with 3×10^5 and 3×10^4 tumor cells was similar to that in the control animals given the same schedule of the intradermal inoculations. It was noted, however, that intradermal papules persisted longer in those animals that had tumor than in those animals that did not have tumor. These observations indicate that the intradermal growth of the tumor papule represents an indirect measure of the concomitant immunity of the host to his tumor at the time when the papule is inoculated and grows. Success or failure of immunotherapy in this system depends mainly on the number of cells in the challenge inoculum. With too high a cell inoculum injected intramuscularly, host-dependent immunotherapy was unsuccessful.

The results of the following experiments confirm the observation that successful treatment of a solid tumor can be accomplished by intradermal immunization with living tumor cells. These experiments also demonstrated that tumor cells antigenically unrelated to the challenge tumor are not effective in immunotherapy.

In both of these experiments the challenge inoculum of line-1 tumor cells was 3×10^4 cells. There were three groups (six animals per group) in each experiment: (i) a control group which received no treatment, (ii) a treated group which received three intradermal inoculations with line-1 ascites tumor cells (3×10^6) on days 5, 12, and 19, and (iii) a treated group which received three intradermal inoculations with an antigenically unrelated tumor line (line-4 in the first experiment and line-7 tissue culture cells in the second experiment) (*4*). The results of these two experiments are summarized in Table 2.

It can be seen from Table 2 that in the first experiment animals which developed tumor had palpable tumors at approximately the same time as the controls and that all animals with tumor died at about the same time. One of the six animals in the group treated with three intradermal injections of ascites line-1 tumor cells never developed tumor and has remained tumor-free for more than 1 year. In the second experiment the six control animals, on the average, had palpable leg tumors by day 23.8 ± 1.1, and the average day of death was 85.3 ± 2.8. Four animals in the group treated with three intradermal injections of ascites line-1 cells had palpable tumor by day 101.3 ± 38.4, on the average, and the average day of death was 159.8 ± 40.5. This was significant to $P < .01$. Two animals in this group did not develop tumor and have remained tumor-free for more than 1 year. Both of these experiments demonstrate that tumor cells antigenically unrelated to the challenge tumor were not effective in immunotherapy. These results also confirm the previous observations of our original experiment. Other experiments done in this laboratory demonstrated that antigenically related tumor cells must be viable in order to be effective in immunotherapy and that passive immunotherapy can be accomplished with peritoneal exudate cells obtained from appropriately immunized animals (*7*).

Immunotherapy has been the subject of several recent reviews (*8–10*). Alexander (*8*) has discussed several different approaches to immunotherapy. The approach we have taken is active immunization of the tumor-bearing host with living syngeneic tumor cells. Regression of tumor papules in the skin was first described by Andervont (*5*) and Gross (*11*). What we have described is an immunotherapy model in which immunization is accomplished by the intradermal injection of living tumor cells. The use of the intradermal site for immunization against the tumor offers several advantages over other sites: (i) papules are always visible, so that tumor growth and regression can be followed easily; (ii) delayed cutaneous hypersensitivity reactions may be observed; and (iii) immunization can be carried out with living tumor cells that grow and sometimes regress spontaneously. If the tumor papule grows progressively it can be removed surgically before metastasis occurs, and it has been found that this also results in the induction of tumor immunity (*4*). Many studies have been done in an attempt to immunize the tumor-bearing host against his tumor (*12*), but no studies have used the intradermal route as their sole means of immunization and living tumor cells as the source of antigen.

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Cerebral Acid Hydrolase Activities: Comparison in "Quaking" and Normal Mice

Abstract. *The activity of several acid hydrolase enzymes was determined in whole brain homogenates of adult "quaking" and normal mice. A striking decrease was found in alpha-mannosidase and, to a lesser extent, aryl sulfatase levels in the samples from the mutant animals. The activities of the other "lysosomal" enzymes were only slightly lowered.*

The "quaking" mouse is an autosomal recessive mutant characterized histologically by a deficiency of central nervous system myelin. Affected animals can be recognized before the 12th day postpartum by an unsteady gait and tremor of the hindquarters (*1*). Tonic-clonic seizures are readily induced by sensory stimulation.

Quantitative studies on the biochemical alterations associated with this myelin deficiency have demonstrated a decreased cerebral content of cerebroside, sulfatide, and sphingomyelin (*2*), as well as the long-chain fatty acids (for example, C_{24}) associated with these lipids. The ganglioside (a predominantly extramyelin lipid) content of "quaking" animals is not significantly altered (*2*). Recent in vivo studies have documented

Table 1. Cerebral acid hydrolase activities. Except for *p*-nitrophenyl β-glucosaminide, which was present at 4 μmole, all the other substrates were at 1.5 μmole. In addition, each incubation mixture contained 500 μmole of acetate buffer (*p*H 3.6) for PNP β-glucosaminide, PNP α-galactoside, and PNP β-galactoside; 500 μmole of acetate buffer (*p*H 4.5) for PNP α-glucoside, PNP β-glucoside, PNP α-mannoside, PNP α-fucoside, and nitrocatechol sulfate; 500 μmole acetate buffer (*p*H 5.5) for PNP phosphate; and 100 and 200 μl of homogenate in a total volume of 1.1 ml. Each mixture was incubated at 37°C with shaking in air for 2 hours. At that time the tubes were chilled in ice, 1 ml of 2*M* NH_4OH was added, and the insoluble material was removed by centrifugation. The absorption of PNP was determined at 420 nm and nitrocatechol at 515 nm in a Zeiss spectrophotometer. Values represent means ± the standard error.

