FEVER FROM PATHOGENIC FUNGI *

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Most studies of fever have dealt with the pyrogenic activity of the bacterial lipopolysaccharides of gram-negative bacteria (1). The pyrogenic properties of pathogenic gram-positive bacteria, on the other hand, have been the subject of only a few reports. It is of interest, however, that intravenous injection of living staphylococci and corynebacteria were found to produce higher fever in rabbits than did gram-negative bacteria (2), and that pronounced elevations of body temperature were found after intravenous injections of both living and heat-killed streptococci, as well as streptococcal lysates (3). The pyrogenicity of another group of gram-positive microorganisms, the pathogenic fungi, has received no consideration in the literature, although Salvin (4) reported that they possess lethal endotoxins for mice. The following study was undertaken, therefore, to determine which of the common pathogenic fungi can induce fever and to compare their pyrogenic effect with that of the lipopolysaccharides of gram-negative bacteria. The presence of pyrogens in saprophytic molds has been reported but the characteristics of the pyrogen and of the fever response were not described (5).

METHODS AND MATERIALS

Source of pathogenic fungi. All but one strain were isolated from human infections. The strain of *Candida albicans* was cultured from the blood of a patient with fatal hematogenous candidiasis (6), and the strain of *Cryptococcus neoformans* from the spinal fluid of a patient with fatal cryptococcal meningitis. Two strains of *Histoplasma capsulatum* were obtained from Dr. Chester Emmons, of the National Institutes of Health. One of these (*H. capsulatum* G-3996) was isolated from soil and the other (H-635) at autopsy. The Hattie Davis and Sautman strains of *Blastomyces dermatitidis* were provided by Dr. Robert Abernathy and *Sporotrichum schenckii*, strain 7019, was obtained from the American Type Culture Collection.

Suspensions of fungi. Fungi were washed from the surface of their culture medium with a pyrogen-free buffer (pH 7.1) containing NaH_2PO_4 1.6 g, Na_2HPO_4 7.5 g, and NaCl 4.4 g per L H_2O, washed 3 times by centrifugation and suspended in 1.0 ml of the buffered saline to give the desired number of fungus cells. The fungus cells were enumerated in a Spencer bright line Neubauer hemocytometer counting chamber after dilution in saline in a red cell or white cell pipet.

Pyrogen studies. Living or autoclaved fungus suspensions were injected into the marginal ear vein of rabbits and temperatures were measured rectally by inserting the glass probe of the thermistor apparatus (Sargent). Autoclaving of fungi was done at 145° C for 15 minutes at 15 pounds pressure, and always sterilized the culture. Glassware and needles were rendered free of pyrogens by heating in an oven at 170° C for 2 hours. Pyrogen-free solutions of physiologic saline and water were purchased from the Abbott Company. Albino rabbits of both sexes, weighing 3 to 5 kg, were kept in an air-conditioned room at approximately 70° F. They were conditioned by measuring the temperatures hourly for 8 hours on the day preceding each experiment and animals were used only if their temperatures remained within a constant range of plus or minus 0.3° F during the period of conditioning. On the day of the experiment, temperatures were measured at 30-minute intervals for 2 hours before injection and hourly for 8 hours thereafter. Temperatures were also taken immediately before injection and 30 minutes afterward. Animals were rejected for the study if their temperatures were not constant during the preliminary period of 2 hours before injection.

EXPERIMENTAL RESULTS

A. Fever

1. Cryptococcus neoformans

The entire growth obtained after culture for 11 days at room temperature on the surface of Sabouraud-dextrose agar was suspended in buffered saline. One ml of a heavy turbid suspension of living cryptococci, containing about 2.5×10^8 yeast cells, was then injected intravenously into three rabbits. No fever resulted on the day of injection or on three subsequent days of observation. The rabbits showed no signs of illness from the injection. The experiment was repeated with a 71 day old culture of *C. neoformans*. An inocu-

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lum of 2.5×10^8 cells again failed to produce fever.

An attempt was made to release a pyrogen by autoclaving and agitating with glass beads the suspension of 10^8 cryptococci per ml until they appeared fragmented, had lost their gram-staining properties, and stained only faintly with safranine. The disrupted cryptococci were then centrifuged at 2,000 rpm for 4.5 hours and 5.0 ml of supernate was injected intravenously into each of five rabbits. Three rabbits developed fever with peaks of 1.2°, 2.4°, and 4.0° F, respectively. Fever began to rise after latent periods of 30 to 60 minutes and the temperatures returned to normal 8 to 10 hours after injection. Two rabbits did not develop fever.

2. Candida albicans

a. Living agar cultures. Intravenous injection of 1 ml of a suspension of a five day culture of *C. albicans* grown on Sabouraud-dextrose agar at 27° C and containing 10^9 yeast cells produced high fever in four rabbits. After a latent period with no fever, lasting 30 minutes, the temperature rose 0.8° to 2.8° F at 1 hour and reached a peak of 3.8° to 5° F at 4 hours. The temperatures then fell to 1.6° to 2° F at 7 hours but rose again to similar peaks by 24 hours. The fever remained elevated until all animals died before 48 hours (Figure 1, C-71).

