Experimental Endocarditis
IV: Tricuspid and Aortic Valve Infection
with Candida albicans in Rabbits

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Candida albicans is a pathogen of increasing significance in man and is the most common cause of fungal infection in patients with underlying disease(1). Endocardial infection with this microorganism usually occurs on previously damaged heart valves or after the placement of prosthetic heart valves(2). The route of entry of candida in these patients is believed to be the blood stream because of the frequency of infection in drug addicts and after the prolonged administration of intravenous medication through indwelling polyethylene catheters(3). Systemic fungal infections are difficult to treat; endocarditis due to C. albicans usually progresses to a fatal outcome(1–3).

Previous efforts to reproduce candida endocarditis in animals have been limited to one study in dogs with surgically induced aortic insufficiency. This is a difficult model to establish and antibiotic treatment appeared to be necessary for the establishment of infection with C. guilliermondii(4).

The discovery of simple techniques for the establishment of staphylococcal endocarditis in the right or left sides of the heart in rabbits prompted the present effort to establish intracardiac infection with C. albicans(5,6). The development of an easily reproducible, small animal model for experimental candida endocarditis would be of considerable value in increasing understanding of the pathogenesis of these infections and would permit detailed study of the effect of chemotherapeutic agents.

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MATERIALS AND METHODS

The method employed for establishing endocarditis depends upon inserting a polyethylene catheter in a peripheral artery or vein and advancing it to a point at the entrance to or within the heart chambers. When the catheters were filled with sterile saline, nonbacterial thrombotic endocardial lesions developed in the region of the tip of the catheter. When bacteria were introduced into the lumen of the catheter, bacterial endocarditis was the result. Catheters filled with bacteria and inserted to a point short of the heart did not produce bacterial endocarditis(5,6).

The details of the methods for placement of the polyethylene catheters in the right heart chambers of rabbits have been described previously(5,6). Catheters were introduced into the venous system through the femoral vein for insertion into the right heart and into the carotid artery for placement adjacent to the aortic valve. The catheters were filled with the desired number of microorganisms, the end folded, tied upon itself with sterile silk, and the skin sutured over the folded end of the catheter.

A strain of *C. albicans*, an isolate obtained in the Bacteriology Laboratory of the Yale–New Haven Hospital, was kindly supplied by Dr. Alexander Von Graevenitz. Identification was confirmed by Dr. Morris A. Gordon of the New York State Laboratories in Albany by conventional morphological and biochemical reactions; pseudohyphae, chlamydomyces (on cornmeal—Tween 80 and zein—Tween 80 agars), fermentation pattern, and virulence for rabbits. The culture was maintained on Sabauraud dextrose agar (SDA) and test inocula prepared from 18- to 24-hr cultures grown in trypticase soy broth (TSB) at 37°.

Candida colony-forming units (CFU) were enumerated by the surface plating on SDA of 0.1-ml samples of serial dilutions in TSB of the overnight cultures.

Examination of the heart and other organs was carried out under sterile conditions. All chambers of the heart were carefully examined for lesions and all lesions were smeared and studied by Gram stain. When Candida were not easily seen, the smears were repeated, and multiple cultures were taken from the heart lesions of such animals. Samples of liver, spleen, and whole or half kidneys were homogenized in TSB and serially diluted prior to surface plating of appropriate dilutions of SDA. The spleen was considered enlarged when its wet weight was greater than 2.0 g. Blood cultures were taken from a lateral ear vein when the rabbits were alive and from the inferior vena cava at the completion of an experiment. Blood was added to SDA and incubated at 37°.

