Candida albicans Infections in Actively and Passively Immunized Animals[1)]

by

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Candida albicans (Robin) Berkhout, 1923, a yeast-like organism, is the causative agent of human moniliasis, a disease syndrome embracing a number of manifestations which have often been considered unrelated. The fungus may produce lesions in the mouth, vagina, skin, nails, bronchi, or lungs and more rarely, septicemia, endocarditis and meningitis. In the domestic rabbit, the experimental animal chosen for this work, the primary organ attacked is the kidney. Lesions are also produced in the liver, spleen, heart, lungs and intestines.

Grossly, the kidney and to a lesser extent the brain, heart and liver of rabbits infected with *C. albicans* show numerous, small, white abscesses scattered over the surface. Perhaps the work of MACKINNON (11) gives the best description of the disease in experimental animals. Intravenous inoculations in large doses produces death in from five days to a week. Autopsy shows small abscesses in all the visceral organs, but especially in the cortex of the kidney. In smaller doses many animals survive 8—10 days and sometimes longer. After 10—15 days, autopsies show exclusively renal lesions affecting the collecting tubules. In general these lesions are triangular in shape and marked out by abscesses 1—2 mm in diameter. These lesions leave traces of fibrosis. CECIL (3) describes the pathology of human monilial lesions as focal areas of necrosis in which are found budding cells with or without mycelium. He says that giant cells may or may not be present. The cellular reaction is one of polymorphonuclear leukocytes, monocytes and lymphocytes.

JACOBSON and co-workers (9) in their text give a rather thorough description of the lesions in animals.

While much has been published on moniliasis, there is a dearth of material concerning actual immunity and the disease. CONNOR (5) working with organisms isolated from a case of osteomyelitis, noted that in a vaccinated animal there were no frank abscesses; rather he found small, acute tubercle-like lesions with *Candida* as the „common invader". Small giant cells were numerous in the lymph nodes and kidney lesions. Whether this difference in the type of reaction was caused by the vaccine or not, the author was not certain; but

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he expressed the thought that a slight relative immunity might be shown in this way.

CATELLANI (2) very briefly suggests that *Monilia* (*Candida*) vaccines are useful in only a few cases but does not say that there is any particular type of moniliasis which responds to the vaccine. According to JACOBSON (9), autogenous vaccines made from organisms have been employed with success by GROSSI and BALOG in the treatment of a series of cases of bronchopulmonary moniliasis. In 1924 MARTIN (12) reported a case of pulmonary moniliasis which was treated successfully with immune serum. In this case there was a negative skin test, a negative agglutination, but a positive FOSHAY test.

HOFF (8) in his studies on *Cryptocossus hominis* (*C. neoformans*) reported immunizing mice by the injection of heat killed organisms. While both sets of mice died in approximately the same period of time, histological examination of lesions in the earlier stages showed immunity epxressed as a hastening of cellular proliferation with subsequent scar tissue reaction and the disappearance of the organisms. ZIMMERMAN (15) in reporting a case of meningitis due to *Candida albicans* suggested that some immunity may have been developed which played a part in the course of the disease.

The purpose of this work was to study the effect of antiserum on the course of the disease in experimentally infected rabbits. Since there is the possibility of sensitizing an animal through the use of vaccines, a method of passive immunization was tried. Special attention was given to histological differences between infected normal animals and infected immunized animals.

Passively and actively immunized animals as well as normal controls were given minimum lethal doses of a suspension of *C. albicans* and comparisons were made between tissue sections of the various groups. Comparisons were also made between groups of animals subjected to different experimental procedures, and consideration was also given to survival time by regrouping the animals and comparing those that survived for the same lengths of time regardless of the experimental procedure so as to indicate whether any differences observed were due to the experimental techniques or whether they might be due to a greater survival time of some of the rabbits.