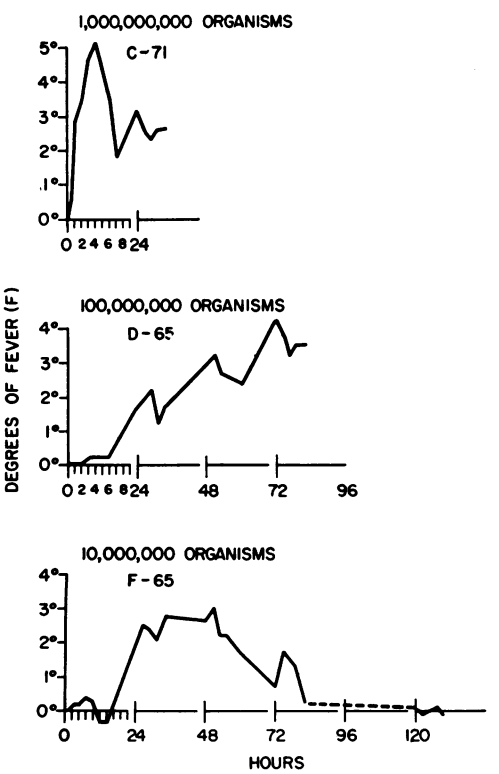

FIG. 1. FEVER RESPONSE TO LIVE *CANDIDA ALBICANS* INJECTED INTRAVENOUSLY.

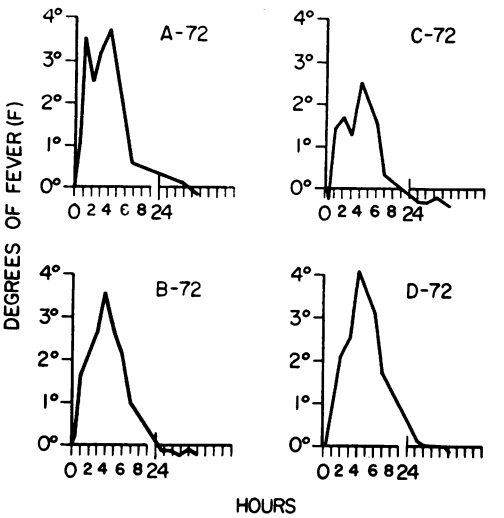

FIG. 2. FEVER RESPONSE FROM 1×10^9 AUTOCLAVED *CANDIDA ALBICANS* (SUHR) INJECTED INTRAVENOUSLY.

b. Living broth cultures. The growth at 27° C in a four day culture in trypticase soy broth was collected on the filter pad during Seitz filtration and washed three times in buffered saline. The organisms were then suspended in the buffered saline to produce a count of approximately 10^9 yeast cells per ml and tenfold serial dilutions were made to give suspensions with 10^8 and 10^7 cells per ml, respectively. One ml of each serial dilution was then injected intravenously. The rabbits given 10^9 organisms collapsed at 3 hours, developed extreme hypothermia to a level 4° F below the initial temperature and died. Two rabbits inoculated with 10^8 candida cells remained afebrile for 2 hours and then developed a rise in temperature of 1.2° and 2.0° F at 3 hours. The temperature in both animals continued to rise to 1.8° and 2.8° F at 5 hours and remained elevated during the four day period of observation. A third rabbit inoculated with 10^8 organisms remained afebrile the first day but developed a high fever at 2.3° F the second day and this continued to mount during the four day period.

Inoculation of 10^7 living candida cells into three rabbits produced essentially no fever on Day 1, but all three had fevers on Day 2 ranging from 0.8 to 2.4° F. The fever continued through the fourth day.

One to 2.0 ml of blood collected during periods of highest fever from the central ear veins of each of the six rabbits, 48 hours after injection, was cultured on Sabouraud's agar, but no growth of candida developed. The dose of 10^8 yeast cells was lethal in about one week, but all three rabbits given 10^7 cells survived.

Representative fever curves for each dose of viable candida are shown in Figure 1.

c. Autoclaved agar culture. A 48 hour culture of *C. albicans* grown on Sabouraud-dextrose agar at 27° C was autoclaved, washed three times, and suspended in buffered saline in a concentration of 10^9 organisms per ml. One ml was then injected into the marginal ear vein of each of two rabbits. Another two rabbits were inoculated intravenously with 10^9 autoclaved candida cells that had been grown in trypticase soy broth at room temperature for 48 hours and washed three times in buffered saline. All rabbits developed high fever and the fever of two was biphasic (Figure 2). There was no difference in fever response to the two types of cultures. In three rabbits there was a latent period of 30 minutes before the temperature began to rise, but fevers of 0.8° to 3.4° F were noted in all four at 1 hour and maximum peaks of 2.4° to 4° F were present at 4 hours. Thereafter, the temperature fell rapidly and in two animals reached normal levels by 7 hours. No fever was noted the following day and the animals showed no ill effects from the inoculation either during the fever or afterwards.

d. Culture filtrates. A culture of *C. albicans,* incubated in trypticase soy broth at room temperature for four days, was passed through a Seitz filter and 10.0 ml of clear sterile filtrate was injected intravenously into two rabbits. Two control rabbits were injected intravenously with 10.0 ml sterile trypticase soy broth. The culture filtrate produced a brisk rise in temperature to 1.5° F in both animals at 1 and 2 hours after an afebrile lag period of 30 minutes. After 2 hours the temperature fell sharply and reached normal by

