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Trapping and Killing of *Candida albicans* by *Corynebacterium parvum*-Activated Livers†

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Corynebacterium parvum vaccination significantly increased the number of leukocytes adherent to hepatic vessels. Perfused *C. parvum*-treated livers killed significantly more *Candida albicans* than did livers not treated with *C. parvum*, an effect reversed by the macrophage inhibitors silica, phenylbutazone, and iodoacetate.

Liver antimicrobial activity is substantially enhanced in *Corynebacterium parvum*-treated animals (1, 2, 4, 5). The organ increases dramatically in size and exhibits a variety of enhanced functional activities. The objectives of this study were to describe some of the morphological changes that occurred in *C. parvum*-treated livers and to evaluate the antimicrobial activity in these organs with respect to the opportunistic pathogen *Candida albicans*.

Sprague-Dawley male rats weighing 300 to 400 g (Spartan Research Animals, Inc., Haslett, Mich.) were maintained under standard laboratory conditions. Purina Laboratory Chow and water were available ad libitum.

The strain of *C. albicans* used in this study has been described previously (13). Stock cultures were maintained on Sabouraud dextrose agar slants at room temperature. For perfusion studies, the inoculum was prepared by incubating a fresh transfer with continuous agitation at 37°C overnight in 100 ml of tryptic soy broth (pH 7.4; Difco Laboratories, Detroit, Mich.) supplemented with 4% glucose. Cells were harvested, washed three times in sterile saline, and standardized with hemocytometer counts and pour plates.

For scanning electron microscopy studies, perfused livers from *C. parvum*-treated rats, with or without infused *C. albicans*, were prepared by the methods of Sawyer et al. (13) and O'Donnell and Hooper (12). Procedures for liver perfusion have been described in detail (6, 7, 10, 13).

C. parvum vaccine was kindly supplied by Richard L. Tuttle, Burroughs Wellcome Co., Research Triangle Park, N.C. Two lots of vaccine (CA 528A and CA 508A) were used throughout this study. They were Formalin-killed suspensions supplied at a concentration of 7 mg of dry bacterial weight per ml with 0.01% thimerosal. Lots were washed free of the preservative by centrifuging three times in sterile saline and were then stored at 4°C. No variation in response between lots of vaccine was observed. Each animal received 350 μg of *C. parvum* intravenously under light pentobarbital anesthesia. Data in the tables were obtained 2 days postvaccination. Scanning electron micrographs taken at 2 or 10 days postinjection showed similar cellular changes. The micrographs presented here were taken 10 days postvaccination.

Phenylbutazone and iodoacetic acid (as free acid) were obtained from Sigma Chemical Co., St. Louis, Mo. Both drugs were used in 1 mM concentrations prepared in fresh M-199 medium (GIBCO Laboratories, Grand Island, N.Y.). The pH was adjusted to 7.3 with 1 N NaOH, and the solution was resterilized by filtration. Both drugs were perfused into the livers immediately before perfusion of *C. albicans*.

Dörenturp silica (DQ12; particle size, 5 μm) was kindly supplied by Richard Friedman, Department of Microbiology and Public Health, Michigan State University, East Lansing, Mich. Formulation of silica for intravenous injection has been previously described (6). Two 5-mg injections were given intravenously 48 h apart, the last injection being given 24 h before experimentation.

Light microscopy of livers from animals given 350 μg of *C. parvum* intravenously showed numerous distinct areas of mononuclear infiltration of hepatic sinusoids and veins (micrographs not shown). Infiltration was evident by 12 to 24 h and peaked by 48 to 72 h. Hematoxylin-eosin staining revealed the presence of monocytes and lymphocytes. Polymorphonuclear leukocytes were absent. A tendency to form small granuloma-like foci in hepatic parenchyma was also observed. Figure 1 shows low-magnification scanning electron micrographs of normal and *C. parvum*-treated livers. Portal veins of normal

