(i) the presence of small amounts of this marker on the inner aspect of the red cell membrane or (ii) partial permeability of the vesicles to the enzymes. The slight inaccessibility of the sialic acid in the bottom band to enzymatic release seems related to the observed tendency of the vesicles in the lower band to continue inverting after removal from the dextran gradient. In any case, by both biochemical and morphologic criteria, more than 85 percent of the vesicles in the upper zone are inside-out.

The conditions for vesicle separation were predicted by a theoretical analysis of the factors determining the buoyant equilibrium of vesicles in density gradients (*7*). According to the proposed model, the prevalence of fixed charges on the inner membrane surface is a major determinant of the vesicle density in discerning gradient systems. The outer surface of the red cell membrane is enriched in sialic acid anions. It was reasoned that internalizing this excess of negative charge would produce vesicles of lower density than the parent ghost. The model further indicates that resolution is optimal in gradients of low osmotic activity, such as dextran. Consistent with the prediction, neither sucrose nor glycerol gradients have afforded satisfactory separation of right-side-out and inside-out vesicles.

Another useful theoretical prediction was that the size of the vesicle membrane should not influence the equilibrium density of the vesicle, within its elastic limits. This was borne out by our observation that intact ghosts and vesicles sharing their membrane orientation achieved buoyant equilibrium at the same density (*7*).

Various treatments have been reported to promote inversion of the red cell membrane (*8*) and the inner mitochondrial membrane (*9*). An electrostatic mechanism appears to be involved in our endocytosis procedure. We have looked for alterations other than topological in the inverted vesicles but have detected no significant perturbation in their fine structure, their sialic acid content, and the specific activity of certain enzymes. The protein electrophoretic patterns of the inside-out and right-side-out vesicles were the same, but both species were greatly depleted of a major, high-molecular-weight protein ["spectrin" (*10*)] found in intact ghosts. The release of spectrin to the medium occurred only under conditions of *p*H, ionic strength, divalent cation concentration, temperature, and incubation time that fostered endocytosis (*11*).

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Immunotherapy of Cancer: An Experimental Model in Syngeneic Guinea Pigs

Abstract. *Successful treatment of a solid tumor was accomplished by repeated intradermal injection of living tumor cells.*

Strain-2 guinea pigs immunized by intradermal injection of living transplantable syngeneic tumor cells acquire a state of systemic immunity. This observation has made it possible to design relatively simple experiments with the aim of establishing conditions for effective immunotherapy. By immunotherapy we mean that immunization is started some time after inoculation of a lethal dose of tumor cells. In these experiments, living tumor cells were injected intramuscularly, and 5 days later the first of three weekly intradermal inoculations with live tumor cells was started. The effect of the intradermal immunization was dependent upon the dose of the tumor cells inoculated intramuscularly. At the two lower doses four of 12 animals were free of tumor and survived for more than a year. It was also noted that the number of tumor cells inoculated intramuscularly may affect the growth of the immunizing inoculum which was injected intradermally.

Age-matched male guinea pigs (Sewall-Wright NIH strain-2) were used in these experiments. The induction of primary hepatomas in random-bred guinea pigs by the administration of the water-soluble carcinogen diethylnitrosamine in drinking water has been described (*1*). We have described the antigenic and biologic properties of transplantable solid and ascites tumors derived from primary hepatomas induced in strain-2 guinea pigs by the carcinogen (2–4). In the experiments described in this report solid tumor line 1, an adenocarcinoma (19th transplant generation) and an ascites variant of tumor line 1 (25th to 27th, 39th to 41st, and 60th to 62nd transplant generations) were used. An ascites variant of tumor line 4 (fifth transplant generation) and tissue culture line 7 (18th ascites transplant generation) were used. Inoculation of tumor cells intradermally resulted in immunization of the host (*3*, *5*, *6*). When normal strain-2 guinea pigs were inoculated with 3×10^6 line-1 ascites tumor cells a papule was formed which increased in size for a few days and then regressed. A second intradermal injection of tumor cells produced a

papule which grew to a smaller size than the first injection and regressed more rapidly. The third injection of living tumor cells showed no papule growth. We also detected delayed hypersensitivity reactions to line-1 tumor cells 24 hours after the second and third intradermal inoculations (*3*). Animals that had received three intradermal inoculations of ascites line-1 tumor cells resisted intramuscular challenge with 4×10^6 tumor cells (*1*).

