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Received July 1, 1957. P.S.E.B.M., 1957, v96.

Effect of *Candida Guilliermondi* Polysaccharide on Transplantable Mouse Sarcoma 37. (23398)

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Previous experiments conducted in this laboratory(1) indicated that several species of *Candida* and *Saccharomyces* exerted a lytic action on Sarcoma 37 ascites and Ehrlich ascites cells *in vitro,* either when incubated together in isotonic glucose solution, or when grown simultaneously in tissue culture; though normal mouse tissues, similarly treated, remained unharmed. These results confirmed and extended those of several earlier workers, already reviewed(1). When we injected mice bearing solid transplantable Sarcoma 37 with living and killed *Candida* species by various routes, sloughing of tumors occurred in varying percentages (approximately 90% with living *C. Guilliermondi* injected intravenously(2,3). The fate of the organisms in tumor and host tissues of intravenously injected mice was followed, and the selectively destructive action of the organisms, administered in non-toxic but tumor-necrotizing doses, was confirmed(4). Heat- and chemically-killed cultures were active, though to a lesser degree(3) and it was therefore decided to test whether some effective agent could be isolated by chemical means from killed or lyophilized cultures.

Our first attempt was to separate a polysaccharide fraction by the procedure of Pillemer *et al.*(5). Since *Candida Guilliermondi* was the most suitable organism for use in the living state because of its high tumor-necrotizing properties with respect to Sarcoma 37, and its nonpathogenicity to mice, cultures of this organism were chosen as our starting material.

Methods. The *Candida* cells were grown in 12 liter flasks on glucose-peptone medium (1% peptone, 2% glucose) and were aerated at room temperature. After 3 days these cultures were centrifuged in a Westphalia apparatus. The yield was, on the average, 300 g of wet yeast cells. These cells were treated according to Pillemer's method for extraction of zymosan, as follows:

Three hundred grams of yeast cells were suspended in 1200 ml of 0.5 *M* sodium monohydrophosphate solution and boiled for 3 hours. To this solution was added 0.17 g of trypsin (Wilson 1:250) on the 1st, 3rd, 6th, and 10th day of incubation at 37°C. Two-tenths ml of toluene was also added on the 1st, 5th, 8th and 11th days. Incubation was continued for 16 days at the same temperature, with occasional shaking, and the pH of the liquor of incubation was adjusted daily to 7.8-8.0 by careful addition of 2 *N* NaOH. The liquor was then centrifuged, the supernate discarded, and the precipitate stirred with 1200 ml of water at room temperature for 1 hour and again separated by centrifugation. The resulting precipitate was suspended with vigorous stirring in 1200 ml of water, maintained at 95-100°C for 1 hour, and recentrifuged while hot. This procedure was repeated twice more. No trace of glucose was found in the final supernate upon testing with Fehling's solution. The precipitate was stirred vigorously with 2400 ml of absolute

ethyl alcohol for 1 hour, centrifuged, and the precipitate treated with 1200 ml of absolute alcohol as before. The residue was refluxed for 3 hours with 150 ml of absolute ethyl alcohol, centrifuged and dried *in vacuo,* with a final yield of approximately 4 g of dried material. Fehling's test, following treatment with HCl, gave a positive reaction for carbohydrate.

Results. Three groups of Swiss mice (100 each) were implanted subcutaneously with Sarcoma 37 and the tumors were allowed to grow for 1 week, or until they averaged 1 cm in diameter. One group was treated with the polysaccharide derived from *C. Guilliermondi;* the second group received Fleischman's zymosan in the same dosage; and the third group received no treatment. Since intravenous injection resulted in an undesirably high mortality rate, administration was made routinely by the intraperitoneal route. Each mouse received 2 doses of 0.4 mg each, 2 days apart. These injections were well tolerated, and there was no local tissue reaction at the injection site.

Twenty days after the initial injection, complete regression of tumors had occurred in 62% of the mice treated with the *C. Guilliermondi* product, in 67% of mice treated with commercial zymosan, and in 6% of the controls. The remaining tumors in each group continued to grow until the death of the hosts (mean survival time, 28 days). Mice whose tumors had regressed were observed for at least one month subsequent to the death of the last control mouse. Regrowth of tumors did not occur. While these experiments were in progress Bradner, *et al.*(6), reported similar regression rates for Sarcoma 180 treated with zymosan.

It will be seen that the polysaccharide extracted from *C. Guilliermondi* at the dose levels employed, and intraperitoneally administered, was not as effective as were the living cells used in previous experiments, which brought about regression of tumors in 80-90% of the treated hosts; however, even living cultures, when injected by the intraperitoneal route, were not as effective as when intravenously administered.

Histological preparations of non-growing tumors at 24 hours, 48 hours, and 72 hours post-injection, revealed progressive destruction of tissue, involving dissociation of cells, destruction of cell nuclei, and extrusion of chromatin filaments, similar to that observed following treatment with bacterial polysaccharides. No hemorrhage was noted either macroscopically or microscopically. At 72 hours the tumor tissue was riddled with necrotic islands composed of enucleated cells and granular debris. There was no mitotic activity and the peripheral stroma was devoid of tumor cells.

Thus far, no effect has been observed on Sarcoma 37 ascites tumors when the host mice were injected intraperitoneally with either of these polysaccharides, and no prolongation of life was achieved. Previous trials had shown that living *C. Guilliermondi,* injected intravenously, caused spontaneous mammary tumors of C3H and Swiss mice to remain static for considerable periods of time, with occasional slight reduction in size, but no effect was observed on such tumors in mice injected intraperitoneally with these yeast polysaccharides.

Summary. Complete regression of subcutaneously implanted Sarcoma 37 occurred in 62% of host mice treated with a polysaccharide derived from *Candida Guilliermondi,* in 67% of those treated with Fleischman's zymosan, and in 6% of the controls. No toxic symptoms developed following intraperitoneal injection of either of these polysaccharides, and no hemorrhagic reaction was noted in tumor tissues. Neither substance had any effect on the ascites form of Sarcoma 37 *in vivo.*

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Received July 1, 1957. P.S.E.B.M., 1957, v96.