Experimental Methods

Three different strains of *Candida albicas* were isolated and identified by following the procedures given by CONANT ET AL (4). After testing for virulence by intravenous injection into the marginal ear vein of a rabbit, a strain isolated from a female patient with chronic oral thrush was chosen for further work. Although this strain was highly pathogenic for rabbits and gave typical kidney lesions, it showed little or no pathogenicity for mice, which is in agreement with other reports on animal pathogenicity of *C. albicans*.

Vaccines prepared from a young culture 24 hours old and an older one of four days were suspended in sterile physiological saline solution (0.85 % NaCl). These suspensions were then heated to 60° C. for thirty minutes, cooled and 1 ml. of 10 % formalin was added to each 100 ml. of suspensions as a preservative. The suspensions were then tested for sterility by streaking modified Sabouraud agar plates. At first a suspension of a density equal to a MCFARLAND number three was used but this was later increased to about a MCFARLAND number six. The dosage varied from 0.5 ml. to 2.5 ml. and was injected into the marginal ear veins of the rabbits. MCKINNEY (13) states that it is more difficult to immunize rabbits against the yeast-like organisms than against bacteria, and the authors of this paper concur with this, hence the increase in density of the suspension and volume injected. DRAKE (6) found hist to be even more striking in work with *Cryptococcus neoformans* since with this organism, large doses of dense suspensions are necessary to assure antibody response in rabbits. The use of small numbers of organisms for immunization has probably been responsible for the erroneous reports that this latter organism is not antigenic.

When the titer was to be tested, the animal was either bled from the ear or anesthetized and bled from the heart. When the titer had reached 1 : 2560, the rabbits were bled under sterile conditions and the serum stored in the refrigerator until needed. No preservative was added.

In testing for agglutinins a method suggested by NORRIS (14) was used at first but this was later replaced by a method described by DRAKE (6) in which the test is read macroscopically on Kline agglutinations slides within 15 minutes after mixing antigen and antiserum. If spontaneous agglutination occurred with the control, the test was repeated.

At death the animals were autopsied and the various organs examined for lesions. Modified Sabouraud agar plates were streaked from the organs having lesions, and parts of the organs were fixed in 10 % formalin for sectioning later. After washing, the tissues were dehydrated either with a dioxane or acetone method and them embedded. Sections were cut from 7 to 10 microns in thickness.

Several methods of staining were tried. One of each of the sections was always stained with a standard hematoxylin-eosin method. In addition a method was sought which would differentially stain the fungus in the tissue. FUENTES' (7) technic, which he considered superior to the Gram method, was tried but without success because efforts to dissolve Sudan IV (scarlet red) in pure lactic acid were unsuccessful. Three different modifications of the Gram stain were tried and used to some extent to supplement the hematoxylin-eosin staining. These were the amended Gram-Weigert, MALLORY's variation (10) and MACCALLUM's modification of GOODPASTURE's method (4).

Experimental Results

The MLD for these experiments was a one ml. suspension of sufficient concentration as to bring death on the seventh day. This was established by preliminary tests using normal rabbits.

The first group of experiments was divided into five parts using a total of 25 rabbits. A series of 10 rabbits was used, each one being given one MLD. In the second series of five animals each was given one ml. of antiserum (titer 1 : 2560) and immediately followed by the injection of one MLD. In the third series of five rabbits, each was given three mls. of antiserum and one MLD immediately afterwards. In a fourth series, which was started some time later, three rabbits were given one MLD and two of these three were given regular intravenous injections of antiserum. In the fifth series the two rabbits which had been used for the production of the antiserum were given one MLD each. Later other experiments were carried out to try to answer some of the questions which arose from the findings of the first group of experiments. These will be briefly outlined in a later section.

