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.

CARL I. THOMPSON*
Department of Behavioral Sciences, Pennsylvania State University, Hershey 17033
JOHN W. KREIDER
Departments of Pathology and *Microbiology, Pennsylvania State University*
PAUL L. BLACK
Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140
THOMAS J. SCHMIDT
Fels Research Institute, Temple University School of Medicine
DAVID L. MARGULES†
Department of Psychology, Temple University, Phildelphia 19122

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* Present address: Department of Psychology, Wabash College, Crawfordsville, Ind. 47933.
† To whom requests for reprints should be sent.

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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

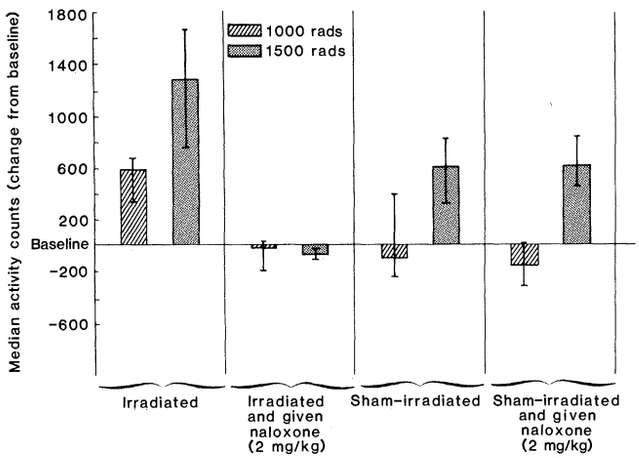

Fig. 1. Median changes in locomotor activity in irradiated and control mice with and without naloxone treatment. Variance indicators represent the quartile range.

exposure, the mice were placed back on the activity monitors and locomotion was again recorded for 30 minutes. Other mice underwent a sham irradiation procedure (like 1000 rads, $N = 10$; like 1500 rads, $N = 10$) in which they were loosely confined in the tubes for as long a time as the experimental animals. The activity of the control animals was monitored in a manner identical to that of the irradiated mice. Each replication of these procedures was conducted at the same time of day to control for any circadian variations.

Exposure to radiation produced a significant, dose-dependent increase in locomotor activity over baseline ($P < .025$, Wilcoxon matched-pairs signed-ranks test), (Fig. 1). Baseline activity (average of two 30-minute periods) did not differ significantly among any of the groups ($P > .05$, Kruskal-Wallis test), but irradiated mice were significantly more active than control mice at both radiation dose levels ($P < .05$, Mann-Whitney U test). In addition, most irradiated mice exhibited a raised tail similar to that observed after an injection of morphine (*5*). None of the control mice displayed this posture.

Since the irradiated mice behaved as though they had been injected with morphine, we attempted to reverse the radiogenic behaviors by administering naloxone. Immediately after mice had been irradiated ($N = 10$) or sham-exposed ($N = 10$), they were given an intraperitoneal injection of naloxone (2 mg/kg). This treatment attenuated the hyperactivity of irradiated mice to such an extent (Fig. 1) that the amount of locomotion was statistically indistinguishable from baseline $P > .05$, (Wilcoxon matched-pairs signed-ranks test). Furthermore, radiation-exposed subjects treated with naloxone were significantly less active than irradiated mice not receiving naloxone ($P < .01$, Mann-Whitney U test). None of the irradiated, naloxone-treated mice exhibited raised tails.

Since irradiated C57BL/6J mice exhibited behaviors similar to those observed after injections of morphine, and since these behaviors were reversed by naloxone, some radiogenic changes in behavior may be mediated by the release of endogenous opiates. These results are consonant with other findings which indicate that morphine-tolerant rats are partially protected from the behavioral incapacitation produced by a large dose of radiation (*9*). Similarly, C57BL/6J mice presumably made tolerant to their endogenous opiates by prolonged exposure to endorphin-producing stressors (such as foot shock and restraint) (*10, 11*) exhibit less radiogenic hyperactivity than do animals that are not stressed before irradiation (*12*).

One control group was moderately hyperactive after sham irradiation. Could this locomotor response have been due to a confinement-induced release of endorphins? Total restraint of rats has been shown to produce a naloxone-reversible analgesia that may be dependent on endorphins (*13*). In the present study, however, restraint was only partial, and the locomotor hyperactivity produced by sham irradiation was not reversed by naloxone. Sham-irradiated mice also failed to exhibit the elevated tail that is consistently observed in C57BL/6J mice injected with a large dose of morphine. Thus it is doubtful that endogenous opiates played a role in the locomotor response of this control group.

The doses of radiation used here exceeded the dose at which 50 percent of the subjects would be expected to die within 30 days of exposure ($LD_{50/30}$) (*8*). Doses lower than these may also cause changes in endogenous opiate systems. For example, 600 rads from ^{60}Co can significantly reduce morphine self-administration in C57BL/6J mice (*14*). These data are consistent with a radiation-induced release of endogenous opiates, in that mice that are experiencing the effects of endogenous opiates might be less compelled to administer exogenous opiates.

Although the studies presented here suggest that endorphins are involved in radiogenic behavioral change, the exact nature of this involvement is still uncertain. Future experiments may better define the specific opioid peptides and physiological mechanisms that produce behavioral alterations in irradiated animals.

