to HSV with HSV can activate the complement sequence (*19*) and may lead to the release of C5a. In addition, the interaction of antibody or sensitized lymphocytes with virus-induced antigens on the membrane of infected cells might lead to cell lysis, release of CGF, cleavage of C5, and attraction of PMN's. This might explain the observation of Oldstone and Dixon (*20*) that PMN's accumulated in the leptomeninges of mice infected with lymphocytic choriomeningitis virus after intrathecal administration of antiviral antibody.

The release of intracellular factors from virus-infected cells might be responsible for other aspects of the inflammatory response, such as monocyte and lymphocyte accumulation and increased vascular permeability. The nature and amount of the factors released may depend upon the specific cell type infected as well as on the characteristics of the virus-induced cytopathology. By use of in vitro systems such as described here, it should be possible to characterize some of these factors and further elucidate the mechanism of inflammation in virus infections.

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6. To abolish the low levels of heat-labile chemotactic activity in normal serum (*7*), after the initial incubation at 37°C for 60 minutes all samples were heated for 30 minutes at 56°C prior to testing for chemotactic activity.
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22. We gratefully acknowledge the technical assistance of J. Kennedy, J. Phillips, and C. Wohlenberg.

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Tolerance to Morphine-Induced Increases in [^{14}C]Catecholamine Synthesis in Mouse Brain

Abstract. *Morphine sulfate increased the incorporation of carbon-14–labeled tyrosine into labeled catecholamines in the mouse brain. Tolerance was manifested by a shift to the right in the dose-response curve for morphine after mice were treated repeatedly with morphine. Naloxone, a specific morphine antagonist, also shifted the dose-response curve for morphine to the right.*

Repeated administration of morphine to mice results in the development of tolerance to such specific effects of morphine as analgesia, increased locomotor activity, and decreased brain catecholamine content (*1–3*). These effects of morphine are prevented by specific antagonists (*2–4*). In contrast, morphine has other effects, such as convulsions and tremors, which are not prevented by specific antagonists. Tolerance does not develop to the convulsant actions of morphine (*5*). Morphine (60 mg/kg, injected intraperitoneally) increases the incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in the rat brain (*6*). We determined the effects of morphine on the incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in the mouse brain under conditions previously found to produce tolerance to other specific actions of morphine. To further evaluate the effect of morphine on catecholamine synthesis, naloxone, a morphine antagonist, was studied.

Table 1. Antagonism by naloxone (1 mg/kg) of the effects of morphine on incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in mouse brain. Each value represents the mean ± S.E. of at least five determinations. The *P* values refer to the significance of differences between each pair of values.

Drug dose (mg/kg)	[^{14}C]Catecholamines (dpm/g)	
	Control	Naloxone treated
	Saline	
Control	1817 ± 139	1787 ± 56 ($P > .4$)
	Morphine	
10	2703 ± 198	2214 ± 103 ($P < .05$)
30	3067 ± 320	2439 ± 103 ($P < .05$)
100	2844 ± 210	2671 ± 124 ($P > .2$)
300	2630 ± 176	2521 ± 250 ($P > .4$)

The incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in brain was determined at various times after the injection of [^{14}C]tyrosine (55 μg/kg; specific activity, 513 mc/mmole, uniformly labeled, Amersham/Searle) into the tail vein of the female Swiss-Webster mouse (20 to 30 g). Brain [^{14}C]catecholamines were isolated on alumina as previously described (*7*).

The maximum incorporation into [^{14}C]catecholamines occurred 40 minutes after the injection of [^{14}C]tyrosine (Fig. 1). A marked increase in incorporation into [^{14}C]catecholamines was observed when the [^{14}C]tyrosine was injected 30 minutes after the injection of morphine sulfate (100 mg/kg, intraperitoneally). After this dose of morphine, the maximum incorporation also occurred 40 minutes after the injection of [^{14}C]tyrosine (Fig. 1). Therefore, dose-response relations for morphine sulfate were determined 70 minutes after morphine administration and 40 minutes after injection of [^{14}C]tyrosine. Morphine (10 to 300 mg/kg, injected intraperitoneally) increased incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines. The maximum increase occurred at a dose of 30 mg/kg (Fig. 2, solid circles). The increased incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines did not result from a morphine-induced increase in the specific activity of [^{14}C]tyrosine in the mouse brain. Morphine (100 mg/

kg) had no effect on the free tyrosine content or on the content of [^{14}C]tyrosine in the brain at 40 minutes after the intravenous injection of [^{14}C]tyrosine (*8*).

