(5) On an equal body weight and gland basis, the mammary glands of mice at end of pregnancy contain approximately 150% as much DNA as do rats.

1. Damm, H. C., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 466.
2. ———, *ibid.*, 1958, v98, 192.
3. Wada, H., Turner, C. W., *ibid.*, 1959, v101, 707.
4. Brookreson, A. D., Turner, C. W., *ibid.*, 1959, v102, 744.
5. Moon, R, C., Griffith, D. R., Turner, C. W., *ibid.*, 1959, v101, 788.
6. Trentin, J. J., Turner, C. W., *Mo. Agr. Exp. St. Bull.* 418, 1948.
7. Damm, H. C., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 472.
8. Newton, W. H., Beck, N., *J. Endocrinology*, 1937, v1, 65.
9. Newton, W. H., Lits, F. J., *Anat. Rec.*, 1938, v72, 333.
10. Cerruti, R. A., Lyons, W. R., *Endocrinology*, 1960, v67, 884.

Received January 9, 1961. P.S.E.B.M., 1961, v106.

Active Immunization of Mice Against *Candida albicans.** (26405)

SAH'L MOURAD† AND LORRAINE FRIEDMAN (Introduced by M. F. Shaffer)
Departments of Tropical Medicine and Public Health, and Microbiology, Tulane University School of Medicine, New Orleans, La.

Despite scanty unequivocal evidence, the possible beneficial role of immunity in human candidiasis has been suggested by clinical experience (*e.g.*, 1, 2) and in one case of pulmonary candidiasis, the use of rabbit antiserum was reported to be efficacious(3). Experimentally, immunologic studies with *C. albicans* have been largely confined to rabbits. Hurd and Drake(4) gave such animals repeated intravenous injections of heat-killed suspensions of a strain described as highly pathogenic for them. When agglutinin titers reached 1:2560 the vaccinated animals, as well as a control group, were given a dose of *C. albicans* defined as "one ml of sufficient concentration as to bring death on the seventh day." The authors concluded that the vaccination was not only ineffective but possibly harmful. Winner(5) found that normal rabbits possessed a high level of natural agglutinins but that actively or passively induced agglutinins did not protect the animals from an inoculum of *C. albicans* lethal for controls.

Since little immunologic work with *C. albicans* in mice was encountered in the literature and since with mice the use of larger groups would be facilitated, this problem was investigated by challenge of such animals which had been previously vaccinated with variously prepared materials, including sonically ruptured cells.

Materials and methods. *C. albicans*, strain 266, of human origin was used throughout this study. The culture was maintained on slants consisting of 1% casamino acids, 1% yeast extract, 2% glucose and 2% agar (CYE agar). Growth less than a week old was used to inoculate 150 ml of CYE broth in 500 ml Erlenmeyer flasks which were incubated on a rotary shaker at 37°C for 24-36 hours. The concentration of yeast particles was first estimated by direct microscopic counts using a hemacytometer and the viables were determined by the pour plate method.

Cells of *C. albicans* were washed and resuspended in saline so as to contain 2×10^8 particles per ml. From this stock, 3 different vaccines were prepared: (a) viable cells; (b) cells killed by exposure to merthiolate for 48 hours, then rewashed with saline; (c) cells killed by subjecting them to sonic vibrations in a 10 kc Raytheon oscillator for nearly 7 hours, the temperature of which was controlled by chilling to approximately 6°C.

* This study was supported by grants from Nat. Inst. Health, U.S.P.H.S.

† Postdoctoral research trainee in epidemiology and mycology.

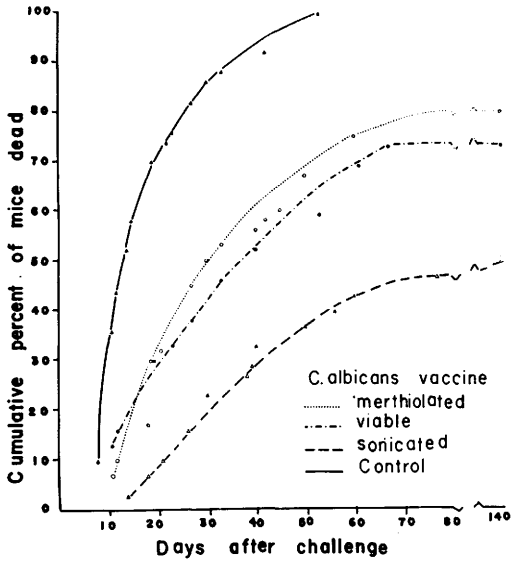

FIG. 1. Cumulative death rates of mice vaccinated with viable, merthiolate-killed or sonicated *Candida albicans* and challenged intrav. with 1×10^6 viable particles of the same strain.

Breakage of the cells was verified by direct microscopic examination, and complete loss of viability was determined by culture. All vaccines were of the same initial cellular concentration.

A preliminary virulence titration of the challenging strain in mice permitted selection of suitable doses for challenge, *viz.*, which would not overwhelm subtle degrees of resistance yet not be of such low virulence that death could not be used as the end-point for comparison of effects.

Six-week-old, white, male mice, obtained from Taconic Farms, were used for the studies. Animals were divided into 4 groups of 80-100 mice each. The first 3 groups were immunized with the living, merthiolate-killed or the sonically-killed vaccine. The fourth group constituted the control which received saline.

