplasmosis

Because of the lack of characteristic clinical symptoms in the course of histoplasmosis, the entire weight of proper diagnosis is based on mycological, serological and allergic examinations. Familiarity with the epidemiological state of the matter as well as the epidemiological investigation in a given case under observation is essential.

In a schematic approach, the course of diagnostic procedure is the following: first, a skin test with histoplasmin is taken. It permits to confirm or reject the infection which has already passed or is still taking place, with the exception of cases which will be discussed later. Secondly, the isolation of the parasite from the suspected material is aimed at simultaneously. The following specimens are most frequently taken: sputum, blood, bone-marrow, pus, exudates, parts of tissues obtained through biopsy and autopsy, as well as samples of soil, dust and specimens from animals which were in the environment of the examined case. From some of the above mentioned specimens, i.e., blood, bone-marrow, tissue sections, preparations are made immediately, which are dyed by the Wright or Giemsa method and characteristic forms of the parasite within cells are sought through microscopic examination.
The culture of Histoplasma capsulatum from infected material is developed in many media and under various conditions. The lack of standardization for the methods of fungus isolation in this case reflects well the difficulties which are faced here. Various centers and the laboratory with which the author worked attempted media and conditions under which isolation is to be conducted. Therefore, in order to establish the various the chance of finding the parasites, the cultures are incubated in two temperatures: in 37°C and in room temperature as well as in Sabouraud media and other media containing entomites from liver, spleen, brain, with the addition of human blood, horse blood or from other animals. Undoubtedly, the best media constitute those containing entomites from internal animal organs or blood ingredients. For example, Howell (48), examining the productivity of isolation from experimentally infected guinea pigs, confirmed positive cultures of 70% on media containing entomites from brain and heart, and 10% defibrinated horse-blood through incubation in room temperature. Using another medium and developing cultures in two temperatures (37°C and room temperature), he was able to obtain 100% positive isolation.

The growth of the micro-organism, especially in the mycotic phase, is very slow. It is, therefore, necessary to prevent the media from drying out and from being overgrown by other, fast-developing micro-organisms. This difficulty can be eliminated to a certain degree through the addition of penicillin and streptomycin in the proportion of 20 to 40 units to 1 ml of medium. The culture is considered negative only after three or four weeks of observation. On media containing large quantities of albuminous and sugary components, the growth takes place generally in the form of mycelium of small quantity or in complete lack of chlamydospores, indispensable to the identification of the fungus. It is necessary in these conditions to transplant the culture on media containing potato flour, agar and glucose, in order to confirm the presence of spores and especially chlamydospores.

In spite of the improvements in fungus isolation methods, confirmation of histoplasmic infection in man through positive culture is very difficult. According to Curry and Wier (17), for example, in 65 cases of doubtless histoplasmic of the lungs confirmed by finding of the fungus in repeated tissues, Histoplasma capsulatum was isolated in only one case. Isolations are more difficult when the disease has been prolonged. When examinations are made in the early stages of infection - two, three weeks from the moment of infection and when the disease has a more acute course, in the disseminated form, the frequency of positive isolations increases substantially. A great advancement in the diagnosis of histoplasmosis was the introduction by Koffit and coll. (61) of the infecting of
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Experimental animals with the examined matter. White mice proved to be especially useful in this case. By peritoneal introduction of adequately prepared, examined specimen, together with antibiotics, the frequency of parasite isolation was substantially increased.

The mouse, very sensitive to infection by Histoplasma capsulatum, in this case fulfills the role of a filter which eliminates the impurities of the examined matter with other saprophytic fungi. The infection of the mouse is a sensitive method, it allows to detect minimal quantities of fungi; its essential defect, however, is the protraction, almost for a month, of the time of isolation of Histoplasma Capsulatum.

Sero logical reactions, especially the fixation reaction with various antigens obtained from the yeast phase and myotic Histoplasma capsulatum, have also a great value for the diagnosis.