To produce tolerance, mice were injected with morphine (100 mg/kg, intraperitoneally) every 6 hours for seven injections. Six hours after the seventh injection, either morphine (10 to 300 mg/kg, intraperitoneally) or saline was injected, followed 30 minutes later by the administration of [^{14}C]-tyrosine. This treatment schedule produces tolerance to both the locomotor activity-increasing and the brain catecholamine-depleting effects of morphine in mice (*2*). Incorporation of [^{14}C]-tyrosine into [^{14}C]catecholamines was not changed significantly in mice receiving saline after treatment with repeated morphine injections. Low doses of morphine (10 and 30 mg/kg) did not significantly increase the incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in mice treated with morphine (Fig. 2, solid triangles). The maximum increase in incorporation occurred at a dose of 100 mg/kg and was equal in magnitude to the maximum increase produced by a morphine dose of 30 mg/kg in nontolerant animals. The shift to the right in the dose-response curve for morphine in mice treated repeatedly with morphine indicates development of tolerance to the effects of morphine on catecholamine synthesis.

When naloxone (1 mg/kg, intraperitoneally) was injected 5 minutes before the injection of morphine, low doses of morphine caused increases in the incorporation of [^{14}C]tyrosine into brain [^{14}C]catecholamines which were much less than those increases seen in nontolerant, control mice (Table 1). The maximum response produced by a morphine dose of 100 mg/kg after naloxone administration was less than the maximum response produced by a morphine dose of 30 mg/kg in nontolerant, control mice. This decrease in the maximum response to morphine was not statistically significant. The shift to the right in the dose-response curve for morphine after naloxone administration is typical of a competitive type of antagonism. However, the slight decrease in the maximum response to morphine after naloxone administration might suggest a more complicated type of antagonism. Naloxone, injected 5 minutes before the injection of saline, did not change the incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in nontolerant, control mice.

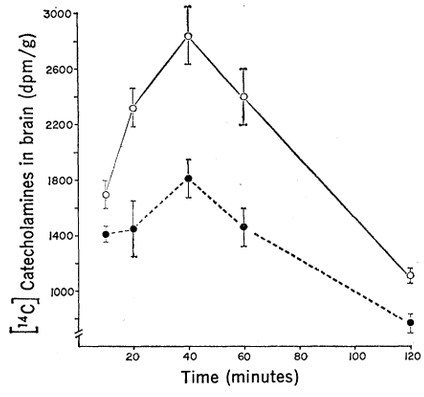

Fig. 1. Incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in the brains of control (solid circles) and morphine-treated mice (100 mg/kg) (open circles) at various times after injection of [^{14}C]tyrosine (55 μg/kg; 513 mc/mmole, intravenously). Each point represents the mean of six observations. Vertical bars represent standard errors. Disintegrations per minute, dpm.

The present study shows that morphine increases the incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in the mouse brain. Since this increased incorporation of [^{14}C]tyrosine is accompanied by a decrease in brain catecholamine content (*2*), morphine increases both synthesis and turnover of brain catecholamines. This effect possesses the two features characteristic of the specific effects of all narcotic analgesics—tolerance develops to this effect, and naloxone, a specific antagonist, blocks it. Clouet and Ratner (*6*) reported that tolerance does not develop to the effects of a large dose of morphine (60 mg/kg) on the incorporation of [^{14}C]tyrosine into [^{14}C]catecholamines in rat brain. In that investigation only one dose of morphine was studied, and it apparently produced maximum increases in catecholamine synthesis in both tolerant and nontolerant animals. In our study, tolerance resulted in a shift to the right in the dose-response curve for morphine rather than a reduction in the magnitude of the maximum response obtained. This study emphasizes the desirability of determining complete dose-response relations when evaluating the development of tolerance to a drug or blockade by an antagonist.

