tabolism tomograms. The tomographic reconstructions obtained 90 minutes later demonstrated restitution of perfusion and partial restoration of accumulation of palmitate. The myocardial salvage seen after reperfusion induced with tPA was greater than that seen in dogs in which thrombolysis was induced with streptokinase, perhaps because the earlier lysis resulted in diminished ischemic time. Despite the overall improvement in myocardial metabolism in the previously ischemic zone, a residual metabolic defect in the center of the initially ischemic zone generally persisted, consistent with death of some cells after ischemia of this duration (Table 1) (*4*).

Neither tPA nor streptokinase led to significant depletion of fibrinogen. The lack of a decrease of fibrinogen induced by streptokinase is typical of results in dogs at the dose used and is in contrast to the marked fibrinogen depletion seen in patients given corresponding doses (*3, 13*).

Analysis of fibrin (or fibrinogen) degradation products, plasminogen, and α_2-antiplasmin blood levels (Table 1) indicated that tPA did not induce a systemic lytic state. In contrast, streptokinase induced a mild systemic lytic state reflected by elevation of fibrin (or fibrinogen) degradation products.

Thrombolysis induced pharmacologically is an attractive therapeutic approach whether clot initiates or simply perpetuates acute myocardial infarction. However, prompt restoration of blood flow is essential (*2, 4*). When given by the i.v. route, tPA elicits angiographically documented coronary thrombolysis promptly with tomographically demonstrable salvage of myocardial metabolism and perfusion and without induction of a systemic lytic state. The i.v. route of administration is as effective as the i.c. route because of the activator's avidity and selectivity for binding to fibrin. Since it may be possible to produce tPA by recombinant DNA technology (*14*), this agent offers particular promise for widely applicable, prompt, safe dissolution of coronary thrombi accompanied by restitution of metabolism in jeopardized myocardium in patients.

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15. Numerical values (PET numbers) throughout each tomographic slice were printed out with a Varian matrix printer. With the use of the transmission and $C^{15}O$ scan data as a guide to overall ventricular wall dimensions, regions compromised metabolically by ischemia were identified as those with less than 50 percent of peak intramural ventricular counts corrected for background. Jeopardized zones were defined as the number of pixels identified in the area of risk divided by the total number of pixels per slice.
16. We thank A. Rand, D. Marshall, and the staff of the Radiation Science Division for technical assistance and L. Dales for preparation of the typescript. Supported in part by NIH grant HL 17646, SCOR in Ischemic Heart Disease and from the Geconcerteerde Onderzoeksacties (Project 80/85-3). Presented in abstract form at the 32nd Annual Scientific Sessions of the American College of Cardiology, New Orleans, 22 March 1983. This work is offered in tribute to the memory of Paul Bergmann.
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Genetically Obese Mice: Resistance to Metastasis of B16 Melanoma and Enhanced T-Lymphocyte Mitogenic Responses

Abstract. *The metastasis of B16 melanoma cells differed significantly in obese* (ob/ob) *and lean (+/?) female mice of strain C57BL/6J. When the mice were inoculated subcutaneously with melanoma cells at 10 to 11 months of age, the primary tumor grew more slowly in obese than in lean littermates and the frequency of lung metastasis was greatly reduced. When the mice were injected with the cells at 4 to 7 months, the primary tumor grew at the same rate in obese and lean mice, but the obese mice again showed a significantly reduced frequency of lung metastasis. That this effect was related to an enhanced immunocompetence in obese mice was supported by the finding that splenic lymphocytes of* ob/ob *mice showed three times the proliferative response to the T-cell mitogen concanavalin A compared with the proliferative response of lean control mice. The* ob/ob *mouse may provide a model for the study of enhanced immunocompetence in obese individuals.*

Obese humans often appear to be highly resistant to cancer. Malignancies occur less often in morbidly obese men than in the general population (*1*), and data from the Framingham study indicate that death rates due to cancer decrease steadily with increases in body build for men aged 40 to 69 years (*2*). Obese women show an increased incidence of breast cancer after menopause (*3*) but in younger women the reverse is true, with a negative association between body build and breast cancer risk (*4*). In both males and females, follow-ups of individuals who are initially disease-free indicate that those destined to develop malignancy weigh less at original screening than controls matched for other factors (*5*).