RESULTS

*Intravenous Injection of Candida albicans in Normal Rabbits*

Normal rabbits were challenged with varying numbers of candida to determine the consequences of intravenous inoculation (Table 1). Bacteriological and morphological studies of 16 rabbits was carried out 7–9 days later. There were no spontaneous deaths.
TABLE 1
Bacteriological Findings 7-9 Days after Intravenous Inoculation of Candida albicans in Normal Rabbits

| Inoculum | Rabbit no. | Spleen* | Liver* | Rt. kidney* | Lt. kidney* | Urine* | Terminal blood culture |
|----------|------------|---------|--------|-------------|-------------|--------|-----------------------|
| $10^4$   | 296        | 0       | 0      | 0           | $(10^9)$    | 0      | Neg.                  |
|          | 297        | 0       | 0      | $10^2$      | 0           | 0      | Neg.                  |
|          | 298        | 0       | 0      | 0           | $(10^9)$    | 0      | Neg.                  |
|          | 299        | 0       | 0      | 0           | $10^2$      | 0      | Neg.                  |
| $10^5$   | 289        | $10^5$  | $10^4$ | $10^5$      | $(10^9)$    | 0      | Neg.                  |
|          | 290        | 0       | 0      | $10^4$      | $10^3$      | 0      | Neg.                  |
|          | 291        | 0       | 0      | $10^3$      | $(10^9)$    | 0      | Neg.                  |
| $10^6$   | 282        | 0       | $10^3$ | $(10^9)$    | $(10^9)$    | 10     | ? Pos.                |
|          | 283        | $10^9$  | $10^4$ | $(10^9)$    | $(10^9)$    | 10     | Neg.                  |
|          | 284        | 0       | 0      | $10^4$      | $(10^9)$    | 10     | Neg.                  |
|          | 285        | 0       | $10^2$ | $(10^9)$    | $(10^9)$    | 10     | Neg.                  |
| $10^7$   | 286        | 0       | 0      | $10^4$      | $10^4$      | 0      | Neg.                  |
|          | 287        | 0       | 0      | $10^4$      | $(10^9)$    | 10     | Neg.                  |

* Kidneys with gross abscesses are noted by parentheses.
* CFU/g tissue.
* CFU/ml.

The only lesion visible grossly was that of pyelonephritis. Abscesses were scattered predominantly throughout the renal cortex. Low numbers of fungi were recovered from the urine only after challenge with $10^5-10^7$ Candida CFU. The rabbits receiving lower inocula had sterile urine cultures even though gross renal abscesses were obvious.

Examination of the heart failed to reveal any evidence of endocarditis. The livers and spleens appeared normal despite the recovery of $10^5-10^7$ Candida CFU/g in about half the animals tested. Splenomegaly was detected twice. Blood cultures at the time of bacteriological study were negative in all but two instances; a single colony of candida was recovered in each sample.

Thus, it was apparent that endocarditis did not result from the intravenous inoculation of C. albicans in numbers sufficient to produce pyelonephritis in normal animals.

RIGHT HEART ENDOCARDITIS

Polyethylene catheters were placed in or at the entrance to the right heart in 10 rabbits. The catheters were filled with $10^4-10^6$ candida CFU and bacteriological and morphological studies carried out after intervals of from 6 to 76 days (Table 2). None of the rabbits died, all appeared to be in excellent health throughout the course of the experiment. In addition, 16 blood cultures taken at varying times during the experiment were sterile.

All catheters were located in the right heart at the time of examination. In 9
### Table 2

