The strains used were *Saccharomyces cerevisiae* 5323/A a/αTRP$_5$/TRP$_5$, 5423/B a/αTRP$_5$/trp$_{5-2}$ and 5423/C a/α trp$_{5-2}$/trp$_{5-2}$ which are highly isogenic for the genetic complement around the tryptophan-synthetase coding gene.

*Results and discussion.* 1. Generation time. In each culture medium, generation time differs significantly among the strains tested. As the table shows, the heterozygous strain TRP$_5$/trp$_{5-2}$ possesses, in both medium 1 and medium 2, a generation time significantly lower than that of the two homozygous strains trp$_{5-2}$/trp$_{5-2}$ and TRP$_5$/TRP$_5$.

2. Cell yields. Comparable results were obtained for cell yield. As shown in the table, this parameter differs significantly, for a given culture medium, in the strains tested. A significant difference is also observed, for a given strain, in the culture media tested; the heterozygous strain TRP$_5$/trp$_{5-2}$ always possesses the highest cell yield values.

3. Variability. The variability in generation time and in cell yield vary significantly, for a given TRP: trp gene combination, among the culture media. In particular, the strain which shows the maximum of variability in function of the culture medium, is the heterozygous strain TRP$_5$/trp$_{5-2}$, whereas the variability of the two homozygous strains TRP$_5$/TRP$_5$ and trp$_{5-2}$/trp$_{5-2}$ is not statistically significant.

The data we have obtained are confirmed by the analysis of the growth parameters of the heterozygous strain TRP$_5$/trp$_{5-2}$ SR–I which was selected as a spontaneous revertant from the recessive homozygous strain trp$_{5-2}$/trp$_{5-2}$. The reversion is not due to suppressor mutation (G. Signifredi, personal communication).

As shown in the table, TRP$_5$/trp$_{5-2}$ SR–I, which is in the highest possible condition of isogeny of the genetic complement around the heterozygous marker in comparison with the homozygous strain from which it is derived, possesses a lower generation time and a higher cell yield than the control strains confirming the effect of the heterozygous condition on these growth parameters in yeast and thus the existence of biochemically determined heterosis in this organism.

It was observed that the heterotic effect of the gene combination TRP$_5$/trp$_{5-2}$ is higher in medium 1 than in the other culture media.

This observation and the fact that the natural 'habitat' for yeast is probably similar to the one of the 'stress' media, suggest that there exists a significant probability of optimizing, in function of the different culture media, generation time as well as cell yield.

Further studies now in progress will help in establishing whether heterosis in yeast is gene-specific or allele specific, i.e., if it depends on a specific biochemical block in a given biosynthetic pathway, or on the molecular nature of the mutation, independently of the gene in which it has occurred (Puglisi et al., in preparation).

## Bactericidal Activity in Conventional or Decontaminated Mice Undergoing GVHD or Radiation-Induced Injury

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*Summary.* Oral antibiotic prophylaxis of mice, particularly those with radiation-induced injury or those undergoing GVHD, alters bactericidal activity of the host.

Sepsis contributes to mortality in animals with a severely compromised immunologic system such as that found in irradiated mice and in those undergoing graft-versus-host disease (GVHD). To obviate this difficulty, numerous investigators have used oral antibiotic prophylaxis to reduce gut flora, an important source of bacterial infection[1–3]. However, decontamination may also alter host resistance to infection if microbial agents are reintroduced. This hypothesis is supported by the observation that intracellular digestion is impaired in germfree animals even though phagocytic rates are normal[4–6].

The possible relationship between enteric flora and host resistance after irradiation and during GVHD led to the present investigation of bacterial uptake and killing in conventional and decontaminated mice. More specifically, we wished to determine whether the reticuloendothelial (RE) system of antibiotic decontaminated animals had altered capabilities to eliminate challenge doses of bacterial organisms.