In group one, eight of the ten rabbits which received one MLD died within a week. Seven of these showed the pathology as described by JACOBSON (9). The eight animal (rabbit 12) had a very small, well circumscribed mass in the lung composed of several dozen macrophages enclosing a single blastospore. Small abscesses were seen in the kidney with mycelium scanty to abundant in amount. The macrophages were the predominating cell type. Some of the abscesses contained packed masses of organisms, chiefly mycelium. These lesions were fairly well circumscribed, but not walled off from the surrounding tissue of which there was invasion at times. In three other rabbits of this group the organisms were packed and more abundant in the periphery of the lesion. Rabbit 12 showed masses of organisms with pink material in between, holding together the mass from which organisms were still growing and extending outwards. Such masses gave a definite impression of an actinomycetoid ("sulfur") granule type of growth, although the radiating arrangement was not as yet well developed. Many lesions were present in the brain with macrophages predominating in some and polymorphonuclear leucocytes in others; mycelium was much more abundant than blastospores. Numerous diffuse lesions showed invasion into the surrounding tissue. Phagocytosis was very rarely seen. The predominating form of the organism in the heart lesions was blastospores in rather large numbers. Although many of the organisms were phacocytized, this process was not controlling the invader.

All of the eight rabbits showed numcerous liver abscesses but the organisms were sparse in number and present mostly as blastospores. The spleens showed

a few scattered organisms which had recently invaded or metastasized without noticeable cell response.

In group two, three of the five rabbits, which were given one MLD and one ml. of antiserum, died in from five to six days. The kidney abscesses showed an unusual amount of necrotic phagocytes and contained blastospores which were often very large with thick walls. In some areas a walled-off central portion of the abscess contained polymorphonuclear leucocytes as well as macrophages and there was in the central portion of some an exceedingly well developed and typical actinomycetoid granule which stained very strongly with eosin. Less well developed granules were seen in some of the other abscesses. It should be noted that the actinomycetoid structure had developed before epithelioid cell development. Many lesions had, some place within the central portion, a mass of tightly entwined, twisted mycelium appearing to be the early stage in the formation of "sulfur granules". There was some connective tissue proliferation but it was still minimal. In the brain there were multiple small and large abscesses with mycelium predominating as short strands, often rather extensively branched and there was some invasion into the surrounding tissue. The predominate form of the organism in the heart was thick-walled blastospores; however, a few lesions showed a very early mycelial stage or else an abortive attempt to form mycelium. The blastospores, some of which appeared to have thick, striated walls or else were very finely lobulated, seemed unusually large and some seemed to be phagocytized.

In group three, four of the five rabbits which were given one MLD and three mls. of antiserum died in from five to six days. The kidney of rabbit 18 contained thick walled blastospores in the lesions. The lesions of rabbit 20 suggested a very early stage of granulomatous tissue, often with necrotic centers, and the macrophages, particularly at the periphery, suggested epithelioid cells. The heart lesions of rabbit 20 showed blastospores, scanty in number, with unusually thick walls; some appeared to be phagocytized. The other two rabbits showed the same general findings as described by JACOBSON.

The rabbits in group four (rabbits 26 and 27) were given one MLD and daily injections of antiserum until they died, which was within a week. Rabbit 27 died on the fiftieth day. Many of the larger abscesses in the kidney cortex of this animal showed little evidence of degeneration and often contained bright eosinophilic amorphous masses in which were embedded blastospores and short strands of mycelium. One abscess contained a packed mass of mycelium and blastospores but these were not sufficiently radiate for a sulfur granule. Rabbit 26, which died in seven days, had multiple abscesses throughout the kidney cortex consisting of a rather dense collection of macrophages with some connective tissue proliferation; the lesions appeared to be developing a tubercle-like structure, although no giant or epithelioid cells were seen. Central masses of some of the lesions were composed of strands of mycelium. Although there was more evidence of repair than usual in the heart muscle, a general impression was that the lesions, which contained a moderate number of elongated blastospores and short strands of mycelium, were larger in size and fewer in number than ordinarily observed. Thick walled blastospores were seen in some of the liver lesions of rabbit 26.

