RESPONSE OF MICE TO THE INOCULATIONS OF BOTH *CANDIDA ALBICANS* AND *ESCHERICHIA COLI*

I. The Enhancement Phenomenon

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It has been established that candida in the gastrointestinal tract and on mucous membranes increases appreciably following antibiotic therapy (Huppert *et al.*, 1955). One explanation for this phenomenon may be that the antibiotic inhibits the growth of the normal flora, particularly coliforms, in the gastrointestinal tract. The few candida which may be present as part of the normal flora, and which may be held down by the coliforms, may then multiply to sufficient numbers to produce the disturbances usually found after such antibiotic therapy. Paine (1952) and Rosebury *et al.* (1954) independently reported their findings that *Escherichia coli* inhibited the *in vitro* growth of *Candida albicans*. In the latter work, it was apparent that the concentration of *E. coli* was the limiting factor, not the concentration of *C. albicans*. Freyschuss and his co-workers (1955) described the isolation of a fungicidal antibiotic, coliformin, produced by a "*Bacillus coli* type," which was effective *in vitro* against *C. albicans* and other pathogenic fungi.

This investigation was undertaken to determine whether inhibition of *C. albicans* by *E. coli* could also occur *in vivo*. Two opposing effects were observed, dependent on the concentration of *E. coli* inoculated: (1) a delay in the time of death of mice injected with a lethal concentration of *C. albicans* (protection), and (2) a decrease in the time of death as compared to *C. albicans* controls (enhancement). The influence of the strain of albino mouse used in demonstration of these phenomena will be described elsewhere. This report is concerned with the enhancement phenomenon, and its possible mechanism.

The strain of *C. albicans* was obtained from the collection of Dr. N. F. Conant of Duke University; the strain of *E. coli* was isolated from the stool of a patient with diarrhea. Both organisms were maintained by subculture at monthly intervals in brain heart (BH) infusion broth (Difco). The inocula were saline suspensions of 3 × washed organisms grown in BH broth. Viable counts of the 22-hr cultures were made by serial dilution in saline, and streak plates of the proper dilutions on 3 to 4 bromcresol green (BCG) agar plates. BCG agar contains heart infusion broth (Difco), 1.0 per cent glucose, 2.5 per cent agar, and 0.002 per cent BCG. *E. coli* takes up the dye selectively, and can be easily differentiated from the white *C. albicans* (Rosebury *et al.*, 1954).

Several strains of inbred albino Swiss mice were used: (1) Taconic Farms (TF) mice and (2) Namru (Garber and Hauth, 1950), which is maintained as our breeding colony. A commercial strain of white mice, which were not inbred, was used only in a preliminary experiment. Female animals, 6 to 8 weeks old (16 to 20 g) were utilized. Intravenous inoculations were done in the tail veins.

Each mouse was tagged with dye, and observed for the time of death at 1-hr intervals for the first 12 hr, at 2-hr intervals for the next 6 days, and daily thereafter. All experiments were repeated two to three times, and representative data reported. The mean time of death for each group of mice was calculated, and used for statistical evaluation. This was done by the comparison of the various groups of mice by the Student "*t*" method for the significance of two means in small samples. The standard deviations in the tables were computed for small samples (Fisher, 1948).

A factor which must be considered in these experiments is that the mean time of death may mask two opposing effects which may occur in the same group of mice; e. g., the enhancing effect in 1 mouse, as opposed to a protective effect in 3 to 4 mice. Such a variable is disclosed by the large standard deviations for many of the groups.

The O antigen was extracted from *E. coli* by the Boivin technique, using trichloracetic acid and precipitation with 68 per cent ethanol (Kabat and Mayer, 1948). Coli was grown for 24 hr in 3 L BH broth, and centrifuged. The sediment was washed 10 × with saline to remove soluble broth constituents, and dried *in vacuo*. The yield of dried cells was 1.645 g. After extraction with 0.5 N trichloracetic acid at 0 C, dialysis with running water to remove the trichloracetic acid, and precipitation of the dialyzed material with 68 per cent ethanol, an opalescent material was obtained which was dried *in vacuo*. The yield was 31.0 mg, which was approximately 2 per cent of the original dry cells. This was dissolved in sufficient sterile saline to give a solution containing 5.0 mg per ml. It was toxic to mice in a 500-μg dose, ip (⅘ dead in 18 hr). The BH broth supernatant, in which the coli had been grown, was filtered through a Seitz filter, and concentrated to ⅛ its original volume by evaporation in Visking casings. This material, *A*, was not toxic to mice. As a control, 8-fold concentrated BH broth *B*, was prepared, which was also not toxic to mice.