An animal model would be useful for investigating mechanisms that might link obesity to an increased resistance to cancer. One potential model is the genetically obese (C57BL/6J *ob/ob*) mouse, which develops a syndrome of obesity caused by a single recessive gene (*6*). In the experiments reported here we infected obese (*ob/ob*) and lean (+/?) mice with B16 melanoma cells and found that the former were more resistant to the growth and metastasis of this cancer than their lean littermate controls.

We used 58 pairs of genetically obese (*ob/ob*) mice and their lean (+/?) littermates. All animals were female. Sixteen pairs were injected with melanoma cells at 10 to 11 months of age (experiment 1), and 42 pairs were inoculated at 4 to 7 months of age (experiment 2). Ten days before the injection the mice were

Table 1. Tumor growth, metastasis, and survival of lean and obese mice injected subcutaneously with B16 melanoma cells. Results are expressed as means ± standard error; N.S., not significant.

Group	Day tumor first appeared	Time of survival (days)	Final tumor diameter (mm)	Percentage with visible metastases	Metastases per affected animal
		Experiment 1 (N = *16 pairs*)			
Obese	17.4 ± 1.2	61.6 ± 5.0	18.4 ± 1.5	25.0	5.0 ± 2.0
Lean	16.0 ± 1.0	53.9 ± 1.6	29.0 ± 0.8	93.8	6.7 ± 1.3
*P**	N.S.	N.S.	.001	.001	N.S.
		Experiment 2 (N = *42 pairs*)			
Obese	14.6 ± 0.7	48.6 ± 1.5	29.1 ± 0.8	38.1	3.7 ± 1.0
Lean	13.9 ± 1.1	44.9 ± 1.1	28.8 ± 0.6	85.7	6.3 ± 1.5
*P**	N.S.	.06	N.S.	.001	N.S.
		Totals			
Obese	15.4	52.2	26.1	34.5	4.0
Lean	14.4	47.4	28.9	87.9	6.4
Error	0.5	1.4	0.6		2.4
*P**	N.S.	.01	.001	.001	N.S.

*The statistical significance of the percentage with visible metastases was determined by χ^2 analysis. Other variables were evaluated by *t*-tests in experiments 1 and 2; totals from experiments 1 and 2 combined were evaluated by analysis of variance, *S* (experimenter × size). Within group error estimate: [(MS error)/(harmonic mean of group sizes)]$^{1/2}$.

housed individually in stainless steel shoebox cages (394 cm^2 floor area) covered with Easi Litter (Westminster Scientific Co.) and given free access to food (Charles River Rat, Mouse & Hamster Formula) and water. To facilitate feeding for the obese animals, the food was placed directly on the cage floor and was changed every 4 days.

On the day the mice were to receive tumor cells, (day 0), a B16 C3 stock tumor was removed from a freshly killed C57BL/6J mouse. This tumor is a thrice-cloned line of B16 tumors selected at each cloning for stability and intensity of melanogenesis (*7*). Cells were dissociated by washing once in a 0.04 percent Versene solution for 30 minutes and were separated from the Versene by centrifugation (1000 rev/min for 15 minutes). They were resuspended in phosphate-buffered saline, counted in a hemacytometer, and adjusted to 1×10^6 cells per 0.1 ml of solution. The mice were injected subcutaneously with 10^6 cells in the right posterior-dorsal quadrant.

Tumor diameters were measured with a Vernier caliper at 2-day intervals, from 12 days after tumor-cell injection until death. Measurements on any given day were calculated as the geometric mean of two diameters (GMD), the first being the longest tumor diameter and the second taken perpendicular to the first. The day of the initial tumor appearance was arbitrarily designated as the first day that the GMD was ≥ 5 mm. Tumors reached a GMD of ≥ 5 mm after an average (± standard error) of 16.7 ± 0.7 days in experiment 1 and 14.2 ± 0.4 days in experiment 2. In neither experiment did obese and lean mice differ significantly in the day of initial tumor appearance (Table 1).