In group five, two rabbits, rabbits 1 and 2, were given injections of antigen beginning December 2, 1949, and continued until August 26, 1950, at which time rabbit A had an agglutin titer of 1 : 2560 and rabbit 2, a titer of 1 : 1280. On September 2, 1950, they were given one MLD. They died in less than 48 hours. Morphologically the organisms, frequently seen invading the kidney tissue, consisted chiefly of unusually short and multibranched strands of

mycelium. In some of the heart lesions short strands of mycelium were invading the surrounding tissue. Blastospores, when seen, seemed unusually large and somewhat thick-walled. In some of the lesions of the rabbit which lived a few hours longer, rabbit 1, a number of the organisms appeared to be growing in very tight masses. This rabbit also had some young lesions with elongated blastospores and a portion of the granular and vacuolated mycelium was in many cases pseudomycelium. In the liver lesions were seen thick-walled blastospores and short strands of mycelium which were somewhat distorted and twisted and often varying in diameter. New lesions appeared to be arising out in the cord cells rather than in regions of fibrosis (these fibrotic areas were probably the result of the large number of injections of antigen.)

Group six. Since these two rabbits, 23 and 25, which received one MLD and one ml. of antiserum on July 27, 1950, had not died, they were killed. In the tissues there were areas of fibrosis which contained scattered macrophages and appeared to be healed and entirely inactive lesions. In other areas fibroblasts and macrophages were the predominating cell type, fibrosis was distinct. There were no organisms seen.

In group seven, rabbit 10 was killed on September 3, 1950, since it had not died after receiving one MLD on Juny 27, 1950. Any lesions which were present appeared completely inactive.

In group eight, rabbit 13 which was given one MLD on July 27, 1950, and another on September 2, 1950, died on September 6, 1950. In the kidney were only a moderate number of dense abscesses present in the cortex with macrophages and degenerating polymorphonuclear leucocytes and rather eosinophilic granules. A moderate number of organisms were present in the abscesses either as blastospores, some of which appeared elongated, or as short strands of mycelium often appearing somewhat degenerated with a great variation in size and thickness of strands and vacuolization. The organisms were present in the brain lesions in only moderate numbers as blastospores and short strands of very granular mycelium. Fairly numerous abscesses were seen in the heart muscle and contained macrophages and abundant polymorphonuclear leucocytes, which sometimes appeared to be degenerating. The organisms were present as short strands of mycelium and thick-walled blastospores, some quite large and some phagocytized. The liver lesions appeared rather young and active. The only type of organisms seen were blastospores, some of which appeared either very large or very small; these were frequently distorted and often appeared to be somewhat degenerating as evidenced by the marked variation in size.

Rabbit 19 (Group nine) was kiled on August 6, since it had not died after receiving one MLD plus three mls. of antiserum on July 27. There were only minimum inactive lesions with fibroblasts, connective tissue fibers, usually some macrophages. The lesions appeared to be almost healed.

In group ten, for comparison rabbits 31 and 32 were each given one MLD. Rabbit 32 was killed after 24 hours and rabbit 31 was killed after 48 yours. The kidney of rabbit 32 contained small cortical abscesses which possessed occasional blastospores and moderate amounts of both pseudomycelium and true mycelium. There was some invasion of the surrounding tissue. The cortical abscesses in rabbit 31 contained macrophages and degenerating polymorphonuclear leucocytes with an unusual abundance of organisms present as mycelium and blastospores which in one case formed a rather tightly packed mass. Similar small lesions were seen in the medulla and in some cases organisms could be seen invading the surrounding tissue.