The vaccines described above were administered as repeated subcutaneous inoculations, 0.2 ml per inoculum, as follows: 40, 30, 20, 12, 5 and 1 days before intravenous challenge with 10^4 or 10^5 viable particles. Non-vaccinated mice were challenged concurrently. Mice were observed daily for deaths over a period of 20 weeks. The animals dying during the experiment and those sacrificed at the end of observation period were autopsied, the gross pathology recorded, and smears and cultures made from the suspect organs, usually the kidneys.

Results. When mice were challenged with 10^5 viable particles, 100% of 49 non-vaccinated mice died whereas of those that received the vaccine of sonicated cells only 50% (15 of 30) succumbed. The total number of deaths among those inoculated with the merthiolate-killed or the living vaccines was also less than in the controls (73% of 30 and 77% of 39 mice, respectively). A comparison of all death rates is given in Fig. 1.

When animals were challenged with 10^4 viable particles, essentially the same relative degree of resistance was observed. A total of 62% (28 of 45) of the non-immunized mice died, but incidence of deaths among those vaccinated was as follows: animals which received sonicated cells, 17% (5 of 30); animals pre-treated with merthiolate-killed cells, 36% (11 of 30); animals pre-treated with living cells, 43% (17 of 40).

The autopsies of immunized as well as non-immunized animals that died during the experiments showed various kidney lesions that were indistinguishable from each other and contained viable *C. albicans*. In the surviving animals, sacrificed 20 weeks after challenge, kidneys showed unilateral but extensive abscesses which were sterile upon culture.

Discussion. The data presented in Fig. 1 demonstrate that vaccination of mice with either merthiolate-killed, sonically-ruptured, or living cells of *C. albicans* protected a significant fraction of the animals against subsequent challenge with a homologous strain. Furthermore, the protection conferred by the sonicated vaccine was greatest, as determined by the following computations. Since the difference in total percentages dead between merthiolated and living vaccines was not significant among any of the groups, the results obtained with these 2 vaccines were pooled and compared with mice given sonicated vaccine. For those mice challenged with 10^5

particles, the observed difference between the sonicated group and all other vaccinated animals was greater than the theoretical (X^2 = 6.14; theoretical X^2 1 df (.95) = 3.84). Similarly, comparison of those mice challenged with 10^4 particles, showed the difference between those vaccinated with sonicated material and with the other types of vaccine, to have X^2 of 5.2, again greater than the theoretical, with the same confidence limits. Although complete protection was not attained, there can be little doubt that at least some degree of resistance was demonstrated. Whether or not this resistance was related to the presence of circulating antibody is being investigated.

Our conclusions therefore differ from those of Hurd and Drake(4) and of Winner(5) who, however, not only employed a different species of experimental animal but also possibly challenged with excessive doses inasmuch as both vaccinated and non-vaccinated animals died in less than a week.

Summary. Mice were vaccinated by repeated subcutaneous inoculations of living, merthiolate-killed, or sonically-ruptured cells of *C. albicans* and were then challenged intravenously with 10^4 or 10^5 viable particles of a homologous strain. Although not all of the vaccinated mice survived challenge, a significant degree of resistance was conferred since fewer deaths occurred among vaccinated animals.

1. Castellani, A., *Arch. Dermat. & Syphilol.*, 1928, v17, 61.
2. Zimmerman, S. L., Frutchey, L., Gibbs, J. H., *JAMA*, 1941, v135, 145.
3. Martin, D. S., *J. Invest. Dermat.*, 1941, v4, 471.
4. Hurd, R. C., Drake, C. H., *Mycopath. et Mycol. Applic.*, 1953, v6, 290.
5. Winner, H. I., *J. Path. & Bact.*, 1956, v71, 234.

Received January 3, 1961. P.S.E.B.M., 1961, v106.

Runt Disease in Adult Tolerant Mice Induced by Intravenous Injection of Immunologically Competent Cells.* (26406)

CARLOS MARTINEZ,† J. M. SMITH AND ROBERT A. GOOD‡
Departments of Physiology and Pediatrics, University of Minnesota, Medical School, Minneapolis

It is now well established that mice, rats, chicks, and other species, injected at birth or prior to birth with immunologically competent lymphoid cells from adult animals, often fail to grow, and succumb within a few weeks. This syndrome, which has been variously called runt disease, homologous disease, immunological disease, or wasting disease, has been found to be the result of an immune reaction elicited in the transferred, immunologically competent, adult cells by the antigens of the host. A similar phenomenon has been produced in adult F_1 hybrid mice injected with mature parent cells and in mice receiving a lethal dose of x-rays followed by administration of bone marrow cells taken from unrelated donors (For review see 1.) Finally, Casterman(2) recently described a similar type of syndrome in adult mice treated with large numbers of homologous lymphoid cells. In all of these situations, the clinical and gross pathologic findings have been similar, consisting of initial enlargement of the spleen, failure to grow, development of diarrhea, and terminating, often with infections, in a state characterized by extreme atrophy of the lymphoid tissues, especially the spleen and lymph nodes.

According to Billingham(1), the ability of the injected cells to induce this disease has 3 bases: 1) maturity and immunological competence of the inoculated cells; 2) presence in the host of transplantation antigens not present in the grafted cells; and 3) accep-

* Aided by grants from U. S. Public Health Service, Minnesota Division of Am. Cancer Soc., and the Am. Cancer Soc.

† Am. Cancer Soc. Professor of Physiology.

‡ American Legion Memorial Heart Research Professor of Pediatrics.