Immunological Phenomena in Histoplasmosis

As a result of histoplasmic infection, there appear in the affected organism processes aiming at elimination of the invasion of the pathogenic factor, conditioned by phenomena of resistivity and growing immunity. The occurrence of resistivity in the course of histoplasmosis is, except for very general and not much examined facts, little known. According to Bumr (7), it is associated with the following mechanisms:

1. The natural tissue barrier which prevents the penetration of micro-organism.
2. Production of substances which block the development or kill the parasite before its entrance into tissues.
3. Cell protection of the RES system.
4. The increased flow of lymph and blood through the areas changed by inflammation.
5. Humoral protection (fibrin, probably natural antibodies).
6. A series of environmental conditions, which may not be favorable to the development of micro-organism; for example: temperature, pH, content of oxygen, surplus or lack of metabolites, etc.

Immunological phenomena in the course of histoplasmosis, the moment of appearance of allergy on the matter produced from the cell disintegration of the myotic phase of Histoplasma capsulatum, i.e., on histoplasmin, is essential. Histoplasminic allergy develops in the second or third week of the disease (8) and lasts through many years, probably throughout the entire life. Only in cases of substantial injury of the organism in the form of disseminated histoplasmosis, causing quick death, and in final stages of prolonged mycosis with visible organic changes, is the lack of oversensitiveness observed. Allergy to histoplasmin like allergy to tuberculine in tuberculosis, is accepted as proof of the decrease of sensitivity to
recurring histoplasmic infection. Wilson (35) explains the increase in number of patients suffering from histoplasmosis among the old people by the extinction of the allergic reaction.

The histoplastic test depends on intravenous introduction of 0.1 ml - adequately diluted, mostly 1/1000 or 1/100, histoplasmin. Results can be studied after 24 and 48 hours. Erythema in diameter equal to or greater than 3 cm is considered as a positive result.

Histoplasmin is not characterized by great specificity. In other mycose, coccidioidomycosis and blastomycesis, histoplasmin yields equally positive results. This fact depends on the presence of common allergenic fractions in histoplasmin, coccidioidin and blastomycein.

Interpretational difficulties, in results obtained from dermal reactions are being solved by simultaneous testing with changed mycotic allergens. This reaction, which forms under the influence of the most diluted allergen, is accepted as specific. Here is suspicion that in some areas of U.S.A. other, unknown mycoses appear which can yield positive results with histoplasmin, coccidioidin and blastomycein. For example Palmer and coll. (63) indicate that within some states (of U.S.) frequently doubtful results with histoplasmin and coccidioidin are confirmed; this can raise the suspicion that the infection is caused by a different kind of fungi.

Many scientists, using the method of gradual purification of preparations, attempted to eliminate the phenomenon of histoplasmin reaction, not only in histoplasmosis but also in other mycoses. Until now however, a substance characterized by complete specificity and adequate sensitiveness has not yet been obtained from histoplasmin. Dyson and Evans (25), basing their examination on the fact that histoplasma Capulatum appears in infected organisms exclusively in the yeast phase, aimed at obtaining from that particular phase adequate allergic preparations. Serotic antigens, extracted from the tissue phase of Histoplasma Capulatum, were, however, unsuitable for allergic tests. It was possible, however, to obtain allergic substances of great specificity and sensitiveness (greater than those peculiar to histoplasmin) through deduction of precipitates from the filtrates of fluid culture of the yeast phase with alcohol. Dyson and Evans (25), testing their new substance on pigs experimentally infected with Histoplasma Capulatum, Coccidiodes immitis, Cryptococcus neoformans, Blastomyces dermatitidis, Candida albicans and Sporotrichum schenckii, confirmed the presence of scral reactions only in animals infected with American drosdag (trans. note - drosdag - yeast).