Rabbit 30 and rabbit 29, group eleven, were each given one MLD and five mls. of antiserum. Rabbit 30 was killed after 24 hours and rabbit 29 after 48 hours. Rabbit 30 had diffuse small long abscesses with necrotic centers, degenerating polymorphonuclear leucocytes and a very few macrophages. Even in this early stage the macrophages tended to pack very tightly together. While some of the organisms appeared to be degenerating, others had exceptionally heavy walls as if surrounded by a capsule. An organism appearing in a lung lesion definitely showed a radiating edge with prickles around the blastospores. Rabbit 29 had many abscesses, small, medium and large, in the kidney cortex with macrophages as the predominating cell type. It was very striking that almost every lesion contained a solid mass of organisms often embedded in an eosinophilic matrix. Of these, both blastospores and mycelium intermixed, none showed radiations. Blastospores and pseudomycelium predominated in occasional small abscesses of the medulla, but in others the greatest bulk was in packed organisms. The liver of rabbit 29 showed a large number of small abscesses with the organisms chiefly present as thick walled blastospores and very short strands of mycelium, which did not appear to be particularly abundant although found in all of the lesions. The heart contained numerous small, medium and large sized abscesses with the organisms present as branched mycelium and blastospores.

Discussion of Results

Using the survival time of the various animals as a basis for grouping, we may compare the findings as follows:

One day: If we take rabbit 32 as an example of the usual pathological changes caused by one MLD, then the variations in the rabbit receiving the antiserum are probably brought about by the antiserum. The short branched mycelium in the kidney of rabbit 30 would not be expected as normal finding and neither would the dense, early packing of macrophages in the spleen and lung. The degeneration and eosinophilic nature of the organisms in the lung and liver, the short, branched mycelium and thick walled blastospores in the lung, one of which definitely showed a radiate edge, were all confined to the serum treated animals and were not found in the control.

Two days: While the kidney of the non-immunized rabbit had one rather tightly packed mass of organisms, it is of interest to note that almost every lesion in the immunized rabbit contained a solid pack of organisms often embedded in an eosinophilic matrix. While orgnaisms were present in both, there was a difference in their morphology, rabbit 31 having blastospores and short mycelium, and rabbit 29 (with antiserum) having branching mycelium in some cases, and thick-walled blastospores in others. There appeared a tendency towards smaller lesions in the nonimmunized rabbit.

Five days: Here a non-immunized rabbit showed some of the characteristics one would expect in an antiserum treated rabbit. A few blastospores with thick walls in the lung and the packed organisms giving a "sulfur granule" type of lesion in the kidney were seen. It may be that the animal had some naturally occurring antibodies, DRAKE (6), or that a titer of sufficient potency had been built up to cause these changes. From the findings in the passively immunized rabbits it may be surmised that the antiserum brought about the changes in the morphology of the organism-thick-walled, unusually large blastospores, some of which had striated edges or were very finely lobulated. It was in this group that the actionomycetoid granules were found. It is speculative whether the larger lesions were from the effect of the antiserum or not.

Six days: Again the immunized animals showed the large thick-walled blastospores and the tendency for the organisms to form packed masses as in the beginning of a "sulfur granule". Another difference to be noted is the necrosis occurring in the centers of many of the lesions, a change not noted in the non-immunized rabbits. At this stage, the lesions in the kidney of the rabbits which received three mls. of antiserum closely resembled the lesions described by JACOBSON (9) in that they were of the granulomatous type.

Seven days: In seven days there were solidly packed massed organisms in the kidney of a non-immunized rabbit. One could ask the question, „Was this the result of a normal progress or were there antibodies present in small amounts which may have had an effect"? The typical clubshaped projections, radially arranged and the tubercle-like structures were found only in treated rabbits.

In the animal which lived a month or longer some of the lesions showed the same picture as was found in the immunized rabbits of a week or less survival. Rabbit 13 which lived 41 days and had been given two MLD's showed a positive titer just before death as did four other rabbits which had been given one MLD and lived for a period of time. The two actively immunized animals were seemingly not protected or else their early death might have been due to a tuberculin type of shock (1).