There are several observations that suggest that catecholamines may be involved in the mechanisms by which morphine-like drugs produce analgesia and increase locomotor activity in mice. Reserpine, which depletes catecholamines, may diminish the analgesic

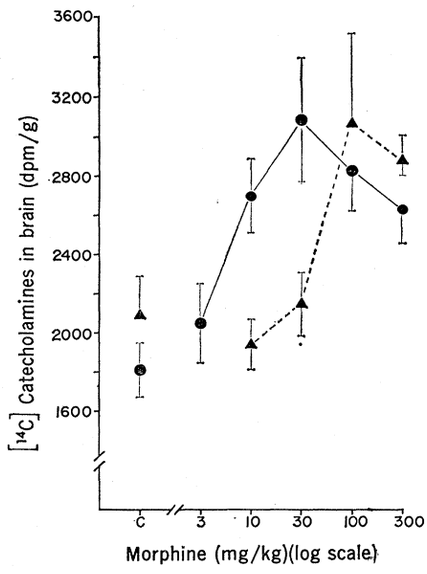

Fig. 2. Morphine-induced increases in the incorporation of [^{14}C]tyrosine into [^{14}C]-catecholamines in the brains of control (solid circles) and morphine-tolerant (solid triangles) mice. (*C*) Determinations on tolerant and nontolerant mice injected with saline. Each point represents the mean of six observations. Vertical bars represent standard errors.

(*9*) and the locomotor activity–increasing (*10*) effects of narcotic analgesics. Drugs that inhibit the oxidative deamination of catecholamines appear to enhance both the analgesic (*9*) and the activity-increasing (*10*) effects of narcotic analgesics. Furthermore, narcotic analgesics decrease brain concentrations of catecholamines (*2*), an effect which has the pharmacological characteristics typical of other specific effects of morphine. Because morphine nearly doubles the rate of catecholamine synthesis in the brain and because this effect has characteristics typical of other specific actions of morphine, our study strongly supports the view that catecholamines participate in the mechanisms of action of narcotic analgesics.

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Antigen Competition: A Paradox

Abstract. *Immunization of mice with pig erythrocytes caused impairment of the antibody response to subsequent immunization with sheep erythrocytes, a phenomenon called "antigen competition." Paradoxically, spleen cells from mice previously injected with pig erythrocytes produced an* increased *response when immunized in vitro with sheep erythrocytes. Augmentation of the in vitro response is due to an increase in one of the interacting cell types. "Antigen competition" is not due to competition for cells. Cell transfer experiments provided evidence that "antigen competition" observed in animals is the result of a humoral factor, presumably antibody, present in the animal but eliminated during preparation of cells for culture.*

The phenomenon of "antigen competition," that is, when the antibody response to one antigen is reduced by a prior injection of a second unrelated antigen, is observed in many species with a wide variety of antigens (*1*). However, no explanation of the mechanism of this phenomenon has been satisfactory. Two alternative hypotheses have been proposed, each with its own implications for present theories of antibody formation. According to one hypothesis, competition occurs for a multipotential stem cell, partial exhaustion of which reduces the response to the second antigen (*2, 3*). According to an alternative hypothesis, suppression is mediated by a humoral factor. A humoral factor required for the response to both antigens could be exhausted by stimulation with the first antigen. Alternatively, a humoral factor produced in response to the first antigen could suppress the response to the second antigen (*4*). Below, evidence is presented that spleen cells obtained from mice which would demonstrate the phenomenon of "antigen competition" are capable in vitro of an augmented response to a second antigen. This augmented in vitro response is the result of an increase in the numbers of at least one of the several cell types required for the in vitro immune response. "Antigen competition," that is, the suppression observed in vitro, is apparently due to a humoral factor, probably antibody.