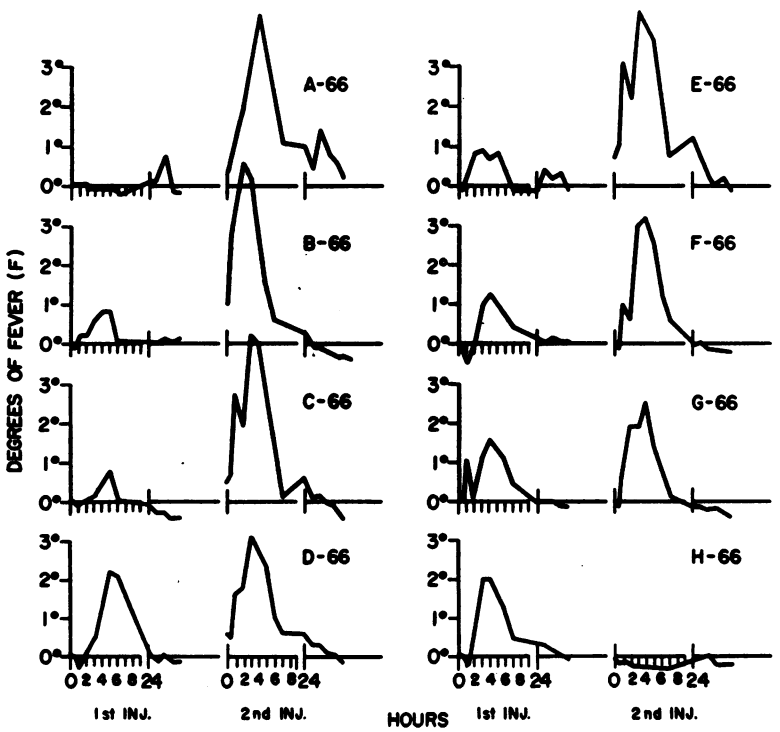

FIG. 3. FEVER RESPONSE AFTER FIRST AND SECOND INTRAVENOUS INJECTION OF LIVE *HISTOPLASMA CAPSULATUM* (AT TWO-WEEK INTERVALS).

4 hours. The animals showed no ill effects from the injection.

No fever was produced by injecting plain sterile broth in control animals.

3. Histoplasma capsulatum

a. Living agar cultures. A culture of *H. capsulatum* (G-3996), incubated at room temperature for 23 days, was removed from Sabouraud-dextrose agar with sterile buffered saline and reduced to an even suspension in a tissue homogenizer. Each of a group of eight rabbits received intravenously 1.0 ml of a saline suspension adjusted to contain 10^6 tuberculate chlamydospores per ml (Figure 3). Seven of the animals developed fever. In two animals the fever was less than 1° F; in the remainder it ranged from 1° to 2.2° F. There was a lag period of 2 hours before the temperature began to rise in five febrile animals and of 1 hour or less in two others; this lag period resembled that observed in animals given 10^8 living candida organisms. The temperature returned to normal in 6 to 8 hours and remained normal the next day. The animals showed no ill effects from the injection.

b. Autoclaved agar culture. A culture of *H. capsulatum* (H-635), grown on Sabouraud-dextrose agar for 45 days at room temperature, was suspended in saline by the method described in 3a and autoclaved. Six rabbits were then injected intravenously with 1.7×10^6 tuberculate chlamydospores in 1.0 ml of the sterile autoclaved suspension. Five rabbits experienced immediate high fevers reaching 1.5° to 4.6° F without the lag period observed in rabbits given the living culture. The temperature rise was started when the first temperature was taken 30 minutes after injection. By 7 to 8 hours the temperature was back to normal.

4. Blastomyces dermatitidis

a. Living agar culture. After growth for 29 days at room temperature a culture of *B. dermatitidis* (Davis strain) was removed from Sabouraud-dextrose agar and a suspension prepared as described in 3a. Four rabbits received intravenously 1.0 ml of a suspension of living organisms containing approximately 3.5×10^7 spores. All animals developed high fevers reaching 1.6° to 3° F (Figure 4). The temperature rose after a lag period of 30 minutes and usually reached a peak in 4 hours. Then the temperature fell abruptly to normal by 6 hours. There was no fever the next day and the animals did not appear ill.

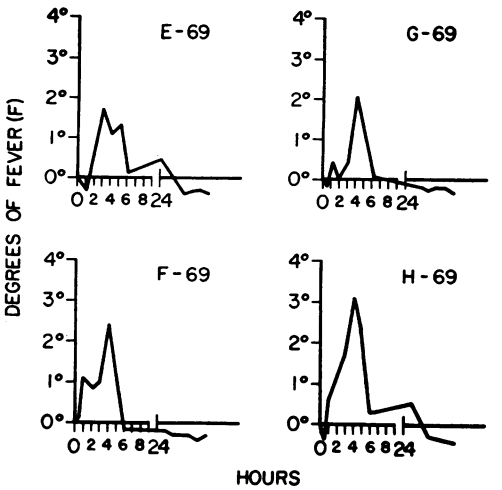

FIG. 4. FEVER RESPONSE TO INTRAVENOUS INJECTION OF 35,000,000 SPORES OF LIVE *BLASTOMYCES DERMATITIDIS.*

b. Autoclaved agar culture. A culture of *B. dermatitidis* (Sautman strain) grown on Sabouraud-dextrose agar for 42 days at room temperature was suspended in saline and autoclaved. Each of five rabbits was then injected intravenously with 1.0 ml of a suspension containing 2.5×10^7 spores. Four of the rabbits developed fevers ranging from 0.6° to 3.6° F (Figure 5, first injection). In each case there was a preliminary drop in temperature at 30 minutes followed by fever peaks at 3 to 4 hours. The injections were well tolerated.