**Bacteriological Findings in Rabbits with Right-Heart Catheters Filled with Candida**

| Inoculum | Rabbit no. | Days followed | Spleen<sup>b</sup> | Liver<sup>b</sup> | Rt. kidney | Lt. kidney | Urine<sup>c</sup> | Catheter<sup>e</sup> Vegetations<sup>b</sup> | Terminal blood culture | Other blood cultures |
|-----------|------------|---------------|-----------------|----------------|-------------|-------------|-------------|------------------------|----------------------|---------------------|
| 10<sup>4</sup> | 242        | 12            | 0               | 0              | 0           | 0           | 0           | 10<sup>5</sup>          | 10<sup>5</sup>         | Neg. — —             |
|           | 243        | 12            | 10<sup>3</sup>   | 0              | 10<sup>3</sup> | 10<sup>3</sup> | 10<sup>2</sup> | >10<sup>4</sup>           | 0 (<10<sup>3</sup>)    | Neg. — —             |
|           | 276        | 21            | 0               | 0              | 0           | 0           | 0           | >10<sup>4</sup>          | 10<sup>5</sup>         | Neg. — —             |
| 10<sup>5</sup> | 210        | 10            | 0               | 0              | (10<sup>5</sup>) | (10<sup>5</sup>) | 10<sup>4</sup> | 10<sup>5</sup>          | 10<sup>4</sup>         | Neg. 9 Neg.          |
|           | 211        | 23            | 0               | 0              | 0           | (10<sup>5</sup>) | 10<sup>5</sup> | >10<sup>4</sup>          | 10<sup>5</sup>         | Neg. 9 Neg.          |
| 10<sup>6</sup> | 212        | 9             | 10<sup>3</sup>   | 10<sup>3</sup> | (10<sup>5</sup>) | (10<sup>5</sup>) | 0           | 0          | —                     | Neg. 8 Neg.          |
|           | 228        | 6             | 10<sup>5</sup>   | 10<sup>2</sup> | 10<sup>3</sup> | 0           | 0           | >10<sup>4</sup>          | 10<sup>5</sup>         | Neg. 6 Neg.          |
|           | 230        | 76            | 0               | 0              | 0           | 0           | 0           | >10<sup>4</sup>          | 10<sup>5</sup>         | — 20,71 Neg.         |
|           | 231        | 76            | 10<sup>3</sup>   | 10<sup>3</sup> | 10<sup>2</sup> | 10<sup>2</sup> | 0           | 10<sup>5</sup>          | —                     | 20,71 Neg.          |
|           | 232        | 20            | 0               | 0              | (10<sup>5</sup>) | (10<sup>5</sup>) | 0           | >10<sup>4</sup>          | 0 (<10<sup>3</sup>)    | Neg. — —             |

<sup>a</sup> Kidneys with gross abscesses are noted by parentheses.

<sup>b</sup> CFU/g tissue.

<sup>c</sup> CFU/ml.
of the 10 rabbits, gross vegetations were easily visible. Candida, however, were seen in only one instance on smear of the vegetations. Nevertheless, between $10^4$ and $10^5$ candida CFU/g were cultured from the vegetations in 7 of the 10 animals. In three instances candida were recovered only from the endocardial lesions and in two animals the vegetations were sterile ($<10^3$ candida CFU/g).

The cardiac lesions were easily seen and measured several millimeters in diameter (Figs. 1 and 2). The lesions usually had creamy white material in their centers.

Splenomegaly was found twice (rabbits 210, 212); one of these animals (212) was the only one without evidence of endocarditis.

Microscopic studies of the right heart vegetations revealed the presence of small numbers of candida organisms. Of particular interest was the finding of yeast in sections of the vegetations of Rabbit No. 232 despite the inability to recover candida with the culture techniques employed (Figs. 2 and 3).

A single rabbit (No. 211) had large lung abscesses in which many yeast cells were easily found on smear. The lungs of all the remaining animals appeared normal. Tissue sections of the lungs were taken from four animals. Abscesses and granuloma-like lesions, some of which contained foamy epithelioid cells and multinucleated giant cells, were seen in the lung sections from rabbits 211 and 228. Rabbits 232 and 242, on the other hand, were normal. Pulmonary emboli and infarcts were not seen.

Renal cortical abscesses were evident grossly in four animals. There was no evidence of infarction of the kidney and there were no definite instances of glomerulonephritis.

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**Fig. 1.** Tricuspid valve vegetation 23 days after placement of catheter containing $10^5$ *Candida albicans* in the right side of the heart. The vegetation contained $10^4$ CFU/g.
Fig. 2. Tricuspid and right-ventricle vegetations 20 days after placement of catheter containing $10^6$ *Candida albicans* in the right side of the heart. No viable candida were recovered from rabbit 232.

Fig. 3. Microscopic view of fungi; section taken from vegetation shown in Fig. 2. Culture revealed < 1000 candida CFU/g. Gridley stain. Rabbit 232.
LEFT HEART ENDOCARDITIS

Catheters were placed at the level of the aortic valve in seven rabbits and filled with $10^7$–$10^8$ CFU of *C. albicans*. Examination was carried out 15–58 days later (Table 3). Only one rabbit died. The catheters were found to have passed between the aortic cusps into the left ventricle in most instances and large vegetations were found in relation to the catheter tip. Often the vegetations appeared to completely fill the aortic cusps and candida were easily seen on smear in all instances. Cultures confirmed this, revealing the presence of $>10^8$ candida CFU/g in five rabbits and $10^4$ and $10^6$ CFU/g in the remaining two. Despite the presence of fungi in the vegetations, blood cultures were all sterile.