G. ANDREW MICKLEY
KAREN E. STEVENS
Department of Behavioral Sciences and Leadership, U.S. Air Force Academy, Colorado Springs, Colorado 80840
GERALD A. WHITE
GREGORY L. GIBBS
Penrose Cancer Hospital, Colorado Springs 80903

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behavioral changes observed within a few hours of exposure to ionizing radiation are quite different from those that occur months later. These early and late effects have been described as different syndromes with different causes. Early behavioral alterations may be primarily due to changes in the nervous system, while later dysfunctions are based more on hemopoietic disruptions [D. J. Kimeldorf and E. L. Hunt, *Ionizing Radiation: Neural Function and Behavior* (Academic Press, New York, 1965) pp. 3–6]. Therefore, endorphins may or may not be involved in long-term behavioral effects of radiation.
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Urinary Phenyl Acetate: A Diagnostic Test for Depression?

Abstract. *The compound 2-phenylethylamine is an "endogenous amphetamine" which may modulate central adrenergic functions. 2-Phenylethylamine is mainly metabolized by monoamine oxidase to form phenyl acetate (PAA). The 24-hour urinary excretion of PAA was measured in normal healthy volunteers and depressed patients. Patients were diagnosed according to the* Diagnostic and Statistical Manual of Mental Disorders, *edition 3. In 70 percent of healthy volunteers of both sexes, the excretion of PAA ranged between 70 and 175 milligrams per 24 hours (mean = 141.1 ± 10.2). Inpatients with major depressive disorder (unipolar type) (N = 31) excreted less PAA (68.7 ± 7.0 milligrams per 24 hours) and 55 percent of them excreted less than 70 milligrams per 24 hours; there were no significant differences in the PAA excretion between untreated patients (N = 13) and those treated with antidepressants that were not effective (N = 18). The PAA excretion was reduced to a lesser extent in 35 less severely depressed unipolar outpatients (drug-free for 1 week) (86.3 ± 11.8 milligrams per 24 hours). These results suggest that low PAA urinary excretion may be a reliable state marker for the diagnosis of some forms of unipolar major depressive disorders.*

The phenylethylamine hypothesis of affective behavior states that 2-phenylethylamine (PEA) is a neuromodulator responsible for triggering or sustaining wakefulness, alertness, and excitement, possibly by the modulation of brain catecholamine synapses (*1*). A decrease in the brain concentrations or turnover of endogenous PEA may therefore play a major physiopathological role in certain forms of endogenous depression, whereas an increase in the concentrations of PEA in the brain or the activation of specific PEA receptors in brain neurons may be responsible in part for the actions of antidepressant and stimulant drugs (*2*).

Structurally, PEA is closely related to amphetamine and to catecholamines; PEA induces behavioral and electrophysiological effects similar to those of amphetamine. These effects are markedly enhanced by monoamine oxidase (MAO) inhibitors because PEA is rapidly metabolized by MAO type B (*3*), forming phenyl acetate (PAA). Pharmacological studies in animals have shown a fairly consistent relation between the affective changes induced by drugs in man and their effects on PEA in the brain (*2*). Behavioral studies suggest that amphetamine mimics and haloperidol blocks the receptors for PEA in brain neurons (*2*). The concentrations of PEA in the brain are increased by all types of antidepressant treatments, including not only classical MAO inhibitors but also tricyclic antidepressants of the imipramine type and electroshock (*2*). This action is remarkably selective in the case of tricyclic antidepressants, which do not modify the brain concentrations of catecholamines or serotonin. On the other hand, MAO inhibitors and electroshock also increase the brain concentrations of other amines. Among antipsychotic drugs, chlorpromazine does not alter concentrations of PEA in the brain, whereas reserpine (which can cause depression in humans) reduces the concentration of PEA in the brain (*2*). The euphoriant agent marihuana (tetrahydrocannabinol) increases brain PEA concentrations and reduces the disposition of PEA in the brain (*2*) with only minor changes in other neuroamines.

Because the amounts of PEA excreted in the urine are very low and highly variable in both control and depressed subjects, clinical studies in which investigators attempted to apply the above concepts have not been successful. We have now studied the 24-hour urinary excretion of its metabolite PAA. After acid hydrolysis of phenylacetylglutamine, the total amount of PAA as its trimethylsilyl derivative was measured by gas-liquid chromatography with flame ionization detection (*4*).

We studied the 24-hour excretion of PAA in three populations of adults of both sexes: (i) 48 healthy adult volunteers, mainly hospital staff members and their relatives, students and their relatives, who were drug-free; (ii) 31 inpatients at admission (of whom 13 had not had antidepressants for two or more days before urine collection); and (iii) 35 outpatients, drug-free for at least 1 week. In each case, the completeness of the collection was stressed, volumes were checked, and several samples were obtained and averaged in many cases. Inpatients were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders,* edition 3 (DSM-III), and the severity of their symptoms was evaluated by means of the Beck depression scale, the Zung depression and anxi-

Table 1. Urinary excretion of phenyl acetate (mean ± standard error).

Subjects			Phenyl acetate excreted (mg/24 hours)	Percentage of group excreting		
Group	*N*	Age		< 70 mg/24 hours	70–175 mg/24 hours	> 175 mg/24 hours
Adult controls	48	16–59	141.1 ± 10.2	15	70	15
Depressed (unipolar) inpatients	18	18–76	68.6 ± 8.8*	50	50	0
Depressed (unipolar) inpatients, untreated	13	22–48	68.8 ± 7.0*	62	38	0
Depressed (unipolar) outpatients, untreated	35	26–59	86.3 ± 11.8*	54	37	9

*$P < .001$ (t-test).