5. Sporotrichum schenckii

A yeast phase culture of *S. schenckii,* grown on trypticase soy agar with 10 per cent rabbit blood at 37° C for six days, was removed in sterile buffered saline, washed three times by centrifugation, and resuspended so that there were 1.75×10^9 organisms per ml. One ml was then injected intravenously into each of four rabbits. All developed high fever reaching 2.0° to 4.6° F (Figure 6). The lag phase varied from 30 to 60 minutes and the peak was reached at 5 hours in all animals. No ill effects were apparent from the injection.

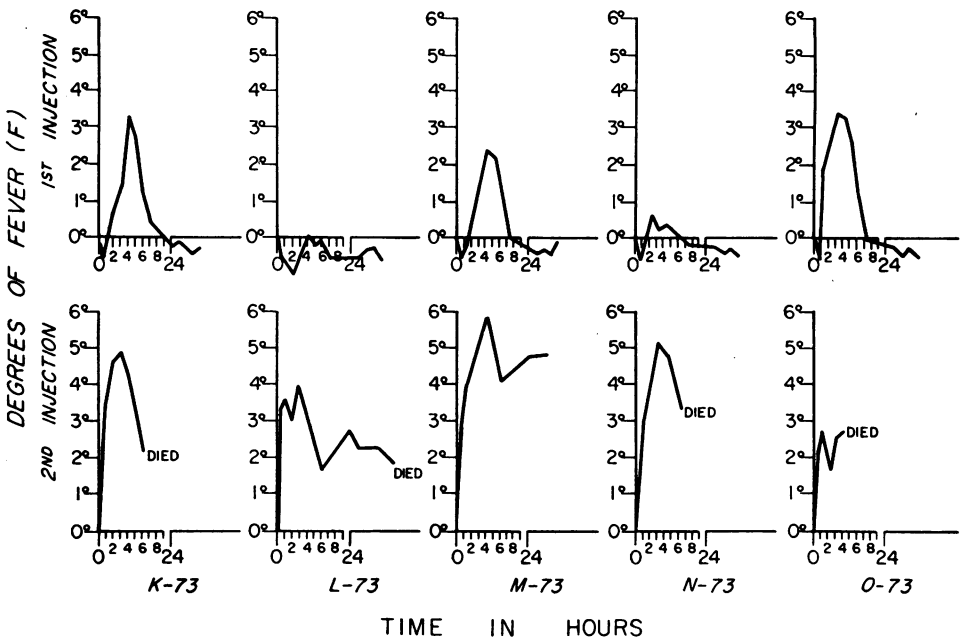

FIG. 5. FEVER RESPONSE AFTER FIRST AND SECOND INTRAVENOUS INJECTION OF 2.5×10^7 AUTOCLAVED *BLASTOMYCES DERMATITIDIS* (AT TWO-WEEK INTERVALS).

Two of the four rabbits developed fever after the first day. One animal showed daily spikes for eight days and then the temperature remained normal. Another sustained a high spike, 4.4° F, on the second day but not afterwards.

B. Hematologic Changes Accompanying Fever

Total and differential leukocyte counts were made on the blood obtained from the ear veins of the four rabbits given 10^9 living *C. albicans* and in the four given 10^9 autoclaved organisms. All

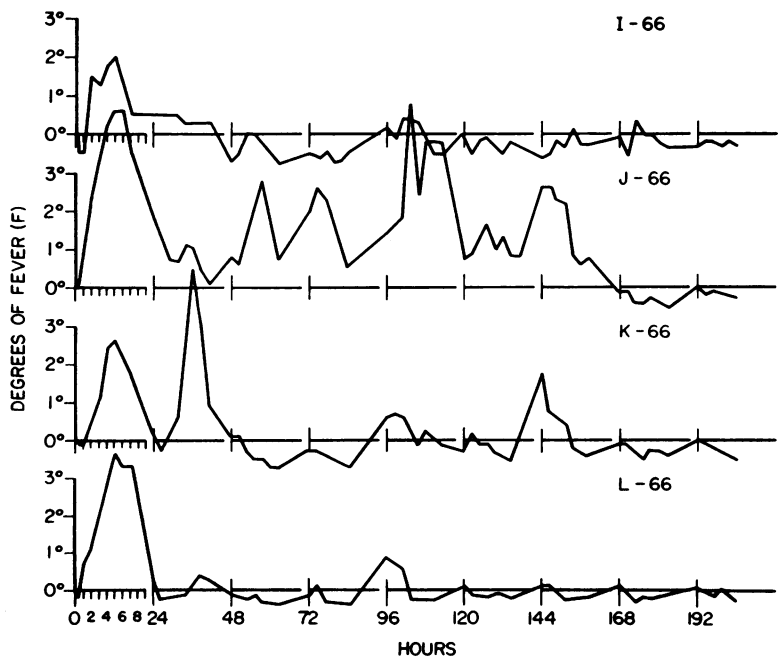

FIG. 6. FEVER RESPONSE TO 1.75×10^9 *SPOROTRICHUM SCHENCKII* INJECTED INTRAVENOUSLY.