Tissue sections confirmed the finding of large numbers of candida in the left heart vegetations (Figs. 4 and 5). Particularly interesting was the obvious localization of candida to specific zones of the abscess; a moderate number were found in the central necrotic portion of the abscess but the predominant concentration was at the edge of the necrotic zone covered by a thin layer of fibrin-like material. Of seven animals examined, two had well-defined lesions of myocarditis, in one instance with well-developed multinucleated giant cells (Fig. 6).

In the lungs of four of six animals examined, tiny focal lung abscesses resembling granulomas were seen (rabbits 263, 271, 274, and 275). Pulmonary emboli and infarcts were not seen but pulmonary arteritis was seen in one rabbit. This lesion was noted in previous studies of right-sided staphylococcus endocarditis(5). Gridley stains of these lung sections did not reveal the presence of candida.

Renal infarcts (rabbits 263, 274, 241, 271, 275, and 277) and abscesses (Table 3) were seen commonly, but no definite instances of glomerulonephritis were observed. Kidney infarcts were also common in previous studies of staphylococcal endocarditis(6). Splenomegaly was found only once (rabbit 263).

SHORT CATHETERS IN THE INFERIOR VENA CAVA

To determine if the presence of the catheter within the heart was necessary for the production of endocarditis with candida, as had been shown previously for staphylococci, catheters were inserted for a short distance into the inferior vena cava of six rabbits. The catheters were filled with $10^3$–$10^4$ candida CFU and the organs examined 16–18 days later.

All catheters were found in the vena cava below the level of the renal veins. The catheter tips and 1–2 cm of the catheter below the tip were usually covered with white friable fibrin-like material. Culture of this material was positive in two instances whereas in the remaining four animals candida were recovered from the spleen, kidneys, or liver. Despite this evidence of egress of candida from within the catheters, the hearts of all six rabbits were normal. Blood cultures at the time of bacteriological study were negative in all instances.
| Inoculum | Rabbit no. | Days followed | Spleen* | Liver* | Rt.* kidney | Lt.* kidney | Urine* | Catheter* contents | Vegetation* | Terminal blood culture | Other blood cultures | Day | Result |
|----------|------------|---------------|---------|--------|-------------|-------------|--------|-------------------|-------------|----------------------|------------------|-----|--------|
| 10       | 263        | 15            | $10^6$  | $10^6$ | ($10^6$)    | ($10^6$)    | —      | $>10^4$           | $10^6$      | Neg.                 | 13               | Neg. |
| 10*      | 274        | 20            | $10^6$  | $10^6$ | $10^6$      | 0           | —      | $>10^4$           | $10^6$      | Neg.                 | 1,15             | Neg. |
| 10*      | 241        | 58            | $10^6$  | $10^6$ | ($10^6$)    | $10^6$      | —      | —                | $10^6$      | Neg.                 | 27,56            | Neg. |
| 10*      | 247 (died) | 22            | $10^6$  | $10^6$ | ($10^6$)    | $10^6$      | —      | —                | $10^6$      | Neg.                 | 21               | Neg. |
| 10*      | 271        | 21            | $10^6$  | $10^6$ | $10^6$      | 0           | —      | $>10^4$           | $10^6$      | Neg.                 | 2,16             | Neg. |
| 10*      | 275        | 21            | $10^6$  | $10^6$ | ($10^6$)    | 0           | —      | $>10^4$           | $10^6$      | Neg.                 | —                | —    |
| 10*      | 277        | 19            | 0       | 0      | $10^6$      | 0           | —      | $>10^4$           | $10^6$      | Neg.                 | —                | —    |

* Kidneys with gross abscesses are noted by parentheses.
* CFU/g tissue.
* CFU/ml.
Fig. 4. Gridley stain of left-heart vegetation, 21 days after placement of catheter containing $10^5$ *Candida*. Note the distribution of fungi in different zones of the vegetation. Culture revealed $10^5$ *Candida* CFU/g. Rabbit 275.