RESULTS

The interaction of these two organisms was tested *in vitro*, using both living and heat killed *E. coli*, respectively, by two methods: (1) growth in liquid media of a mixture of the two organisms, as determined by viable plate counts, compared to that of the respective organisms alone; and (2) results obtained on solid media.

In the first method, 4 tubes were inoculated with aliquots of 18-hr cultures in BH broth, as follows:

(a) 0.2 ml coli plus 19.8 ml BH broth.
(b) 1.0 ml candida plus 19.0 ml BH broth.
(c) 0.2 ml coli and 1.0 ml candida plus 18.8 ml BH broth.
(d) 1.0 ml heat killed coli and 1.0 ml candida plus 18.0 ml BH broth.

At 0, 5, 24, and 48 hr after inoculation and incubation at 37 C, 0.5-ml aliquots were removed, respectively. The aliquots were serially diluted in saline, and viable counts on 4 BCG plates were done with the following dilutions:

(a) Coli alone: 10^{-6} and 10^{-7} dilutions.
(b) Candida alone: 10^{-3} and 10^{-4} dilutions.
(c) Mixture of living coli and candida: 10^{-2} through 10^{-7}, inclusive.

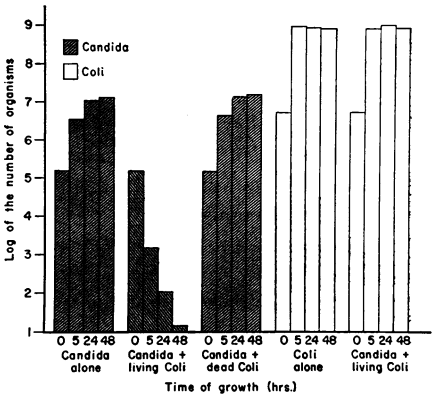

Figure 1. The inhibitory effect of *Escherichia coli* on *Candida albicans in vitro*.

(d) Mixture of dead coli and candida: 10^{-4} and 10^{-5} dilutions.

Figure 1 shows the results obtained. Living coli inhibited the growth of candida more than 100,000-fold, with progressive growth. Dead coli showed no inhibition of candida. Candida had no effect on the growth of coli.

In the second method, 0.2 ml of a candida suspension in saline (6.5 × 10^6 organisms) was streaked by a bent glass rod over the surfaces of 14 BCG plates and allowed to dry. When the candida inoculum was dry, 3 to 4 drops of 10^0 through 10^{-6} dilutions, respectively, of living coli were placed on the surface of each of the plates, using 1 dilution per plate. The drop volume was 0.05 ml, dispensed from a 0.1-ml pipette. Similarly, the same dilutions of heat killed coli were tested, 1 dilution per plate. The 10^0 coli inoculum contained 3.1 × 10^8 organisms per 0.05 ml.

Inhibition of candida occurred under the coli drops in the 10^0, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions, when living coli was used. No inhibition was observed with the dead coli. Such observations were made after incubation of 24 hr, 72 hr and 6 days. However, pinpoint colonies of candida were seen in the coli drops after 6 days' incubation. The greatest number of candida colonies were seen in the 10^{-4} dilution of coli; the least number in the 10^0 dilution. The inhibiting material was not diffusible, since inhibition of candida was observed only within the area covered by the coli drops.

It is evident that this strain of *E. coli* was very effective in inhibiting the *in vitro* growth of

C. albicans. The greater the concentration of coli, the more effective was the inhibition of candida.

The response of mice to the inoculation of both C. albicans and E. coli. In this experiment, the effects of the route of inoculation and of the sequence of inoculation were ascertained. Candida was injected intravenously; coli was injected either intravenously or intraperitoneally into groups of 7 mice (a commercial strain), respectively, in the sequence and at the time intervals indicated in table 1.