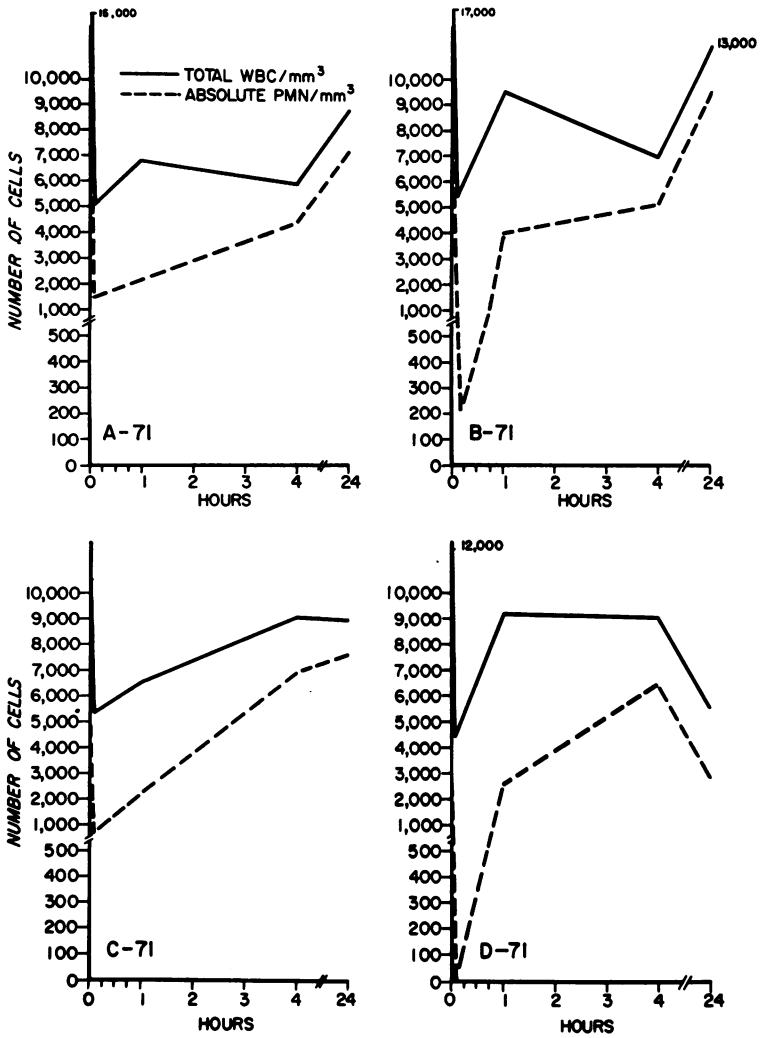

FIG. 7. BLOOD LEUKOCYTE RESPONSE TO INTRAVENOUS INJECTION OF 10^9 VIABLE *CANDIDA ALBICANS*.

leukocyte counts were made in duplicate before injection of yeast and at intervals of 5 minutes, 1 hour, 4 hours and 24 hours after injection. As shown in Figures 7 and 8, both living and dead candida produced a sharp neutropenia within 5 minutes after injection and a rebound to normal or above in 1 to 4 hours.

C. Effects of Repeated Injections on Fever

1. Candida albicans

Two rabbits that had received 5×10^8 autoclaved candida intravenously 24 days earlier were given second intravenous injections with 5×10^8 autoclaved candida. This produced higher and more prolonged fever than that experienced with the initial injection 24 days previously, but no fever was present the following day. Two weeks later a series of five daily injections of the same inoculum was given but no consistent evidence of a gradually diminishing febrile response (tolerance) was found. The animals became weaker and more listless with each injection.

Four additional rabbits, that had received 4.5×10^9 autoclaved *C. albicans* intravenously 14 days earlier, were given second intravenous injections of the same dose. Three of these sustained higher and more prolonged fever than that elicited by the first injection two weeks previously. Daily injections of the same inoculum, given for a

total of five consecutive days, produced no definite evidence of a diminishing response except in one rabbit, and this animal reacted on the fifth day with a fever response greater than that observed with the first injection.

The possibility was considered that tolerance was not demonstrated because the dose of fungus pyrogen may have been too large. An attempt was made, therefore, to determine the minimal pyrogenic dose and repeat the attempts to establish tolerance. Autoclaved suspensions of *C. albicans* were prepared in pyrogen-free saline and serial tenfold dilutions were made so that the final concentrations of fungus cells were 10^6, 10^7 and 10^8 per ml, respectively. A total of nine rabbits was then examined for tolerance by inoculating 1 ml of each concentration intravenously into three rabbits at daily intervals. The lowest dose of 10^6 candida cells proved to be a minimal pyrogenic dose and produced fevers reaching 0.5° to 0.8° F and lasting 4 hours. No sign of a diminished pyrogenic response was observed in any of the nine rabbits after eight daily injections. Instead, the fevers tended to be greater with successive injections.