Fig. 5. Gridley stain of aortic vegetation 15 days after placement of catheter containing 10 *Candida* CFU. Culture grew $10^5$ viable Units/g. Rabbit 263.
DISCUSSION

These experiments demonstrate a method for producing right or left heart endocarditis with *C. albicans* in rabbits. Previous studies have shown that placement of a sterile polyethylene catheter in the chambers of the right or left sides of the heart led to the development of nonbacterial endocarditis which did not become infected unless the catheters were filled with microorganisms or unless microorganisms were injected intravenously (5,6). The ease and reproducibility of the model infection is similar to that reported previously using staphylococci as the test organism (5,6).

Experimental candida endocarditis was remarkably well tolerated; only one animal died and it was in the group with left-sided infection. Between $10^4$ and $10^6$ candida CFU/g were cultured from the vegetations of seven of the nine animals with right sided endocarditis. Fewer than $10^3$ candida CFU/g were cultured from the vegetations of the remaining two rabbits (Figs. 2, 3). On the left side of the heart, even though the catheters were filled with an inoculum 2–5 logs lower than that used to fill the right heart catheters, the number of candida recovered from the vegetations was considerably greater than on the right side. Between $10^5$ and $10^7$ candida CFU/g were recovered from five of seven animals with left-sided endocarditis (Figs. 4 and 5) and the remaining two animals had vegetations containing $10^4$ and $10^5$ candida CFU/g. Thus, it appears that *C.*
Candida albicans multiplied to a greater density in lesions on the left side of the heart than on the right side of the heart.

In studying the evolution of staphylococcal endocarditis after removal of the polyethylene catheter required for the initiation of infection, it had been found that endocarditis on the right side of the heart had a tendency to heal more rapidly than endocarditis on the left side of the heart (7). The explanation for these differences in host–microbe relations on different sides of the heart remains a problem for future study. This difference suggests, however, that the preponderance of left-sided endocarditis in man may not be, as is commonly believed, solely the result of increased frequency of underlying valvular disease on the left side of the heart, since in these experiments microorganisms seemed to multiply to a greater density (candida) and persist better (staphylococci) on the left side of the heart despite their growth in comparable vegetations produced in similar ways.

Candida endocarditis resulted in the death of only one of seven rabbits with left-sided infections and in none of the nine animals with right-sided endocarditis. This contrasts markedly with the outcome of staphylococcal endocarditis reported previously; 3 of 15 rabbits with right-sided endocarditis and all eight of the rabbits with left-sided infections died (5, 6).

The explanation for the dependence of the frequency of fatal outcome upon the location of the infection (right or left side of the heart) and the nature of the infecting microorganism is a most interesting area for speculation. It had been reasoned previously that the greater mortality with left-sided as compared with right-sided staphylococcal endocarditis might be due to some property of the staphylococcus growing more vigorously on the left side of the heart (7). This position was based on the finding that right-sided infection healed more rapidly than left-sided infection after removal of the polyethylene catheter. The peripheral embolic consequences of left-sided infection were not thought to explain the greater frequency of death with left-sided disease since sterile vegetations on the left side of the heart were not fatal and yet seemed to produce the same frequency of peripheral emboli as infected left-sided endocarditis.

The present studies with candida are pertinent to this problem of the cause of death in left-sided staphylococcal endocarditis. The greater density of candida in left-sided vegetations support the previous studies derived from observations of the rate of healing of the staphylococcal lesions. Furthermore, only one of seven rabbits with left-sided candida endocarditis died. Thus, despite the frequency of renal abscesses and infarcts comparable to that found with staphylococcal infection, infection with candida was well tolerated.