Male B6CBF$_1$ mice were irradiated with 850 rads delivered at 40 rads/min by a 300 kVp General Electric Maxitron X-ray[7]. This dose is 100 rads greater than the LD$_{99}$. B6CBF$_1$ animals destined to undergo GVHD received i.v. injections containing $5 \times 10^6$ allogeneic CBA spleen cells within 4 h after irradiation[7,8]. Irradiated mice die around day 14 while mice with GVHD only survive 7 days. Mice to be decontaminated were placed in a laminar air flow environment with sterile cages, food, bedding and given bacitracin and neomycin in acidified (pH 4) drinking water[7,9]. Fecal pellets were cultured in Thioglycollate broth to insure that decontamination was successful.

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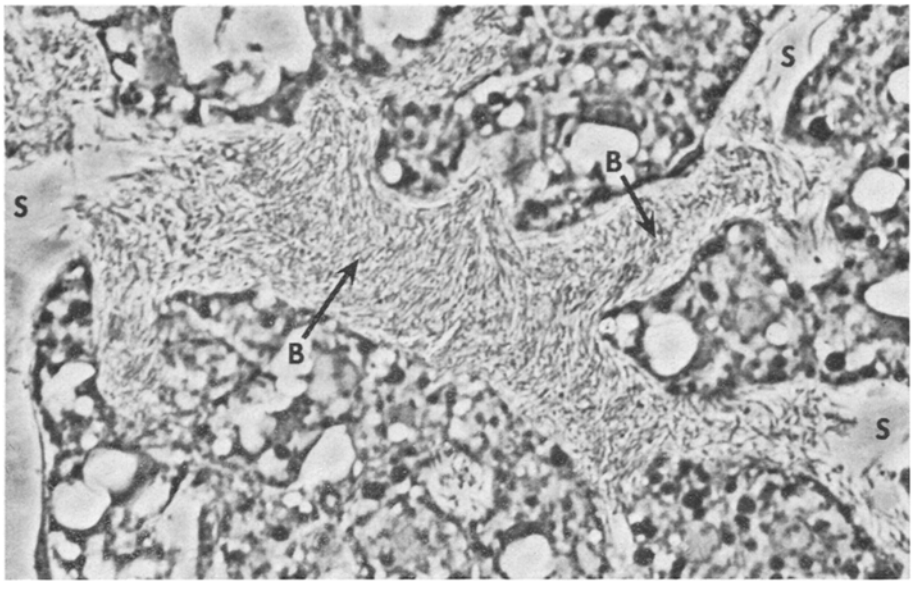


Fig. 1. Phase contrast micrograph of mouse liver 30 min after perfusion with $1 \times 10^9$ *Salmonella typhimurium*. Note that organisms (B) are trapped extracellularly in the sinusoids (S).