2. Histoplasma capsulatum

a. Live agar culture. Eight rabbits that had received 10^6 live tuberculate chlamydospores (see Section 3a) were each challenged again intrave-

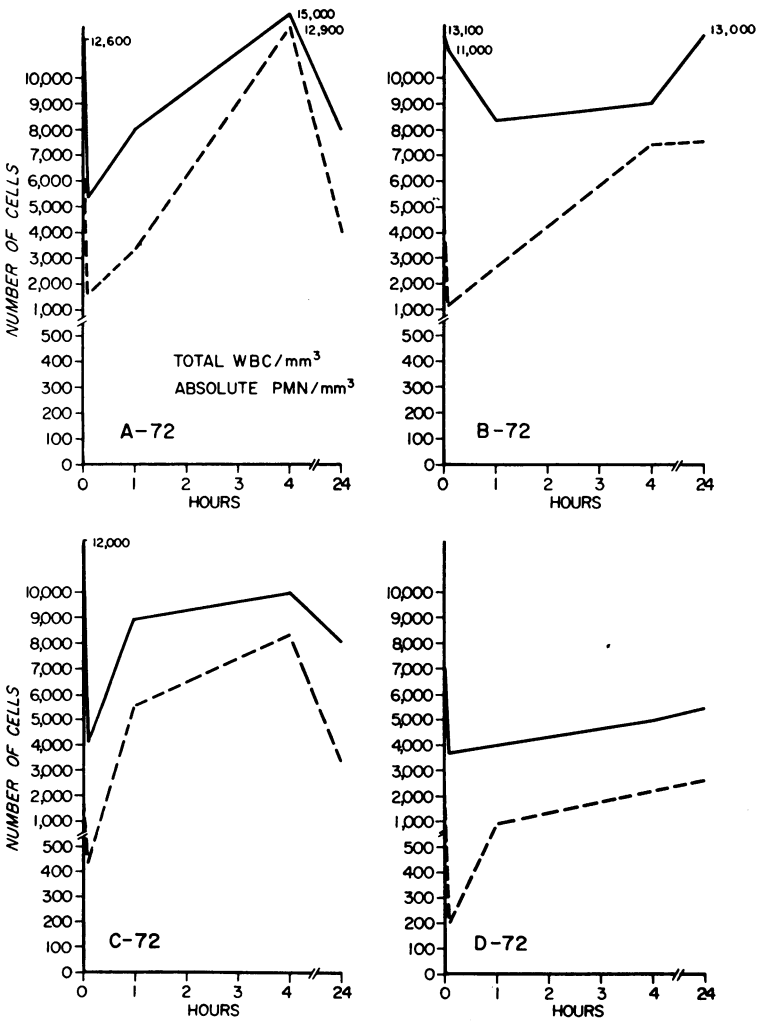

FIG. 8. BLOOD LEUKOCYTE RESPONSE TO INTRAVENOUS INJECTION OF 10^9 AUTOCLAVED *CANDIDA ALBICANS*.

nously two weeks later with 10^6 live histoplasma grown for 18 days on Sabouraud-dextrose agar at room temperature. The fever in seven of the rabbits was much higher and prolonged than at the time of the first injection, when the same number of chlamydospores was inoculated. In all seven rabbits the fever rise was more than 2°, in five, more than 3° and in four, more than 4° F. At the time of the first injection, on the other hand, the temperature of only one animal rose above 2° F (2.2). The second curves also tended more than the first to be biphasic (Figure 3).

b. Autoclaved agar culture. Five rabbits given the autoclaved agar culture of *H. capsulatum* described in 3b were given a second intravenous injection of the same inoculum containing 1.7×10^6 tuberculate chlamydospores on the next day. Three died within a few hours after the injection and the remaining two were found dead the next morning. Only one animal exhibited a significant fever.

In order to determine the role of hypersensitivity in the rapid deaths that followed the second injection of *H. capsulatum,* skin tests were performed with histoplasma filtrates. The autoclaved fungus suspension used for intravenous injection was centrifuged at 2,000 rpm and 0.1 ml of the clear supernate was injected intradermally in the mid-portion of the back of three rabbits 24 hours after each had received 1.7×10^6 chlamydospores intravenously. An intradermal injection of 0.1 ml normal saline served as a control in the same rabbits. Identical tests with histoplasma filtrate and saline were performed in three control rabbits that had received no intravenous injection of fungi. No dermal hypersensitivity was found in the rabbits inoculated intravenously with the histoplasma cells 24 hours before skin testing. The reaction produced by injecting the histoplasma filtrate was the same in test animals and controls. During the first hour the swelling at the test site was no greater than that at the saline site; and at 24 hours slight erythema persisted at the test sites in both groups of animals.

3. Blastomyces dermatitidis

Five rabbits were given a second intravenous inoculation of 2.5×10^7 spores of autoclaved *B. dermatitidis* (Sautman strain) 14 days after the first. The comparative febrile responses to the two injections are shown in Figure 5. Four of the animals sustained fevers higher than 4.0°, and three, higher than 5.0° F. In all animals the lag period was abolished and high fevers were present at 30 minutes. Unlike the first injection, the second killed four animals. The outstanding changes postmortem were pulmonary hyperinflation and engorgement with blood and edema fluid.

No immediate or delayed dermal hypersensitivity to Blastomycin[1] or to the supernate from the autoclaved blastomyces suspension was noted in four additional rabbits subjected to skin tests 14 days after intravenous injection of autoclaved *B. dermatitidis.* The techniques for preparation of the supernate and for skin testing were the same as those described above for histoplasma supernates. Three normal rabbits that had never received fungus injections served as controls.