Obese mice survived longer than lean mice after tumor cell injection (Table 1). This difference was statistically significant when data from both experiments were combined in a two-factor analysis of variance: body size versus experiment, $F(1, 112) = 6.84$, $P < .01$ (Table 1). The 10.1 percent (4.8 day) average increase in survival time for the *ob/ob* animals runs counter to data for normal life expectancy; under standard laboratory conditions the average lean animal survives about 375 percent longer than its obese counterpart (747 as opposed to 157 days) (*8*). When the data for obese and lean mice in experiment 1 were combined, it was evident that this group survived longer than the mice in experiment 2 [57.8 days and 46.7 days, respectively; $F(1, 112) = 25.46$, $P < .0001$]. Although the present experiments were not specifically designed to evaluate differences due to age, the mice in experiment 1 were about 5 months older than those in experiment 2 when tested; thus, it is possible that resistance to B16 melanoma may increase with age in mice.

Growth of the primary tumor after its initial appearance (≥ 5 mm) was slower for obese mice than it was for lean mice in experiment 1. Thus, despite the fact that obese mice lived longer than the lean mice, their final tumor diameters preceding death were an average of 63 percent smaller (Table 1). No such difference in final tumor diameter occurred in experiment 2 (Table 1), and the data for body size interaction were statistically significant [$F(1, 112) = 29.41$, $P < .001$].

When the animals died their opened carcasses were preserved in 10 percent buffered Formalin and organs (brain, lung, heart, liver, pancreas, spleen, adrenal, kidney, and gastrointestinal tract) were examined under ×12 magnification to identify metastases. Visible metastases were confined to lung tissue in all animals. Metastatic lung nodules occurred in fewer obese mice than lean mice, and differences were highly significant ($P < .001$) in both experiments. In Table 1 we show data for mice that had at least one metastatic nodule, regardless of its size. In both experiments combined, 20 of 58 obese mice (34.5 percent) developed one or more visible nodules, compared to 51 of 58 lean mice (87.9 percent), χ^2 (1) = 32.67, $P < .001$. Differences in the proportion of affected animals remained statistically significant when the criterion for metastasis was set to include only mice bearing at least one nodule ≥ 1 mm in diameter. For both experiments combined, 12 of 58 obese animals (20.7 percent) met this criterion, compared to 33 of 58 lean animals (56.9 percent) χ^2 (1) = $P < .001$. Again, the differences between obese and lean mice were significant when the data from each experiment were analyzed individually ($P < .01$). Thus, obese mice were less likely to develop a lung metastasis regardless of whether the primary tumor was smaller than that of the lean mice (experiment 1) or identical in size (experiment 2).

Metastasis was confirmed by microscopic examination of representative histological sections. No morphological differences were apparent in the metastases of obese and lean animals. Among animals with at least one metastatic nodule, each obese mouse showed an average of 4.0 ± 0.8 nodules and each lean mouse an average of 6.4 ± 1.1 nodules (Table 1), a difference that was not statistically significant.

This study shows that the *ob/ob* mouse provides a model system for studies of the interactions between obesity and tumor growth. The obese animals may be obtained with littermate controls that differ genetically only in the homozygous allele, and the latent period for the growth of transplantable tumors in this model is brief.

The mechanism responsible for the inhibition of metastasis in obese mice is not obvious, but could at least in part be associated with various energy-conserving reactions (*9, 10*), such as increased endogenous opioids (*11*) in the pituitary (*12*) and the blood (*13*), reduced body temperature, and decreased thyroid hormone that have previously been documented in *ob/ob* mice (*12–14*). All of these factors are associated with reduced cancer growth or increased immunocompetence (*15–18*). The endogenous opioid