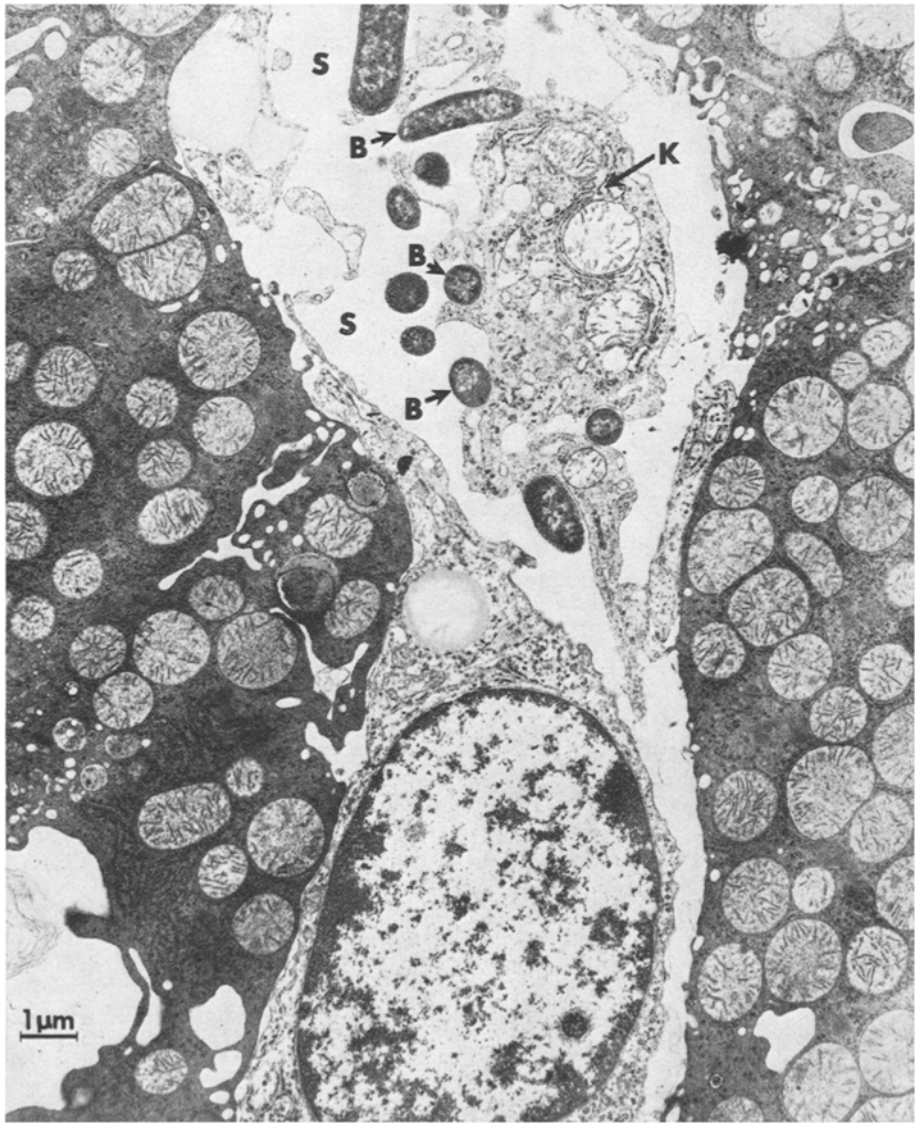


Fig. 2. Electron micrograph of mouse liver 30 min after perfusion with $1 \times 10^9$ *Salmonella typhimurium*. Although surrounded by bacteria (B), the Küpffer cell (K) in the liver sinusoid (S) appears unable to phagocytize them.

The techniques of mouse liver perfusion were similar to those used by other authors[10] and have recently been described in detail[11]. Intact mice were injected i.v. with $1 \times 10^9$ *Salmonella typhimurium* in a volume of 0.1 ml. After 20 min, mice were euthanatized by cervical dislocation, and the numbers of bacteria in liver, spleen and carcass samples were quantiated[11]. The differences between the total bacteria recovered and the number injected was assumed to reflect the number of bacteria killed by the host in the 20 min.

To determine if hepatic trapping of bacteria was altered in decontaminated animals, live bacteria were perfused directly into the experimentally isolated mouse liver in situ. The ability of the perfused liver to trap bacteria (Table 1) is not statistically different in either unirradiated, irradiated or GVHD affected mice, whether or not they were conventional or decontaminated.

Bacteria trapped by the perfused organ were still viable. Bacteria apparently are trapped extracellularly within the sinusoids of the perfused organ in large numbers (Figure 1). The absense of opsonic components[11] in the perfusion fluid prevented phagocytosis of bacteria by the Kupffer cells, as long as 30 min after perfusion of the microorganisms (Figure 2). Intracellular bacteria were only observed in liver monocytes following i.v. injection of the intact animal but not after perfusion of the isolated liver.