It is apparent that with 1.8×10^6 candida and 3.7×10^7 coli:

(1) Intravenous inoculation of candida alone killed 80 per cent of the mice in 18 hr, and all the mice within 24 hr.

(2) Prior inoculation of coli, 30 min before the lethal candida injection, afforded some protection, regardless of the route by which the coli was injected. The prior intravenous inoculation of coli was more effective than the intraperitoneal inoculation, since 70 per cent of the mice were alive 48 hr after inoculation, as against 30 per cent for the latter group.

(3) Intravenous inoculation of coli after the candida injection was ineffectual in delaying the time of death or in affecting the number of deaths due to the candida injection.

(4) Some protective activity was produced by the intraperitoneal inoculation of coli after the candida, at both time intervals.

The effect of the concentration of intraperitoneally injected E. coli on the lethal activity of C. albicans. Although prior inoculation of coli was more effective in protecting mice against a lethal concentration of candida than the injection of coli after the candida inoculation, it was decided to investigate the effect of intraperitoneal inoculation of coli after the candida, for two reasons: (1) any therapeutic to treat candidiasis would be utilized after candida infection had occurred; and (2) intravenous inoculation of coli showed no different results than those obtained with candida controls.

Groups of 7 mice (TF strain), suitably tagged with dye, were inoculated with 1.8×10^6 candida intravenously, followed at the various time intervals and concentrations of coli as shown in table 2 and figure 2. Mice were also injected with a high concentration of coli alone, and with candida alone, as controls.

When 10^7 coli was injected 30 min after candida, a statistically significant protective action was seen, as compared to the candida controls. Such delay in the mean time of death was also observed with the 2-hr interval between inoculations, but not to the same degree. With a

TABLE 1

*The interaction of Candida albicans and Escherichia coli in vivo**

Group No.	First Inoculation	Second Inoculation	Time Interval between Inoculations	Results†	Survivors
			min		
1	Candida iv	None	0	4/7 (8); 6/7 (17); 7/7 (24)	0
2	Coli iv	None	0	1/7 (10)	6
3	Coli ip	None	0	0/7	7
4	Candida iv	Coli ip	30	2/7 (8); 5/7 (17)	2
5	Candida iv	Coli ip	150	1/7 (9); 4/7 (18); 5/7 (42)	2
6	Candida iv	Coli iv	30	6/7 (18); 7/7 (46)	0
7	Candida iv	Coli iv	150	6/7 (10); 7/7 (24)	0
8	Coli iv	Candida iv	30	1/7 (24); 2/7 (28); 4/7 (120)	3
9	Coli ip	Candida iv	30	1/7 (24); 2/7 (26); 5/7 (42)	2

* Candida concentration, 1.8×10^6 organisms; coli concentration, 3.7×10^7 organisms. All surviving mice of this commercial strain were sacrificed after 6 days.

† Deaths/total; (time of death, hr).

10-fold *decrease* in the concentration of coli, protection was apparent at the 2-hr interval, but not at the 30-min interval. A 10-fold *increase* in coli concentration (10^8 organisms) produced a significant decrease in the mean time of death as compared to that of candida controls at the 30-min and 1-hr intervals. No such effect was seen with the increasing time intervals.

It therefore appeared that the protective phenomenon was exerted by the coli within a narrow range of concentration. In addition, the time interval between inoculations markedly influenced the results obtained. The course of the effect was determined within 2 hr after the inoculation of candida.

TABLE 2

The effect of intravenously inoculated Candida albicans and intraperitoneally inoculated Escherichia coli in mice

Group No.	Candida Inoculated iv	Coli Inoculated ip	Time Interval between Inoculations	Mean Time of Death ± SD*	*P*†
			hr	*hr*	
1	1.8×10^6	None	0	33½±13½	—
2	1.8×10^6	1.2×10^8	0.5	13½±4½	<0.01
3	1.8×10^6	1.2×10^8	1	14½±5½‡	<0.01
4	1.8×10^6	1.2×10^8	2	31½±22	0.8
5	1.8×10^6	1.2×10^8	3	39 ±24½	0.5
6	1.8×10^6	1.2×10^8	4	36½±27	0.8
7	1.8×10^6	1.2×10^7	0.5	96½±44½	<0.01
8	1.8×10^6	1.2×10^7	2	51 ±21	0.1
9	1.8×10^6	1.2×10^6	0.5	27 ±10	0.3
10	1.8×10^6	1.2×10^6	2	58½±11½	<0.01
11	None	1.2×10^8	0	>144	—

The mice used were the TF (Taconic Farms) strain.