In the beginning, skin tests were conducted with various preparations of histoplasmin, which was responsible for many interpro-
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Technical difficulties and did not allow for comparison of results obtained during investigations. Thanks to the studies of Shaw and coi. (31), it became possible to avoid these divergencies: namely, in 1933 the standard preparation of histoplasm in H-15 was introduced to routine application. This histoplasm, up to the present, has not lost any of its value and it serves as an example for other histoplasmin preparations. Histoplasm is a filtrate of several months' culture from the syngastic medium of the mycotic phase of Histoplasma Capsulatum. It is composed of polynucleated and allantoinous fractions. The elimination of the allantoinous part does not deprive histoplasm of the ability to react in skin reaction (16). The full histoplasm serves as an antigen in the fixation reaction, precipitation and hemagglutination. However, in serological reactions, histoplasm shows a great degree of non-specificity of reaction. Therefore, in diagnostic examinations, in the search for antibodies in examined serum, except histoplasm, other antigens from the yeasty and mycotic phases of Histoplasma Capsulatum are also applied.

The fixation reaction has a great significance for diagnostics as well as for prognostics of histoplasmosis.

The antibodies taking part in this reaction, appear in the third or fourth week of the disease and remain in serum from several months to several years. A characteristic feature is the high titer of antibodies in the disseminated forms of histoplasmosis. In the mild form of the disease, antibodies are not found, or they appear in small quantity. The titers of antibodies reach their maximum in the fifth - sixth week of the disease. Sometimes, they can be found in the dilution of serum 1 to several or even several thousands (46, 50). There is a great probability, based on examinations in the course of coccidioidomycosis as well as on few observations in histoplasmosis, that the quantity of antibodies present in serum, taking part in the fixation reaction, is directly proportional to the quantity of active cells of the parasite in the organism.

Quite an essential problem in the discovery of Guadry-Milhau (trans. note - histoplasmosis?; antibodies, active cells?), is the matter of using the proper antigens. Experiments with a series of nuclei in the fixation reaction indicate that it is erroneous to apply only one antigen to discovery antihistoplastic antibodies (46). It is recommended to use antigens obtained from cells of the yeasty phase and from culture filtrates of both phases. This can be deduced from the fact that in the course of various periods of the disease and in various cases of histoplasmosis, the presence of antibodies for only one or a second antigen is sometimes confirmed.

The problem of why and when certain antibodies appear, has not yet been solved. Lashoffsky and coll. (50) while concerning
themselves with these problems showed, that for eight antigen fractions obtained by them from Histoplasma capsulatum, the antibodies in sera of immunized rabbits appear in various periods of time. The antigenic fractions IV, VI and IX distinguished themselves by a clear specificity, the rest reacted with histological sera as well as with sera of rabbits infected with other diphasic fungi. Similar examinations made with human sera give an opportunity to introduce specific antigen for discovery of antihistoplasmic antibodies. Such antigens would be characterized not only by great specificity, but would allow to determine the time of injection, the course of the disease and the prognosis. (90).

The precipitatory reactions have a great significance for the identification of histoplasmosis, especially in the early stages. Such a reaction was applied for the first time by Van Pernis and coll. (84), and later by Schaff (79), Peters. (69), as well as by Salvin and Bottlo (75). The latter confirmed that precipitins, in the course of experimental histoplasmosis, show up shortly after the appearance of allergic reaction and maintain themselves in short-lasting sera. Observations made by Salvin and Bottlo (75) were confirmed several years later on humans. (77). Precipititative reactions with histoplasmic sera occur positively in the first or second week of the disease and remain in serum from 3 weeks to 10 months.

By comparing the results of the fixation reaction and precipitation, no correlation between them was confirmed. The precipitation reaction is especially helpful for identification of acute cases of histoplasmosis, and those cases in which the fixation reaction was negative. The precipititative tests with histoplasmic sera occur positively not only with sera of histoplasmosis cases, but also in other mycoses. Titors, however, in specific reactions are substantially higher and they make possible the actual identification of the pathologic unit.

Recently, the precipitation reaction in agar gel according to Cautherton was introduced to the diagnosis of histoplasmosis. Thanks to this technique, Keiner (45) gained with positive human sera and histoplasmic ten precipitatitive lines of which one is characteristic to the pathologic process, and the other only proves the existence of the state of super sensitivity to histoplasmic.

An attempt was also made to introduce the reaction of passive agglutination to the discovery of antihistoplasmic antibodies. Collid particles (58) and red blood corpuscles (63) sensitized with histoplasmic were applied to the reaction. This method, however, was not commonly accepted.
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