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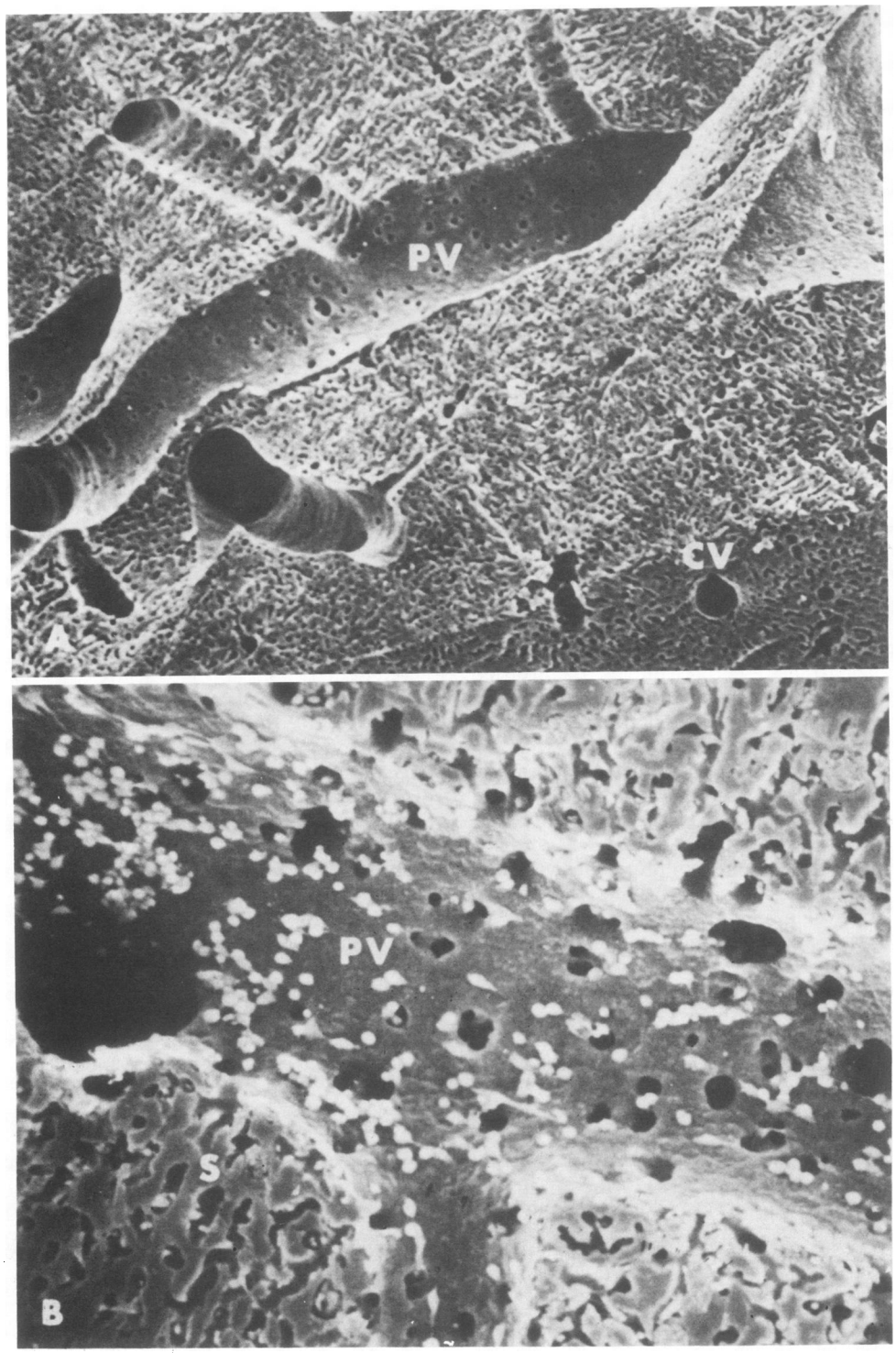

FIG. 1. *Scanning electron micrographs of normal and C. parvum-treated livers. (A) Normal liver with branching portal veins (PV), sinusoids (S), and central vein (CV) (×42). (B) Portal vein (PV) and sinusoids (S) of C. parvum-treated liver showing adhering leukocytes (×100).*