* Standard deviation computed for small samples.

† The Student *t* test for the significance between two means, for small samples. Comparison of all groups to group I (candida controls). Level of significance is at *P* of 0.05.

‡ 6 mice, all other groups 7 mice. All survivors were sacrificed after 6 days.

The effect of heat killed E. coli and C. albicans in vivo. Heat killed coli had no inhibitory effect on candida *in vitro* (figure 1). What activity might it have *in vivo*?

Groups of 7 mice each (TF strain) were injected with 6.8×10^6 candida intravenously, and the concentration of living or dead coli described in figure 3, 30 min later. The results obtained were compared to those with candida alone, and between each pair of the same concentration.

A significant difference was seen between the effect of the inoculation of living coli and that of dead coli, with the 10^7 and 10^6 concentrations. The mean time of death due to dead coli was

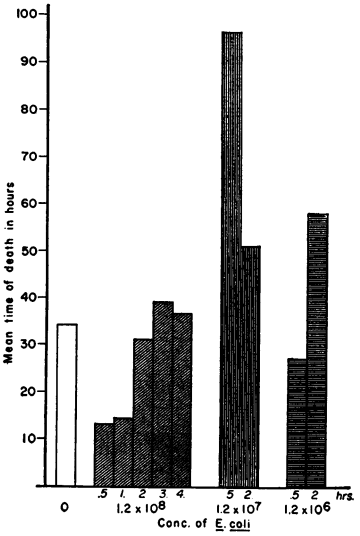

Figure 2. The effect of varying concentrations of *Escherichia coli* injected intraperitoneally at various time intervals on the lethal activity of *Candida albicans* in mice.

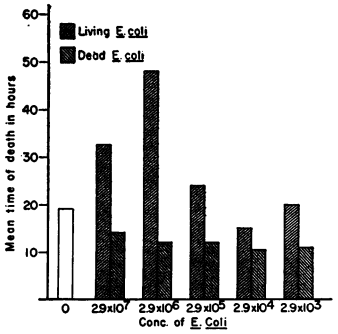

Figure 3. The effect of heat killed *Escherichia coli* on the lethal activity of *Candida albicans* as compared to that of living *E. coli*.

less, for all concentrations of coli, than that due to candida alone. The time of death due to the lower concentrations of living coli was similar to that of the candida alone. It is interesting to note that the dose of 2.9×10^8 dead coli without prior inoculation of candida was not lethal, but 2.9×10^3 dead coli with prior candida injection was markedly lethal.

It may be concluded that dead coli is no more inhibitory to candida *in vivo* than it was *in vitro*. The enhancement of the time of death, however, was seen in the mice injected with dead coli in all concentrations tested.

Several questions were raised by these findings. What is the enhancement effect? Is the decreased time of death due to lethal action by the candida or by the coli? Is the protective activity an antibiotic effect of the coli due to colicines or coliformin?

A working hypothesis to explain the enhancement phenomenon is that the prior inoculation of candida may interfere with the defense mechanisms of the host. Living coli may then multiply to the threshold concentration which would be lethal because of the production of endotoxin; dead coli would be lethal because of its endo-

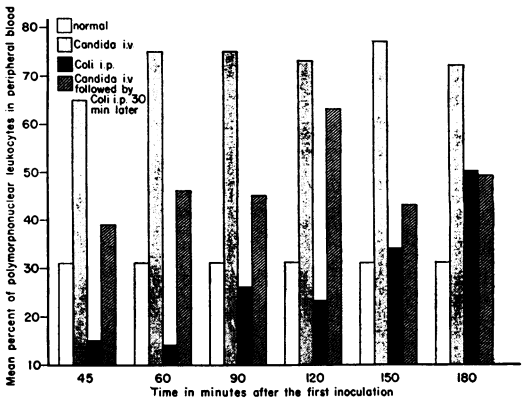

Figure 4. The leukocytic response in the peripheral blood of mice inoculated with a lethal concentration of *Candida albicans* and a nonlethal concentration of *Escherichia coli*.