20 minutes after i.v. injection of $1 \times 10^9$ viable bacteria into conventional unirradiated mice, 50% of the organisms were killed. No significant differences in the percent of bacteria killed were noted if mice had been irradiated or were undergoing GVHD (Table 2). Likewise, the distribution of viable *S. typhimurium* among the liver, spleen and carcass was essentially similar in all three treatment groups. When normal mice or mice with GVHD were decontaminated with antibiotics, no significant differences were noted between them either in the total percent recovery or the distribution of bacteria which remained viable within the tissues. Irradiated mice were affected more by decontamination. In this group bactericidal activity in the carcass was notably lower than in the other two groups of decontaminated animals. Judging by these findings, we conclude that bactericidal amounts of antibiotic do not enter the blood stream.

Although bacterial killing remains normal after radiation and during GVHD, extrahepatic bactericidal systems, which accounted for approximately 50% of the bacteria killed within 20 min, are reduced significantly by the decontamination process. Depressed bactericidal activity was most apparent in irradiated animals. In unirradiated animals, normal granulocyte levels may partially mask a similar decontamination-induced depression of bactericidal activity. Likewise, in animals undergoing GVHD, engraftment with spleen cells may compensate partially for decreased antibacterial activity[12].

Table 1. Percent trapping of injected *Salmonella typhimurium* by the perfused liver of conventional and decontaminated mice receiving either no treatment or irradiation or undergoing GVHD

| Percent trapping | Unirr | Irr[a] | GVHD[2] |
|---|---|---|---|
| Conv.[b] | 65.5 | 69.7 | 73.2 |
| Decon.[b] | 62.8 | 76.5 | 72.6 |

[a] All experiments were done 3 days before the expected time of death for each group of mice. Irradiated mice usually die on day 14, hence experiments were performed on days 11, 12 and 13 postirradiation; mice with GVHD die on day 7 and were studied on days 4, 5 and 6. [b] 6 livers were perfused in each group.

Table 2. Percent of recovery of viable *Salmonella typhimurium* 20 min after intravenous injection of $1 \times 10^9$ bacteria into both conventional and decontaminated, unirradiated, irradiated and mice with GVHD

| | Percent recovery[a] | | | | | |
|---|---|---|---|---|---|---|
| | Conventional | | | Decontaminated | | |
| | unirr | irr | GVHD | unirr | irr | GVHD |
| Liver | 25.5 | 17.6 | 20.7 | 29.2 | 23.3 | 20.6 |
| Spleen | 0.5 | 0.6 | 0.5 | 0.6 | 0.3 | 0.3 |
| Carcass | 25.0 | 23.8 | 29.7 | 38.6 | 59.0[b] | 40.9[b] |
| Percent recovered | 51.0 | 42.0 | 50.9 | 68.4 | 82.6 | 61.8 |
| Percent killing | 49.0 | 58.0 | 49.1 | 31.6 | 17.4[b] | 38.2[b] |

[a] No flora other than *S. typhimurium* were found in tissues at the dilutions studied. [b] Statistically different from appropriate control. 6 mice were used to obtain each value shown.

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## Gonadectomy and Growth of *Taenia crassiceps* (Cestoda) Cysticerci in Mice

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*Summary.* Gonadectomy of SWR of both sexes significantly reduced the number of cysticerci of *Taenia crassiceps*, 60 days post infection. There was a significant decrease in the total number of larvae and the number of nonbudding individuals, corresponding with increased number of budding larvae. This indicates that the asexual multiplication of cysticerci in populations from gonadectomized mice was inhibited.

In two experiments 80 SWR mice of both sexes, 6 months old, were used. Half of them were gonadectomized a month prior to infection. All were infected with 25 nonbudding cysticerci each and killed 60 days post infection. At autopsy the number of nonbudding individuals, budding individuals, and the total number of larvae were counted.

In both experiments control females had more larvae than control males, the mean number of larvae from peritoneal cavities being 82 $\pm$ 4.33 and 73 $\pm$ 4.14 in