The same accelerated and increased fever response was seen in rabbits given this inoculum of autoclaved *B. dermatitidis* 14 days after the inoculation of living *B. dermatitidis* (3.5×10^7 spores of the Davis strain). Four such infected animals developed fevers of over 5° F and died. These animals also showed severe pulmonary engorgement and overdistention. In addition, there were blastomycotic abscesses in the lungs and kidneys.

4. Cryptococcal lysate

Five ml of the cryptococcal lysate prepared by disrupting the cells with glass beads (as described in A1) was injected intravenously daily into each of five rabbits. All developed fever with no evidence of a diminished response after one week of consecutive daily injections. Instead, all showed gradually increasing fever with succeeding injections of the yeast cell lysate.

D. Passive Transfer of Circulating Pyrogen

Nine rabbits were inoculated intravenously with a suspension of 5×10^8 *C. albicans* prepared as described in Section A2a. One died before 24

[1] Blastomycin was obtained from Parke, Davis and Co., Detroit. It is described as a sterile filtrate from the culture of the mycelial phase of *B. dermatitidis* grown on liquid synthetic medium. Each rabbit received 0.1 ml of Blastomycin intradermally.

hours, five were exsanguinated at 24 hours, and three were exsanguinated at 48 hours. The temperature elevations at the time of bleeding at 24 hours ranged from 1.9° to 6.2° with an average of 3.7° F; at 48 hours they ranged from 1.1° to 2.7° with an average of 1.8° F. The sera obtained at 24 hours and 48 hours were pooled separately. Three ml of the 24 hour pool and 3.0 ml of the 48 hour pool were cultured on trypticase soy agar and trypticase soy broth, and both pools proved to be sterile. The 24 hour pool was divided into five portions of 30 ml and the 48 hour pool into two 25-ml portions. Each portion of pooled serum was injected intravenously into a normal rabbit. Three rabbits receiving the 24 hour pooled serum developed fevers lasting 3 hours with single peaks in each at 1 hour of 1.0°, 1.6°, and 1.7°, respectively. No fever occurred in the two rabbits given 48 hour pooled serum. *C. albicans* was recovered by culture in enormous numbers from the kidneys of all nine donor rabbits sacrificed at 24 and 48 hours after injection. These infected kidneys were studded with microscopic mycotic abscesses which became grossly evident in other rabbits followed for 76 hours (Figure 9). The absence of a pyrogen in the 48 hour serum may have been related to the fact that the fevers were lower than at 24 hours when serum was found to contain a pyrogen.

E. Pyrogen Controls

No rise in temperature resulted from the injection of 1.0 ml of each of the following materials intravenously in rabbits: *1*) Buffered saline. *2*) Trypticase soy broth. *3*) Agar washings prepared from Sabouraud-dextrose agar by flooding it with buffered saline and scraping bits of agar loose from the surface. *4*) Agar washings prepared in a similar fashion from trypticase soy agar containing 10 per cent rabbit blood. These washings developed a strong tinge of color from the hemoglobin.

DISCUSSION

The pyrogenic properties of the pathogenic fungi examined in this study were similar in many respects to those of the gram-negative bacteria and their lipopolysaccharide pyrogen. The pyrogens of both fungi and gram-negative bacteria resist heat, pass through Seitz filters, induce neutropenia and elicit similar fever curves. These fever curves are characterized by: *1*) a lag pe-

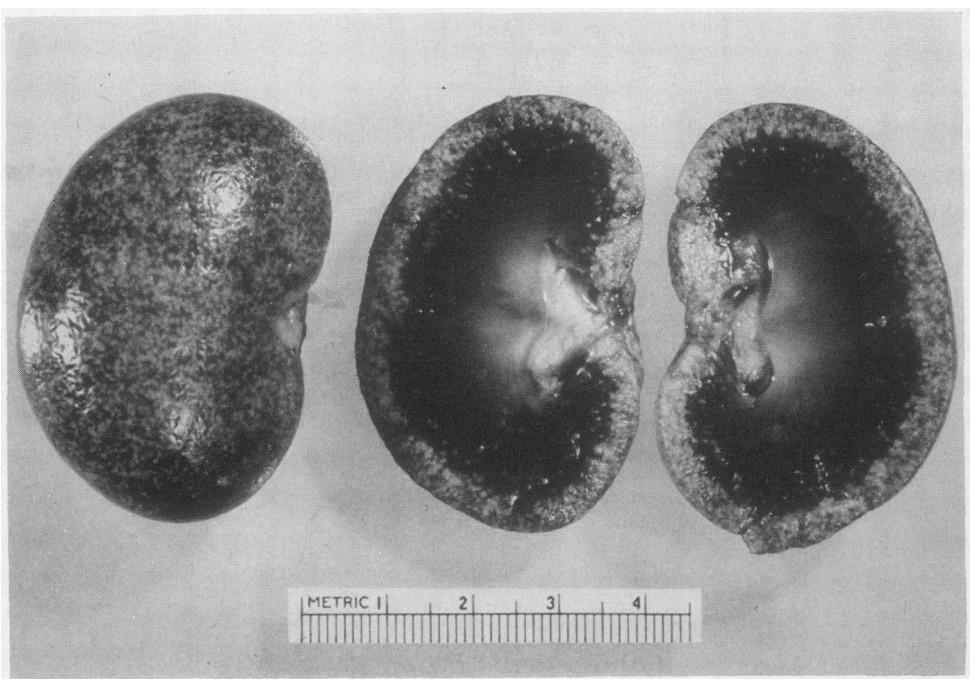

FIG. 9. MYCOTIC ABSCESSES OF RABBIT KIDNEYS 96 HOURS AFTER INTRAVENOUS INOCULATION OF 5×10^8 VIABLE *CANDIDA ALBICANS*.