TABLE 3

The activity of the O antigen of Escherichia coli in the candida-coli interaction in vivo

Group No.	Candida Inoculated iv	Coli or Coli Materials Inoculated ip, 30 Min Later	Mean ± SD	P*
			hour	
1	2.2×10^6	None	45 ± 12½	—
2	2.2×10^6	O antigen, 100 μg†	8½ ± 2½‡	<0.01
3	None	O antigen 100 μg†	—	—
4	2.2×10^6	A†	10½ ± 6	<0.01
5	None	A†	>144	—
6	2.2×10^6	B†	31½ ± 16½§	0.2
7	None	B†	>144	—
8	2.2×10^6	1.3×10^8 living	11½ ± 7	<0.01
9	2.2×10^6	1.3×10^8 dead	11 ± 6½	<0.01
10	2.2×10^6	1.3×10^9 dead	9 ± 5§	<0.01
11	None	1.3×10^9 living	9 ± 4½	—
12	None	1.3×10^9 dead	>144	—
13	None	1.3×10^8 living	>144	—
14	None	1.3×10^8 dead	>144	—

* Student *t* test for the significance of two means, small samples. All groups, where applicable, compared to group I. Level of significance at *P* of 0.05.

† O antigen of *E. coli*, extracted by the Boivin technique. A is the 8-fold concentrated brain heart (BH) broth in which the *E. coli* was grown. B is the 8-fold concentrated BH broth control.

‡ 2/5 mice dead in 36 hr, the 3 survivors lived more than 144 hr.

§ 6 mice, all other groups 7 mice (TF strain).

toxin content. The leukocytic response is one of such defense mechanisms of the host.

The leukocytic response in mice injected with candida intravenously (iv) and a high concentration of coli, intraperitoneally (ip). Three groups of 10 mice (TF strain) were inoculated as follows:

Series I: 5.5×10^6 candida, iv
Series II: 1.0×10^8 coli, ip
Series III: 5.5×10^6 candida, iv; followed 30 min later by 1.0×10^8 coli, ip

At the time intervals shown in figure 4, 5 mice in each series were bled from the tail veins, each mouse being bled for all the time intervals. Differential smears were prepared and stained with Giemsa stain. The percentage of polymorphonuclear leukocytes per 100 cells and counts of the number of organisms present in the peripheral blood were obtained simultaneously. The results are summarized as follows:

(1) Uninoculated, normal mice have a mean leukocyte count in their peripheral blood of 31± 10 per cent. The inoculation of candida alone (series I) produced a marked leukocytic response, reaching a plateau at 60 min after inoculation. The coli control mice (series II) showed a leukopenic response until 120 min after inoculation. In series III, the leukocytic response reflected a possible interaction of candida and coli. It is difficult to determine whether the coli inoculation significantly lowered the leukocytic response to candida, or whether the prior injection of candida significantly increased the leukocyte response to coli.

(2) Candida was present to 150 min after inoculation in series I (candida alone), but in decreased numbers after the first 30 min; coli was found in small numbers in the peripheral blood to 30 min after inoculation, and sporadically thereafter (series II, coli alone). In series III, candida was cultured in small numbers to 90 min after the first inoculation; coli was present in large numbers to 180 min after the first inoculation and sporadically thereafter. It would seem that the prior inoculation of candida effected a coli bacteremia.

(3) The mean time of death of the bled mice in the three groups was: series I, 24 hr; series II, 66½ hr; series III, 5 hr.

The effect of the lipopolysaccharide O antigen of E. coli on C. albicans in vivo. If the enhancement phenomenon were due to the endotoxin of coli, then intraperitoneal inoculation of the O antigen (endotoxin) derived from coli, 30 min after the intravenous candida injection should show similar enhancing results.