Substrate	Micromoles of substrate cleaved per gram of tissue (fresh weight) per hour	
	Control mice	"Quaking" mice
PNP α-glucoside	2.130 ± 0.081	1.610 ± 0.099
PNP β-glucoside	4.290 ± 0.312	3.620 ± 0.573
PNP α-galactoside	1.384 ± 0.056	1.110 ± 0.011
PNP β-galactoside	2.722 ± 0.044	1.960 ± 0.285
PNP β-glucosaminide	0.835 ± 0.081	0.847 ± 0.039
PNP α-mannoside	1.020 ± 0.006	0.336 ± 0.059
PNP phosphate	29.790 ± 1.425	28.890 ± 1.923
PNP L-fucoside	1.260	0.975
Nitrocatechol sulfate	78.720 ± 0.887	47.880 ± 0.327

the ability of these mutant animals to incorporate labeled precursors into the sphingolipids at a rate comparable to that of normal animals (*3*).

In order to evaluate the possibility of altered catabolic activity a study was carried out to quantitate the level of certain "lysosomal" acid hydrolases. Altered hydrolytic enzyme levels have been reported in several inborn metabolic errors (*4*).

Homogenates of cerebral tissue were prepared in five volumes of 0.25*M* sucrose and $10^{-3}M$ ethylenediaminetetraacetic acid and used directly. Preliminary studies in which several of the substrates were used indicated that neither freeze-thawing, sonification, nor the addition of detergents (*5*) influenced the amount of detectable enzymatic activity of the homogenate under the conditions employed. Synthetic substrates (*6*) used in these studies included: *p*-nitrophenyl (PNP) derivatives of α-glucoside, β-glucoside, α-galactoside, β-galactoside, β-glucosaminide, α-L-fucoside, α-mannoside, and PNP-phosphate in addition to nitrocatechol sulfate. Determination of the levels of hydrolytic enzymes, in which these substrates were used, were made by using procedures already published (*7*). Duplicate portions, as well as increasing amounts of homogenates, were incubated with each substrate.

Boiled enzyme and buffer controls were routinely included in each determination. Preliminary experiments were performed to establish optimum incubation conditions for time, *p*H, and enzyme stoichiometry. Samples were dialyzed overnight in the cold in order to obtain enzyme proportionality with nitrocatechol sulfate. Adult "quaking" and control animals, strain C57B1/6J, were used (*8*).

There is no elevation of hydrolytic activity, but several of these activities are decreased (Table 1). It is evident that α-mannosidase activity is most severely affected, being reduced to one-third of control values. Incubation mixtures containing portions of homogenate from both the normal and "quaking" animal gave additive results. This would argue strongly against the possibility of an inhibitor in the mutant animal. The reduction in activities toward other substrates is less marked. Bowen and Radin (*9*) in an examination of several hydrolytic enzymes in young "quaking" mice did not detect any differences in hydrolytic activity toward galactose cerebroside, PNP β-glucoside, PNP β-galactoside, or nitrocatechol sulfate. These authors did not report on α-mannosidase levels in their studies.

Attenuated levels of hydrolytic enzyme activities have been demonstrated in the sphingolipidoses and glycogen storage diseases (*10*). These pathological conditions are characterized by the accumulation of specific compounds in the organs of affected individuals.

The nature of the relation, in "quaking" mouse cerebral tissue, between decreased myelin content, sphingolipid content, and lowered α-mannosidase activity is a matter of speculation. The simplest explanation would be that the organism reduces the level of enzymes in response to substrate reduction, that is, enzymatic activity is inducible. This would stand in contrast to the accumulation of substrate with decreased catabolic enzyme activity as seen in the sphingolipidoses. An example of altered levels of lysosomal enzyme activity has been provided by Kampine *et al.* (*11*) who demonstrated increases in splenic and hepatic glucocerebrosidase and sphingomyelinase in experimentally induced erythrocytorrhexsis. In addition, hepatic lysosomal hydrolase activities increase during starvation of rats.

Recently a storage disorder related to Hurler's syndrome, which has been termed mannosidosis, has been reported in which a low molecular weight substrate composed of glucosamine and mannose accumulates. A decrease in mannosidase activity has been observed in this condition (*12*). It is of interest to note that a mannose-containing glycoprotein has been isolated from brain tissue (*13*).

The striking decrease of α-mannoside activity seen by us would suggest that there may be a specific localization of such linkages in myelin macromolecules. Mannose has not thus far been detected in cerebral lipid; however, a recent publication has reported the incorporation of radioactive mannose from guanosine diphosphomannose into a lipid fraction by brain tissue (*14*).

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