β-endorphin, for example, potentiates the proliferative response of T lymphocytes from rats by as much as three times (*17*). Beta-endorphin also enhances natural cytotoxicity (*18*). In each of two experiments in vitro, we found that the proliferative response of splenic lymphocytes from *ob/ob* mice to the T-cell mitogen concanavalin A was at least three times that of splenic lymphocytes from lean littermate controls (+/?). In contrast, the proliferative response of spleen cells from *ob/ob* and (+/?) mice to the B-cell mitogen bacterial lypopolysaccharide was identical (data not shown). The increased mitogenic responses occurred in spite of the increased concentrations of plasma corticosterone in *ob/ob* mice (*14*). Although increased corticosterone concentrations have been associated with reduced lymphocyte number, decreased spleen size, and increased susceptibility to cancer (*19*), the spleens of our *ob/ob* mice showed no decrease in size compared with spleens from lean mice. This indicates that they might be less sensitive to the lympholytic action of corticosterone at least in vivo. The insensitivity of T lymphocytes to corticosterone could have evolved gradually in the *ob/ob* mouse by natural selection of T lymphocytes with few corticosterone receptors in response to the gradually increasing plasma corticosterone. In some experiments we found that the mitogen responses (in vitro) of enriched splenic T lymphocytes (nylon wool passed) from obese mice were less sensitive to the immunosuppressive effects of glucocorticoids (triamcinolone acetonide, $10^{-8}M$, and corticosterone, $10^{-8}M$ and $10^{-6}M$) (data not shown). Therefore, reduced T lympholysis and increased T lymphocyte production could contribute jointly to the enhanced immunocompetence of the obese.

The decreased growth of the B16 melanoma in the *ob/ob* mouse might be interpreted alternatively as a result of an inhibitory effect of glucocorticoids acting directly via the glucocorticoid receptors of the tumor. Although the B16 clone used in our study has not been tested for glucocorticoid sensitivity, these hormones were found to inhibit the growth of another B16 melanoma clone in male and female 6- to 8-week-old C57BL/6J mice (*20*). In that experiment, however, glucocorticoids failed to reduce lung metastases in the mice and in some cases even increased them (*20*). Thus, even if our B16 clone has fully functional glucocorticoid receptors, the finding of a reduced rate of metastasis in the obese mice cannot easily be attributed to direct effects of glucocorticoids on the tumor. Further work will clarify how the interaction of endocrine and immunological systems enable the obese to resist metastasis.

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Endogenous Opiates Mediate Radiogenic Behavioral Change

Abstract. *Exposure of C57BL/6J mice to ionizing radiation caused stereotypical locomotor hyperactivity similar to that produced by morphine. Naloxone administration prevented this radiation-induced behavioral activation. These results support the hypothesis that endorphins are involved in some aspects of radiogenic behavioral change.*

When given a sufficiently large dose of morphine, most animals exhibit lethargy, somnolence, and a reduction in behavioral responsiveness (*1*). Similar symptoms have been observed in several species after exposure to ionizing radiation (*2*). Since endorphins have been implicated in the defensive response of organisms to some (*3*) but not all (*4*) stressors, it occurred to us that radiogenic behavioral changes might involve endorphins and that this might account for some of the similarities in the behavioral effects of irradiation and morphine treatment.

The C57BL/6J mouse does not become lethargic after a large dose of morphine, but exhibits a dose-dependent, stereotypical, locomotor hyperactivity and an elevated tail (*5*). Similar behaviors have been observed in another strain of mouse after an intraventricular injection of enkephalins (*6*). If ionizing radiation causes the release of endogenous opiates, then the C57BL/6J mouse should not exhibit lethargy after irradiation, but should become hyperactive. Similarly, this radiation-induced hyperactivity should be reversible by the opiate antagonist naloxone (*7*). The data presented here support these predictions.

Male C57BL/6J mice (15 to 25 g) were maintained on a 12-hour light-dark cycle (lights on at 0600 hours). The animals were housed individually and had constant access to food and water. An Automex D system (Columbus Instruments) was used to record baseline locomotor activity for two 30-minute periods immediately before irradiation. Twenty mice were then placed individually in polyethylene tubes, where they received 1000 rads ($N = 10$) or 1500 rads ($N = 10$) of ^{60}Co irradiation (*8*). Immediately after