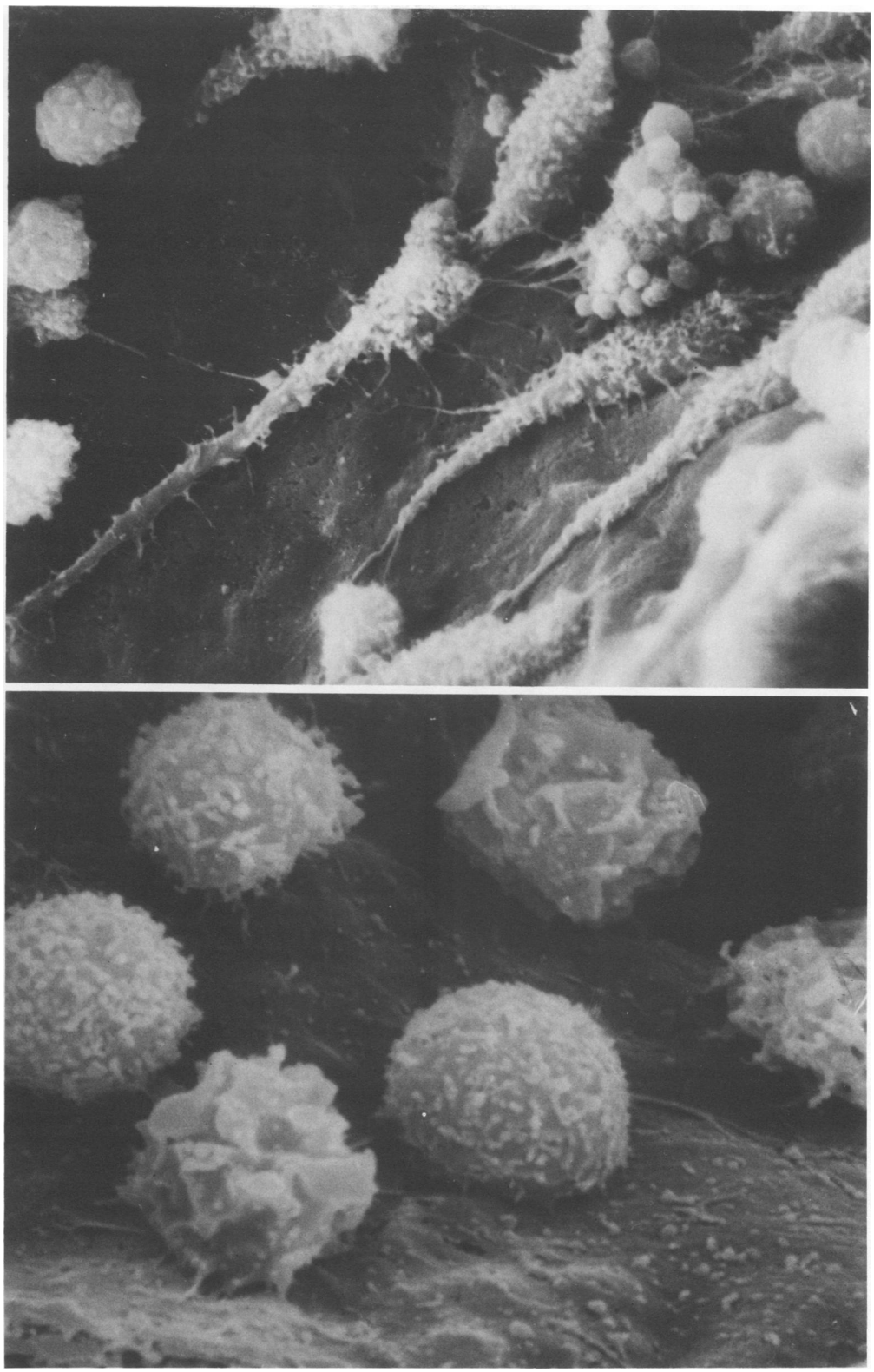

FIG. 2. *Scanning electron micrographs of various leukocyte types observed in C. parvum-treated livers. Top, ×200; bottom, ×700.*