riod, *2*) one or two distinct peaks, and *3*) a total duration of 7 to 8 hours with large (but not overwhelming) doses. These similarities between pyrogenicity of the gram-positive fungi and gram-negative bacteria provide additional evidence that endotoxic properties are not limited to the latter group of microorganisms (3, 4). The only apparent difference between the fungus pyrogen and the gram-negative pyrogen was found upon repeated injections. Repeated injections of the pyrogens of gram-negative organisms lead to diminished febrile reactions and greater resistance to the other toxic effects (1). No convincing evidence of such tolerance was found upon repeated injections of fungi; instead the pyrogenicity and toxicity were usually enhanced. For example, a second injection of autoclaved histoplasma organisms 24 hours after the first was lethal. While two consecutive injections of bacterial lipopolysaccharide can also kill rabbits through the effects of the generalized Shwartzman reaction, preliminary evidence indicates that the phenomena are not related. The generalized Shwartzman reaction is not readily produced in adult rabbits and does not elicit the rapid (often instantaneous) deaths observed with a second daily dose of *Histoplasma capsulatum* (*7*). A similar susceptibility to injury by fungi was reported by Henrici (8) who produced anaphylactic reactions in rabbits given a second injection of an extract of *Rhizopus nigricans.*

The only organism examined in this study that failed to produce fever was *Cryptococcus neoformans.* This observation is of interest because, in contrast to other disseminated fungus infections, there is but little fever in disseminated cryptococcosis (9). The cryptococcus also differs from the other fungi in this study in two other respects: *1*) its failure to form mycelium, and *2*) its production of an enormous capsular polysaccharide. There is a possibility that the capsule may interfere with the pyrogenicity of the yeast cell by enclosing the pyrogenically active material. This idea is supported by reports that another encapsulated gram-positive organism, the pneumococcus, is also not pyrogenic upon intravenous injection of animals (10); and by our observation that a pyrogen appeared in supernates of cryptococcal suspensions after the cryptococcal cells were disrupted.

In addition to the classical pyrogenic response beginning after a short afebrile period, living fungi also produced a delayed response that sometimes appeared after a 24 hour incubation period. Such delayed fever was observed mainly with *Candida albicans* and persisted until the animal recovered or died. The kidneys and heart of these animals were studded with mycotic abscesses described in fatal human cases (6). This progressive fever following a protracted lag (or incubation) period appears to be unique to infection since it was never observed with dead fungi. The relationship between the progressive fever associated with fungus infection and the fever following injection of dead fungi remains to be determined. Because the protracted fever of candida infection (Figure 1) was accompanied by sterile blood cultures, and because a transferable pyrogen was present in sera from febrile animals, it is possible that either the fungus pyrogen or an endogenous pyrogen (11, 12) is released from the heavily infected abscesses of the kidneys and other organs.

SUMMARY

The mechanism of fever in fungus infections was studied by systematically examining pathogenic fungi for their capacity to induce fever in rabbits. Intravenous injections of viable *Candida albicans, Blastomyces dermatitidis, Histoplasma capsulatum,* and *Sporotrichum schenckii* produced fever, but cultures of *Cryptococcus neoformans* did not. The fever from these live cultures took two forms, depending upon the dose and the species of fungus: *1*) An immediate fever resembling that produced by gram-negative bacterial lipopolysaccharides. This fever began 1 to 3 hours after injection, lasted 7 to 8 hours, and sometimes produced *biphasic* curves with fever peaks of 1.5 to 4° F at 1 and 3 hours. *2*) A delayed fever, beginning as late as 24 hours after inoculation and persisting until death or recovery from infection.

The first type of fever curve was attributed to a heat-stable filterable fungus pyrogen, since the fever curve could be reproduced with autoclaved fungus cells or Seitz filtrates of fungus cultures. Unlike gram-negative bacterial pyrogens the fungus pyrogens did not cause tolerance to fever upon repeated daily injections and single massive doses did not produce a shock-like state. Instead,

one or more injections of the fungus pyrogen led to increased susceptibility to its toxic effects that produced death from hemorrhagic pneumonia. Aside from pyrogenicity, the main similarity between fungus pyrogens and the pyrogens of gram-negatives, was the marked instantaneous neutropenia and subsequent neutrophilia that followed intravenous injection.

The second type of fever curve (i.e., the delayed persistent type) was believed to be a consequence of infection of the tissues. It was observed with experimental candidiasis and sporotrichosis and was not accompanied by positive blood cultures of the infecting fungus. For this reason, and because a circulating pyrogen was found on the second day of infection, it is believed that either the fungus pyrogen or an endogenous tissue pyrogen is released from the infected tissues.

The absence of fever after injection of cryptococci coincides with the clinical observation that this fungus disease alone is often characterized by little or no fever in the course of severe systemic human infections.

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