Groups of 7 mice each (TF strain) were inoculated intravenously with 2.2×10^6 candida, followed in 30 min by the materials and concentrations of living and dead coli shown in table 3. Injection of 100 μg O antigen was as enhancing as the high concentration of living or dead coli. The Seitz-filtered, concentrated BH broth in which coli had been grown, *A*, also showed lethal activity with the prior inoculation of candida (enhancement), but no such lethal action when inoculated alone. The BH broth control of same concentration, *B*, exhibited no such effect. It is probable that the lethal activity of material *A* was due to small amounts of O antigen. There seemed to be no evidence for a diffusible antibiotic produced by coli during its growth.

A determination of the least amount of O antigen required to show the enhancement phenomenon was done in Namru mice, as shown in table 4. As little as 1.0 μg O antigen was sufficient to show the effect.

TABLE 4

*The effect of varying concentrations of the O antigen of Escherichia coli on the intravenous inoculation of Candida albicans in mice**

Group No.	Candida Inoculated iv	0 Antigen Inoculated 30 min Later	Mean ± SD	P†
		μg	hr	
1	5.3×10^6	0	6±0	—
2	5.3×10^6	100	3¼±¾	<0.01
3	None	100	—‡	—
4	5.3×10^6	10	4±¾	<0.01
5	None	10	>144	—
6	5.3×10^6	1.0	5±¾	<0.01
7	None	1.0	>144	—
8	5.3×10^6	0.1	6½±2½	0.7
9	None	0.1	>144	—
10	5.3×10^6	0.01	8½±2	0.13
11	None	0.01	>144	—

* Namru strain of white mice.

† Student *t* test. All groups where applicable compared to group 1. Level of significance at *P* of 0.05.

‡ ⅗ dead in 16 hr, 2 survivors lived after 144 hr.

Evidence has been presented to show that the decrease in mean time of death in the mice inoculated intravenously with a lethal concentration of candida followed by the intraperitoneal inoculation of high concentrations of living or dead coli (the enhancement phenomenon) is due to the toxemia caused by the endotoxin of *E. coli*.

DISCUSSION

It is well established that interactions between microorganisms occur *in vivo*, best exemplified by the interference phenomenon observed with many viral agents (Schlesinger, 1952). Schlesinger (1952) also points out that virus exaltation, the ability of one virus to make a host abnormally susceptible to the effects of another virus, may also be seen.

Examples of interactions between different kinds of organisms *in vivo* are not as numerous as in the virus-virus interactions. Most of the reports are concerned with increased resistance to infection by one agent, which is influenced by the route of inoculation and concentration of the modifying agent. Horsfall and McCarty (1947) reported the modifying effect of a nonhemolytic streptococcus on the course of PVM virus infection (pneumonia virus of mice), both administered intranasally. Scherr (1953*a*) described increased resistance of mice to encephalomyocarditis virus injected intranasally, by *Cryptococcus neoformans* inoculated intraperitoneally, 9 days previously. Salvin and Bell (1955) demonstrated inhibition of infection of mice by *Rickettsia typhi* or *Rickettsia tsutsugamushi*, following the intraperitoneal inoculation of a suitable concentration of *Histoplasma capsulatum*. Nyka (1956) reported enhanced resistance to experimental tuberculosis in mice which had been infected previously with either virulent or avirulent *Brucella abortus*. The route of administration of brucella influenced the degree of resistance of the animals.

Another aspect of the interaction of microorganisms *in vivo* is the proimmunity (a nonspecific, rapid immunity, not related to the formation of antibodies), described by Oerskov (1940), Field *et al.* (1955), Brandis (1954), and others. These authors have found that the inoculation of killed or living organisms, the same or similar to the challenge organisms, protected mice against death, if administered 4 to 48 hr before the lethal challenge dose of virulent organisms. Oerskov (1940) reported his findings that the proimmunity effect of the pretreatment of mice with *E. coli* vaccine inoculated 24 hr before a lethal dose of *Shigella shiga* was caused by a rapid phagocytosis and inhibition of lysis of the Shigella. The proimmunity had no effect on death due to injected endotoxin, or to killed *Shigella shiga*.