These observations suggest that death in experimental staphylococcal endocarditis in rabbits results from some substance or substances produced by microorganisms multiplying in the vegetations. In this way vegetations supporting more vigorous microbial multiplication would be more likely to kill the animals and the fatal nature of the infection would also depend on the ability of microorganisms to produce these injurious substances (toxins). Considering how little we know about the mechanisms by which infections cause death, this model ap-
pears to be a promising method by which to study microbial toxin production and the outcome of endocardial infection in the rabbit.

An interesting feature of the vegetations containing *C. albicans* is the orientation of the yeast as seen in the microscopic sections. The previous studies of staphylococcal endocarditis revealed microscopic solid masses of cocci enmeshed in laminated acellular material. In the present study, however, candida were oriented differently. They were found either scattered through necrotic tissue or more commonly in a ring at the periphery of a mass of necrotic tissue (Figs. 7 and 8).

Microscopic studies did not reveal the presence of pulmonary emboli or infarcts in the lungs of animals with right heart infection despite the frequent finding of renal infarcts in animals with left heart infections. In previous studies with staphylococci similar findings obtained. Pulmonary arteritis was seen after right heart staphylococcal infection but in the present study occurred only once with left heart infection. The pathogenesis of these arterial lesions is not clear; the possibility of either an immunologic or an infectious basis is obvious.

The finding of pulmonary and myocardial lesions resembling granulomas, as well as renal infarcts and abscesses, is consistent with blood stream dissemination of yeast and thus calls into question the meaning of the negative blood cultures in these animals. Intermittent dissemination of candida would provide an explanation, but one would have expected to find a positive blood culture at least occasionally.

After intravenous challenge, candida were found rarely in the urine despite evidence of multiplication and tissue destruction in the kidney. Previous studies of experimental pyelonephritis have demonstrated that bacteria are not normally filtered by the kidney but appear regularly in the urine when bacterial multiplication takes place in the kidney. This suggests either that urine inhibits the growth of candida or that the mode of access of candida to the urine from tissue infection is different for candida than it is for bacteria. There is evidence to support the latter view.

Candida endocarditis is usually associated in man with a source of blood stream contamination superimposed on underlying valvular disease. The present model reproduces these circumstances in the rabbit. Although many patients with nosocomial candida infections have immunological abnormalities, acquired through disease or induced by drug treatment, and others have been under treatment with antibiotics or corticosteroids, there is uncertainty as to the role these factors play in the development of candida infection. Those aspects of underlying illness and therapy which have been implicated in the initiation of candidiasis include providing a portal of entry for infection by intravenous medication and altering the flora of the host by antibiotic treatment to favor the growth of fungi.

The present model closely resembles the disease seen in man; factors predisposing to infection, the histology of the vegetations, the finding of inflammatory foci and granulomatous lesions in the heart as well as renal infarcts and abscesses with a relatively low frequency of positive urine cultures are all typical of the
human disease (2, 10–12). The absence of large vessel emboli, splenic enlargement, or positive blood cultures are features which differentiate the experimental from human infection. However, the blood culture technique in the present study was quantitative and the occurrence of large vessel emboli clinically (about 35% of cases) was noted predominantly in patients with endocarditis of some months’ duration (2). Thus, these discrepancies may relate to questions of bacteriological technique and duration of infection rather than to any difference in the fundamental nature of the disease. There is no obvious explanation for the infrequent finding of splenomegaly with candida infection in the rabbit. Splenomegaly was common with experimental staphylococcal endocarditis; its absence in candida infection may represent a difference in host immunological response. Questions as to the effect of antibiotics, corticosteroids, and immunosuppressive agents on the course of this infection are of obvious importance and will serve as subjects for future study.

SUMMARY

Methods are described for the production of Candida albicans endocarditis in rabbits. Infections were easily produced in the right or left sides of the heart in association with placement of indwelling intravascular polyethylene cannulas. The infections were well tolerated and were not accompanied by positive blood cultures or splenomegaly despite the finding of large numbers of microorganisms in the endocardial vegetations and foci of metastatic infection in the kidneys. Multiplication of fungi was greater in the left side of the heart than in the right side. This model reproduces circumstances similar to those in which infection is seen in man and appears suitable for the study of the pathogenesis and therapy of C. albicans endocarditis.

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