Summary and Conclusions

1. The literature on the pathology and immunology of *Candida albicans* is briefly reviewed.
2. Experimental methods and techniques used in this study are presented.
3. Experimental observations described here show that:
   a. Many of the lesions found differed from those described by CECIL and JACOBSON.
   b. The immunization did not seem to preserve the life of the animal nor did it extend the survival time over that of the controles, the general impression being that it was not only ineffective but possibly deleterious.
   c. The antiserum seemed to have some effect on the morphology of the organisms shown by the multibranched mycelium, the thick-walled, distorted blastospores, the heavy packing of the organisms in some cases and the presence of typical "sulfur granules", the actinomycetoid form of the organisms, in a number of animals.
   d. The antiserum seemed to have little or not effect on the tissue reactions.

Sommaire et Conclusions

1. La littérature concernant la pathologie et l'immunologie de *Candida albicans* est brièvement passée en revue.
2. Les méthodes d'experimentation et les techniques employées dans cette étude sont presentées.
3. Les observations faites pendant les experiences montrent que:
   a. Beaucoup des lésions trouvées differaient de celles décrites par CECIL et JACOBSON.
   b. L'immunisation n'a pas semblé préserver la vie de l'animal ni prolonger la période de survie au dela de celle des animaux de contrôle. L'impression générale est que l'immunisation fut, non seulement inefficace, mais peut-être nuisible.
   c. L'antisérum semble avoir eu, sur la morphologie des organismes, quelque effet démontré par le mycélium multibranche, les blastospores tordus

aux parois épaisses, dans certains cas un conglomérat très dense des organismes et la présence de typiques "sulfur granules", la forme actoinomycetoide des organismes chez un certain nombre d'animaux.

d. L'antisérum semble avoir eu peu ou pas d'effet sur la réaction tissulaire.

Literature Cited

1. BOYD, W. C. Fundamentals of Immunology. (revised and re-written). Interscience Publ., New York. 1947.
2. CASTELLANI, A. Fungi and Fungous Diseases. Amer. Med. Assoc., Chicago. 1928. Also Arch. of Dermatol. Syphilol. **16**: 383—425, 571—604, 714—740; **17**: 61—92, 194—220, 354—379. 1927—1928.
3. CECIL, R. L. and F. KENNEDY. A Textbook of Medicine. Saunders, Philadelphia. 1944.
4. CONANT, N. B., D. S. MARTIN, C. T. SMITH, R. D. BAKER and J. L. CALLOWAY. Manual of Clinical Mycology. Saunders, Philadelphia. 1934.
5. CONNOR, C. L. Monilia from osteomyelitis. Jour. Infect. Dis., **43**: 108—116. 1928.
6. DRAKE, C. H. Naturel antibodies against yeast-like fungi as measured by slide-agglutination. Jour. Immunol., **50**: 185—189. 1945.
7. FUENTES, C. A method for differentiating *Candida albicans* in tissue. Jour. Bact., **51**: 245—246. 1946.
8. HOFF, C. L. Immunity studies of *Cryptococcus hominis* (*Torula histolytica*) in mice. Jour. Lab. Clin. Med., **27**: 751—754. 1942.
9. JACOBSON H. P., J. F. SCHAMBERG, and R. MORROW. Fungous Diseases. a Clinico-Mycological Text. Thomas, Baltimore. 1932.
10. LILLIE, R. D. Histopathologic Technic. Blakiston, Philadelphia. 1948.
11. MACKINNON, J. E. Zimología Médica. Siglo Ilustrado, Montevideo. 1946.
12. MARTIN, D. S. The practical application of some immunologic principles to the diagnosis and treatment of certain fungus infections. Jour. Invest. Dermat., **4**: 471—482. 1941.
13. MCKINNEY, M. J. Yeast-like organisms of human origin. Jour. Infect. Dis., **44**: 47—45. 1929.
14. NORRIS, R. F. and A. J. RAWSON. Detection of serum agglutinins for Monilia and other yeast-like organisms. Sci., **105**: 105. 1947.
15. ZIMMERMAN, S. L., L. FRUTCHEY and J. H. GIBBES. Meningitis due to *Candida albicans* with recovery. Jour. Amer. Med. Assoc., **135**: 145—147. 1941.