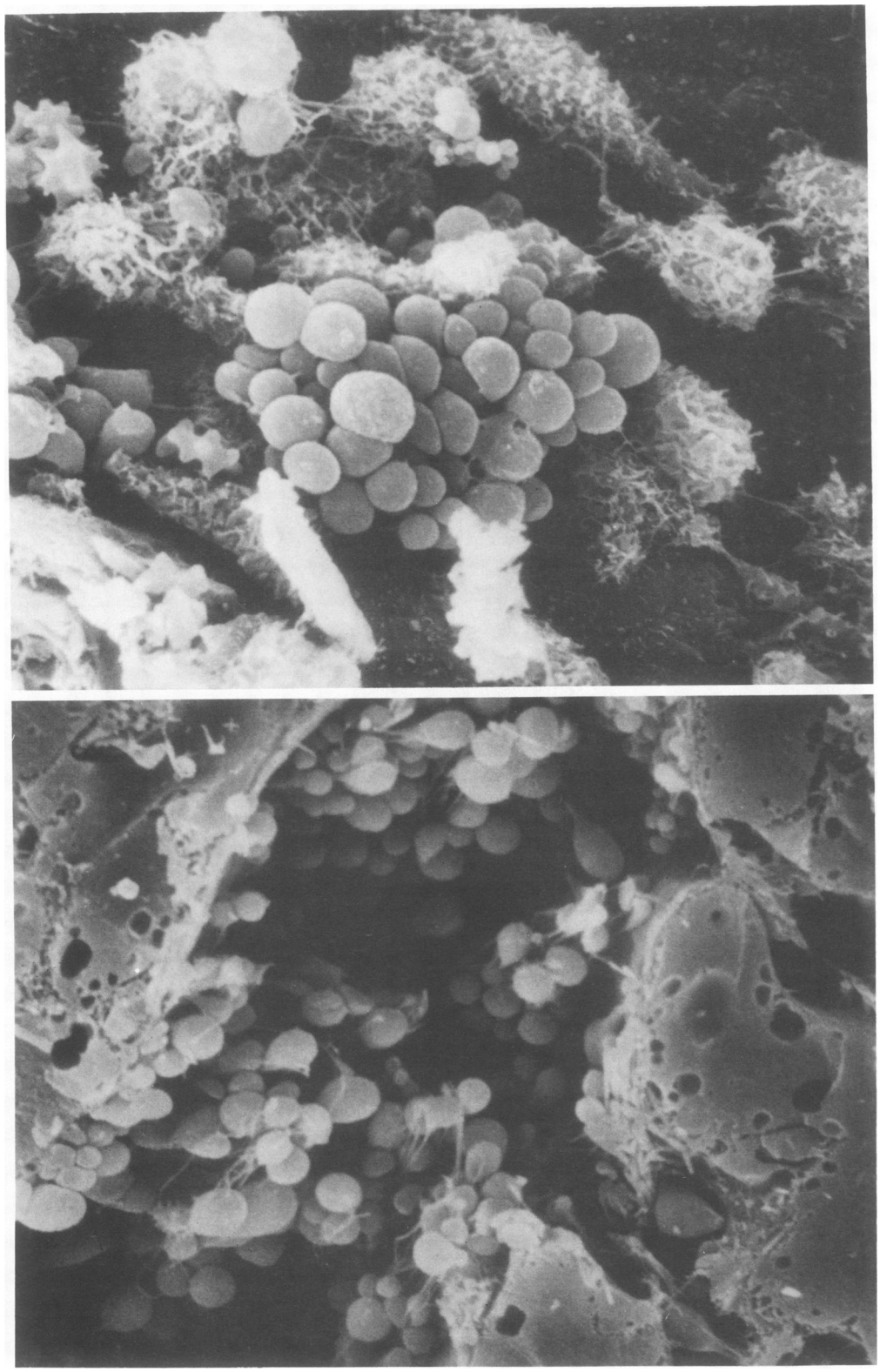

FIG. 3. *Scanning electron micrographs of C. albicans trapped in perfused livers of C. parvum-treated rats (×1,000).*

livers were free of adhering leukocytes (Fig. 1A). Veins in *C. parvum*-treated livers had numerous cells adhering to vessel walls (Fig. 1B). Figure 2 is a higher-magnification micrograph showing the adherent cells in more detail. It appeared that sinusoids also had an increased number of adherent cells but they were difficult to distinguish from normal Kupffer cells.