Rowley (1955) observed the effect of the injection of cell walls of *E. coli* either intravenously or intraperitoneally, on the subsequent infection of white mice with *E. coli* suspended in mucin. Enhanced susceptibility to the infection occurred if the time interval between inoculation was less than 2 hr. Increased resistance was demonstrated when the challenge dose of *E. coli* in mucin was delayed 24 hr after the inoculation of the cell walls of *E. coli*. These findings have been correlated with the immediate rapid fall of serum-properdin levels to 20 per cent of their normal value, and the subsequent rapid restoration of the properdin levels to greater than 4 × normal level, 48 hr after the inoculation of mice with cell walls of *E. coli* or with "zymosan" (a yeast polysaccharide), as shown by Pillemer and his associates (1955). Injection of mice with zymosan produced the same results to challenge with *E. coli* in mucin as the injection of cell walls of *E. coli*. It is of interest that Scherr (1953*b*) showed that the pathogenicity of intraperitoneally injected *C. albicans* for mice was enhanced by either living or formolized washed cells of *Saccharomyces cerevisiae* injected intraperitoneally at 48-hr intervals into the candida inoculated animals.

In our experiments, the enhancement phenomenon is very similar to the enhanced susceptibility of mice to *E. coli* within 2 hr after the inoculation of cell walls of *E. coli* or of zymosan (Rowley, 1955). It is likely that the properdin system (Pillemer *et al*, 1954, 1955) may play a part in the mechanism of this phenomenon, since cell walls of a *Candida* species were very effective in removing properdin from serum *in vitro*. Moreover, infection promoting materials like hog gastric mucin (Gale and Elberg, 1952) and levans (Hestrin *et al.*, 1954) produced a marked rapid fall in the properdin level in the serum of mice injected intravenously with these mate-

rials, within 2 to 3 hr (Pillemer *et al.*, 1954). The prior inoculation of *C. albicans* in our experiments may also have lowered the properdin level in the mice so that the living *E. coli*, in high concentration, could multiply uninterruptedly to the threshold level which was lethal to mice. A coli bacteremia was observed in the mice inoculated with candida and the high concentration of coli.

However, the properdin system does not provide the entire answer. It is difficult to see how this immunity factor can influence the death due to the toxemia caused by endotoxin. The time of death in the mice inoculated with very small numbers of dead *E. coli* after the prior injection of *C. albicans* was very much decreased from that of 10^8 dead *E. coli* alone (no deaths). Moreover, the time of death of mice injected with 1.0 μg O antigen (lipopolysaccharide) after the prior inoculation of *C. albicans* was greatly decreased from that of 1,000 μg "O" antigen alone. Other defense mechanisms of the host must also be involved.

The leukocytic response in the peripheral blood of the mice following the inoculation of the two organisms was significantly different from that of either organism alone, for the first 3 hr. Whether the change was due to the influence of the candida inoculation on the coli response, or to the effect of the coli injection on the candida leukocytosis, cannot be assessed from the data. However, it is probable that the response observed may have resulted from the effect of the prior inoculation of candida on the coli leukopenia.

With regard to the protective effect, no explanation for the mechanism involved can be attempted at the present time. The only information available from the data above is that the effect was reproducible both *in vitro* and *in vivo*, and that it was not due to a diffusible soluble antibiotic similar to the colicines. There seems to be no similarity to the increased resistance to infection to one organism by the modification of another unrelated organism, since the concentration differential of the coli inoculum is so narrow. It may be related to the proimmunity studies described, and may thus be influenced by the properdin system. Experiments are now in progress in an attempt to elucidate such a mechanism.

SUMMARY

Prior inoculation of a nonlethal concentration of living *Escherichia coli* either intravenously or intraperitoneally protected mice from a lethal concentration of *Candida albicans*, injected intravenously.

The intraperitoneal inoculation of nonlethal concentrations of *E. coli* after the intravenous injection of a lethal concentration of *C. albicans* produced two contrary effects dependent on the coli concentration: (1) a delay in the mean time of death (protective), and (2) a decrease in the mean time of death (enhancing) as compared to candida alone.

Dead coli of any concentration tested showed only the enhancing effect. As little as 1.0 μg of O antigen extracted from *E. coli* by the Boivin technique, was enhancing. The enhancement effect seemed to be caused by the toxemia due to the endotoxin of *E. coli*.

The possible mechanism of action of the enhancement phenomenon was discussed with reference to the properdin system.

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