For the immunotherapy studies an experiment was designed to see if the intradermal inoculation of line-1 ascites tumor cells affected the growth of a previously inoculated tumor. Line-1 solid leg tumor was digested with pronase and deoxyribonuclease according to the procedure previously described (*2*). Three groups of animals (12 per group) were injected with the following doses of enzymatically digested line-1 tumor cells: 3×10^6, 3×10^5, and 3×10^4. Six of the 12 animals per group were treated with intradermal inoculations of line-1 ascites tumor cells on days 5, 12, and 19 following the intramuscular challenge inoculation of line-1 solid tumor cells. The data are summarized in Table 1.

Table 1. The effect of intradermal immunization upon the growth of an intramuscular tumor, three dose levels examined (tumor line 1).

Treatment	Intramuscular tumor dose inoculated on day 0	Number of animals that died of tumor	Average day that tumor was palpable*	Average day of death of animal*	Number of animals surviving over 1 year
No treatment	3×10^6	6	16	65.2 ± 3.6	0
Three intradermal immunizations with line-1 ascites on days 5, 12, 19	3×10^6	6	16	66.5 ± 3.1	0
No treatment	3×10^5	6	21	69.8 ± 3.1	0
Three intradermal immunizations with line-1 ascites on days 5, 12, 19	3×10^5	4	21	105.5 ± 12.15	2 †
No treatment	3×10^4	6	22.7 ± 0.8	84.5 ± 3.8	0
Three intradermal immunizations with line-1 ascites on days 5, 12, 19	3×10^4	4	41.7 ± 9.9	102.0 ± 13.7	2

* Values expressed as mean ± S.E.M. † Tumors were palpable on day 25, but had regressed by day 39. Animals have remained free of tumor for more than 1 year.

Table 2. The specificity of the effect of intradermal immunization upon the growth of an intramuscular tumor (line 1).

Treatment	Intramuscular tumor dose inoculated on day 0	Number of animals that died of tumor	Average day that tumor was palpable*	Average day of death of animal*	Number of animals surviving over 1 year
No treatment	3×10^4	6	25.1 ± 1.5	77.8 ± 3.8	0
Three intradermal immunizations with line-1 ascites on days 5, 12, 19	3×10^4	5	32.8 ± 5.5	91.2 ± 6.0	1
Three intradermal immunizations with line-4 ascites on days 5, 12, 19	3×10^4	6	24.7 ± 1.6	73.1 ± 1.33	0
No treatment	3×10^4	6	23.8 ± 1.1	85.3 ± 2.8	0
Three intradermal immunizations with line-1 ascites on days 5, 12, 19	3×10^4	4	101.3 ± 38.4	159.8 ± 40.5	2
Three intradermal immunizations with line-7 tumor cells †	3×10^4	6	27.3 ± 0.8	83.7 ± 4.4	0

* Values expressed as mean ± S.E.M. † Immunization was done with line-7 tissue culture cells. Ascites line-7 cells grow progressively when inoculated intradermally. Line-7 tissue culture cells do not grow progressively when inoculated intradermally. Line-1 tissue culture cells were protective in this experiment.

The development of tumors in animals injected intramuscularly with 3×10^6 line-1 tumor cells was unaffected by intradermal immunization. Leg tumors were palpable on day 16, and the average day of death for the untreated animals was 65.2 ± 3.6 and for the treated animals was 66.5 ± 3.1.

The 12 animals injected with 3×10^5 cells intramuscularly had palpable leg tumors by day 21. By day 39 two of the tumors in the treated group were no longer palpable. The two animals in which the tumor regressed remained tumor-free for over 1 year and are still without tumor. The four treated animals which developed tumor had an average day of death of 105.5 ± 12.2 as compared with 69.8 ± 3.2 for the controls. This difference was significant to $P < .01$.

Twelve animals received a challenge inoculum of 3×10^4 line-1 tumor cells intramuscularly. The six control animals had palpable leg tumors by day 22.7 ± 0.8. Four animals in the treated group developed leg tumors and the average time of onset of palpable tumor was 41.8 ± 9.9 days. This difference was significant to $P < .01$. Two treated animals never developed a palpable tumor and have remained tumor-free for more than 1 year. The four animals in the treated group that developed tumors had an average day of death of 102 ± 13.7; the controls had an average day of death of 84.5 ± 3.8. This difference was not significant. Table 1 summarizes the experimental data.

Animals that were inoculated with 3×10^6 cells intramuscularly were incapable of suppressing the growth of the first intradermal papule. The growth of the second and third intradermal injections of tumor cells was also not rejected and the papules persisted until the death of the animals. In control animals spontaneous regression of the papules of the first intradermal injection and an accelerated regression of the papule of the second and third injections was seen.

The growth and regression of papules in animals inoculated intramuscularly

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