Figure 3 shows micrographs of livers from *C. parvum*-treated rats after perfusion with 10^6 *C. albicans*. Yeast clusters trapped in portal veins and frequently associated with adhered cells were readily observed. Many fine cytoplasmic filaments (possibly fibrin) entangled the clusters.

The fate of *C. albicans* after perfusion is shown in Table 1. In normal livers perfused with 10^6 *C. albicans* in the absence of plasma only 4% of the organisms were recovered in the effluent, suggesting that over 95% were trapped in the liver. Because the total recovery was 95%, only negligible killing of the trapped yeasts occurred. The presence of plasma did not significantly alter results in normal livers. Livers from rats treated with *C. parvum* in the absence of plasma trapped similar numbers of yeast cells, but in this case only 73% were recovered in the homogenate, for a total recovery of 78%. This suggests that 22% of the yeast cells were killed after 60 min; this represented the first instance in our experiments in which perfused livers killed *C. albicans*. Addition of fresh plasma to the perfusion medium of *C. parvum* livers resulted in 39% of the yeasts being killed (Table 1). Plasma alone did not kill *C. albicans* after 1 h in vitro.

Pretreatment of *C. albicans*-activated livers in vivo with silica or in situ with phenylbutazone or iodoacetic acid (9, 11) completely abolished killing. Table 2 shows that in all instances over 100% of the perfused yeast cells were accounted for in the effluent plus homogenate, even in the presence of plasma.

Considerable evidence suggests that *C. parvum* enhances the nonspecific antimicrobial defense properties of hepatic tissue (5, 14). Consistent with these observations, our microscopic data demonstrate a significant enhancement in the number of fixed mononuclear cells resident in the liver 48 h after *C. parvum* treatment. A previous publication from our laboratory extensively describes clearance parameters of *C. albicans* in normal perfused livers (13). In that study yeasts were found trapped both in sinusoids and portal veins. This present study represents the first instance in which trapped yeasts were killed. That the fungicidal cells in the liver are, in fact, mononuclear cells is strongly suggested by the data in Table 2. Dörenturp silica (DQ_{12}), a specific macrophage toxin (3, 8), completely abolished *C. albicans* killing. Phenylbutazone and iodoacetic acid had the same effect. It should also be noted that none of these materials significantly altered trapping. Cumulatively, we conclude that, whereas resident Kupffer cells were unable to kill *C. albicans*, even in the presence of plasma, the newly arrived monocytes which appeared after *C. parvum* treatment were functionally unique such that the trapped yeasts were killed. The functional differences between these monocyte populations awaits further clarification.

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TABLE 2. *Effect of phenylbutazone iodoacetic acid, and silica on candidacidal activity of C. parvum-treated perfused livers in the presence of plasma*

Macrophage inhibitor	% Recovery[a]			% Killing
	Liver	Effluent	Total	
None (control)	59 ± 8	2 ± 1	61 ± 8	39
Phenylbutazone	93 ± 10	7 ± 3	100 ± 7	0
Iodoacetic acid	111 ± 12	2 ± 1	113 ± 12	0
Silica	83 ± 2	19 ± 10	102 ± 5	0

[a] Average value ± standard deviations from at least five separate experimental determinations.

TABLE 1. *Trapping and killing of C. albicans by normal and C. parvum-treated rat livers in the absence and presence of plasma*

Liver	Solution	% Recovery[a]			% Killing
		Liver	Effluent	Total	
Normal	M-199	91 ± 3	4 ± 1	95 ± 4	5
	Plasma	90 ± 10	9 ± 6	99 ± 5	1
C. parvum treated	M-199	73 ± 2	5 ± 2	78 ± 5	22[b]
	Plasma	59 ± 8	2 ± <1	61 ± 8	39[b]

[a] Average value ± standard deviations from at least five separate experimental determinations.
[b] $P < 0.001$ versus normal control.

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