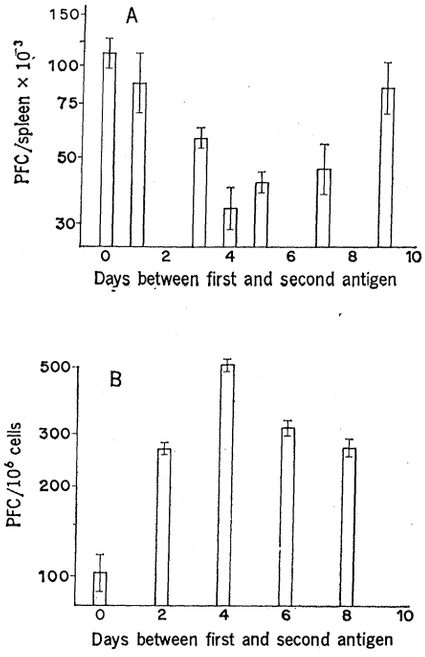

Fig. 1. (A) The in vivo 4-day plaque-forming-cell (PFC) response of mice immunized with sheep erythrocytes at various intervals after injection of pig erythrocytes. (B) The in vitro PFC response of spleen cell cultures immunized with sheep erythrocytes at various intervals after in vivo immunization with pig erythrocytes. Each bar represents the average of at least three animals or three cultures, respectively. The standard error of the mean is indicated.

DBA mice, 7 to 12 weeks old, were used. Pig erythrocytes and sheep erythrocytes were chosen as the two antigens because of their low reciprocal cross-reactivity (*4*). The response to these erythrocytes was assayed by the direct hemolysis-in-gel technique (*5*).

When 10^8 sheep erythrocytes were given intravenously 4 days after an injection of 5×10^8 pig erythrocytes, the 4-day plaque-forming-cell (PFC) response to sheep erythrocytes was suppressed about fivefold (Fig. 1A). With longer or shorter intervals between injection of the two antigens, suppression was less. When the two antigens were injected simultaneously, there was no suppresson of response to either antigen. These results are similar to those of Radovich and Talmage (*4*).

An augmented PFC response to sheep erythrocytes was observed, however, when spleen cells from animals immunized 2, 4, 6, or 8 days previously with pig erythrocytes were immunized in vitro with sheep erythrocytes. The culture method of Mishell and Dutton was used with 10^7 spleen cells per culture, and 10^7 sheep erythrocytes as antigen (*6*). The PFC response to sheep erythrocytes was measured at 4 days. The prior in vivo exposure to pig erythrocytes caused an increase rather than a decrease in the in vitro response to sheep erythrocytes (Fig. 1B). The maximum increase in

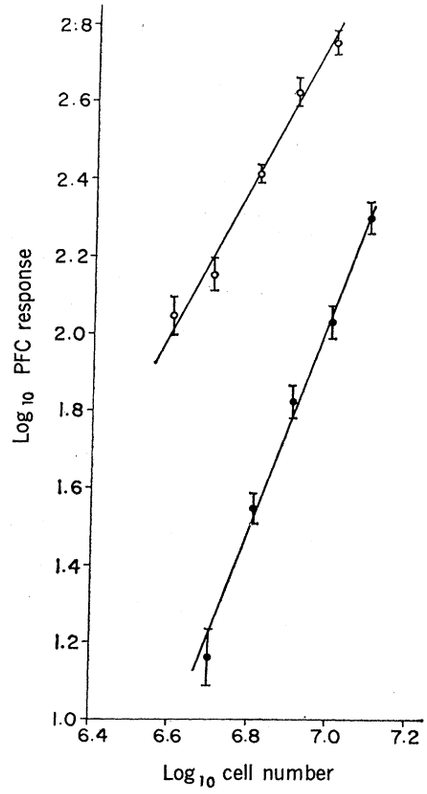

Fig. 2. The $\log_{10}$ of the plaque-forming-cell response versus the $\log_{10}$ of various numbers of spleen cells immunized in culture with sheep erythrocytes: normal cells (●) and cells from mice injected with pig erythrocytes 4 days prior to culture (○). By linear regression analysis the slope of the response of normal cells = 2.83 ± 0.16; the slope of pig erythrocyte–primed cells = 1.97 ± 0.10. The slopes are significantly different ($P < .01$).