

leini or cottony cultures with well-developed aerial hyphae. Microscopically these cultures were composed of a filamentous, branching, septate mycelium with intercalary, lateral and terminal chlamydospores. When this growth was transplanted to blood, Sabouraud's dextrose, beef infusion or beef extract agar and incubated at 37°C, after varying periods of time the fungus converted to a waxy and smooth or cerebriform growth. Microscopically this growth was composed of multiple-budding, yeast-like cells, identical with the forms seen in tissue.

Since these 2 fungi behave in a similar manner in tissues, in cultures at room temperature and in cultures at incubator temperature, it would seem that the differences noted above should be considered of specific rather than of generic importance. It is the writers' opinion, therefore, that only a single genus should represent the several isolations of fungi from North American blastomycosis and the several isolations of fungi from South American blastomycosis. For this reason the new combination, *Blastomyces brasiliensis*, is proposed for the fungi which produce South American blastomycosis. Although *Blastomyces* is not a suitable name for this genus because of the rules of priority in botanical nomenclature, it should be retained for these fungi until some generally accepted name is agreed upon. As only one species causes North American blastomycosis and the various species reported from South American blastomycosis^{10, 18} differ only slightly in cultural aspects and morphology, only 2 species need to be considered, namely, *Blastomyces dermatitidis* Gilchrist and Stokes 1898 and *Blastomyces brasiliensis* (Splendore) Conant and Howell, comb. nov.

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Effect of Injections of Nuclei on "Take" of Implants of a Lymphoma in Mice.

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In these experiments we have attempted to determine whether or not, by the injection of nuclei of liver and of tumor cells, it is pos-

¹⁸ Moore, M., *Rev. biol. hyg.*, 1935, **6**, 148.

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sible to immunize mice against a lymphoma which, on implantation, invariably grows till the host is killed. Stoneberg and Haven¹ have reported inhibition of growth of the Walker carcinoma 256 after injection of serum of rabbits which had received injections of nuclei of the tumor. Since spontaneous regression and failure of transplants to "take" occurs rather commonly when this tumor is used, it seemed doubtful that definite conclusions could be drawn from their experiment.

Materials and Methods. The Gardner-Lawrence² lymphoma used in these experiments has been transplanted into about 40 generations of the highly inbred Strong "A" strain of mice.

Four types of suspensions were injected subcutaneously: (1) Approximately 500 viable lymphoma cells; (2) fragmented lymphoma cells prepared by successively freezing in liquid air, grinding and thawing 3 times; (3) nuclei of lymphoma cells; and (4) nuclei of livers of A strain mice. The method of isolating nuclei has been described.³ The material of (1) and (2) was suspended in 0.9% saline. The nuclei of the first 8 injections of (3) and the first 5 injections of (4) were suspended in 5% citric acid. Since this produced cutaneous ulceration, the last 6 injections of nuclei were suspended in isotonic McIlvaine buffer at pH 7.0 which produced no local reaction.

Fragments of the lymphoma were implanted subcutaneously into 400 control A strain mice and in no case did the tumor fail to grow; 2500 A strain mice received intraperitoneal injections of 15,000,000 lymphoma cells and in all cases a lymphoma developed and grew. No regression of this tumor has ever been observed.

Results and Conclusions. In Table I are summarized the number and type of "immunizing" injections administered and the number of survivors of 3 subsequent tumor implants given 1 month apart. The "immunizing" treatment in (1) and (2) killed over half of the animals, most dying of lymphoma, some of toxemia. The injections in (3) and (4) resulted in a mortality rate little more than would be expected in an untreated group in 4 months (9.5%). None developed tumors following injections of nuclei.

Of the 100 mice receiving a single injection of 500 viable lymphoma cells only 2 survived. Of these, one survived 2 implantations before succumbing to pneumonia and the other survived 3 implantations and at the time of this writing is still alive without tumor. Since

¹ Stoneberg, C. A., and Haven, F. L., *Am. J. Cancer*, 1940, **38**, 377.

² Lawrence, J. H., and Gardner, W. U., *Am. J. Cancer*, 1938, **33**, 112.

³ Marshak, A., *Science*, 1940, **92**, 460.

TABLE I.

	1 Viable lymphoma cells to 100 mice	2 Fragmented lymphoma cells to 90 mice	3 Lymphoma nuclei to 95 mice	4 Liver nuclei to 90 mice
No. injections 5/15/40-9/15/40	1	10	14	11
Amt. injected per mouse	500 cells	50,000 cells	.003-.077 cc nuclei	.001-.01 cc nuclei
No. of survivors of "immunization" treatment	2	43	83	84
No. of survivors of 3 successive implants following "immunization"	1	4	2	4

the number of survivors is so small no deductions can be drawn regarding the efficacy of this treatment as means for "immunizing" the animals to the tumor. In group (2) receiving fragmented cells, 4 of the 43 survivors (9.3%) showed "immunity" to 3 successive implantations. Group (3) receiving tumor nuclei showed 2.4% "immunity" while in group (4) receiving liver nuclei there was 4.8% immunity.

Since no failures to "take" were observed in 2900 control animals inoculated with the tumor, the incidence of immune animals in this strain must be less than .035%. As a result of the treatments described, the incidence of immunity has been raised to 2.4-9.3% (which seems to be a significant increase). The injections of nuclei produce about as much "immunity" in this population of mice as the other two methods used (injection of viable and of ground frozen cells), without the risk of killing a large proportion of the animals entailed in these latter procedures. Furthermore the "immunization" obtained in treatments (3) and (4) must be attributed to a response to nuclear material only. This suggests that the "immunization" obtained by injection of various types of whole cells and macerated tissues⁴ may be through response to the nuclei they contain.

⁴ MacDowell, E. C., *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 84; MacDowell, E. C., *et al.*, *Proc. Nat. Acad. Sci.*, 1935, **21**, 507; Rhoads, C. P., and Miller, D. K., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 817.