Cocaine Inhibits Production of Murine Hepatitis Virus by Peritoneal Macrophages *In Vitro* (44117)

S. S. LEFKOWITZ,*[,1] D. J. BROWN,† K. GRATTENDICK,* AND D. L. LEFKOWITZ†

Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430; and Department of Biological Sciences,† Texas Tech University, Lubbock, Texas 79409*

Abstract. **Our previous studies have demonstrated a number of effects of cocaine on macrophage (Mø) functions. The present studies were initiated to ascertain the effects of cocaine on virus production *in vitro*. C57BL/6 mice were injected intraperitoneally with thioglycollate broth, and the peritoneal Mø collected 4 days later were cultured in 96-well microtiter plates as continuous monolayers. Various concentrations of cocaine were incubated with Mø for up to 5 days. Mouse hepatitis virus (MHV) was added to these cultures, which was followed by a methyl cellulose overlay. Cocaine caused a dose-dependent inhibition of viral plaques after 48 hr of incubation. The inhibitory activity was transferable to fresh cultures of Mø, inhibiting both plaque size and number. Specific polyclonal antibodies to α + β-interferon but not tumor necrosis factor or transforming growth factor–β partially reversed the inhibition of both plaque size and plaque number. It appears that MHV induced interferon in cultured Mø, an effect that was enhanced by cocaine. Since cocaine has been reported to interfere with calcium mobilization, studies were done using ionomycin, a calcium ionophore, in order to reverse possible effects on intracellular calcium that could affect virus production. The presence of ionomycin completely reversed the inhibition of virus production by cocaine. The antiviral effects of cocaine appear to be caused by modulation of intracellular calcium and, to a lesser extent, by the enhancement of interferon production.** [P.S.E.B.M. 1997, Vol 215]

Cocaine has been shown to have immunomodulatory effects on certain immune parameters (1). Studies by others (2) have described a number of immune abnormalities in mice exposed to cocaine, including an enhancement of neutrophil phagocytosis, a reduction of T-cell responses to the mitogen phytohemagglutinin, and a depression of cytotoxic activity of immune spleen cells. In a series of studies, Lefkowitz *et al.* (3–6) reported that cocaine can have profound effects on macrophage (Mø) functions. These investigators demonstrated an increase in the respiratory burst with a concomitant increase in phagocytosis of opsonized zymosan and a decrease in Mø-mediated cytotoxicity (MMC) with a reduced ability to produce nitric oxide. We have demonstrated that exposure to cocaine *in vivo* can cause a temporary loss or alteration of certain cell surface receptors (unpublished observations). A number of recent studies have demonstrated that mouse Mø exposed to cocaine, either *in vitro* or *in vivo*, can inhibit the production of cytokines (7–9). In a series of publications, Peterson *et al.* (10–12) reported an enhancement of HIV replication in cultures of human monocytes *via* an induction of transforming growth factor–β (TGFβ) and tumor necrosis factor–α (TNF-α). An increase in p24, one of the internal viral proteins, was used to quantify viral replication. A recent study (13) also associated TGFβ, as well as interleukin-6 (IL-6), with Mø exposed to cocaine. These investigators reported that supernatants from cocaine-exposed Mø cultures modulate mesangial cell proliferation *in vitro*. At the present time, the influence of cocaine on various disease states is somewhat ambiguous, and studies need

[1] To whom requests for reprints should be addressed at Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

This work was supported by National Institutes of Health Grant 5 R01 DA07298-02.

Received September 3, 1996. [P.S.E.B.M. 1997, Vol 215]
Accepted November 15, 1996.

0037-9727/97/2151-0087$10.50/0

to be done to ascertain the relative role of cocaine on induction and/or enhancement of certain latent infections.

The present study was undertaken to ascertain the effects of cocaine on virus production by murine peritoneal Mø. The system utilized was the replication of murine hepatitis virus (MHV) in cultured thioglycollate-induced Mø. The results obtained suggest that under the conditions employed, cocaine can inhibit production of MHV and that this inhibition can be transferred by culture supernatants. Evidence that this inhibition was caused by modulation of intracellular calcium and an enhancement of interferon production is presented.

Materials and Methods

Experimental Animals. Male or female, 6- to 8-week-old C57BL/6 mice were purchased from Jackson Laboratories (Bar Habor, ME). All mice were housed in the Texas Tech University Health Sciences Center Vivarium or at the Biological Sciences Building of the Texas Tech University. These animals were cared for as stated in the policies and regulations of the Institutional Animal Care and Use Committee.

Culture Media and Reagents. Dulbecco's modified Eagle's medium (DMEM) (Gibco, Long Island, NY) was supplemented with 2% fetal bovine serum (FBS) (Intergen, NY), 25 m*M* N-2-hydroxyethyl-piperazine N′-2-ethanesulfonic acid (HEPES) (Research Organics, Cleveland, OH), and 50 mg/l gentamycin sulfate (U.S. Biochemicals, Cleveland, OH). Phosphate-buffered saline (PBS) at pH 7.2 was also used.

Rabbit polyclonal anti-α+β-interferon (IFN-α+β) (55,000 U/ml) and anti-IFN-β (10,000 U/ml) were obtained from Lee Biomolecular, Inc. (San Diego, CA). Polyclonal anti-TGFβ1 was obtained from R&D (Minneapolis, MN). Polyclonal anti-TNF-α was received as a gift from Dr. George Gifford, Department of Microbiology at the University of Florida (Gainesville, FL). Ionomycin and dimethyl sulfoxide were obtained from Sigma Chemical Co. (St. Louis, MO). Cocaine hydrochloride was obtained from National Institute of Drug Abuse (NIDA). Cocaine was dissolved in DMEM with 2% FBS and filtered through a 0.2-μ filter prior to use.

Mø Collection and Preparation. Mice, 8–16 weeks of age, were injected i.p. with 1 ml of thioglycollate broth (Baltimore Biological Laboratories, Baltimore, MD). After 4 days, the animals were sacrificed by cervical dislocation. Peritoneal lavage was performed using 10 ml of PBS. Peritoneal Mø were centrifuged at 250*g* for 10 min, and the sedimented cells were resuspended at 1.5×10^6 cells/ml in DMEM containing 2% FBS. One hundred microliters of cell suspension were added to each well of a Costar (Cambridge, MA) 96-well tissue culture plate. After 2 hr of incubation, cells were washed with DMEM to remove nonadherent cells, and 200 μl of DMEM containing 2% FBS were added to each well. After overnight incubation, supernatants were removed, and media containing various dilutions of cocaine, from 1 to 100 μg/ml, were added to wells containing adherent Mø. Incubation intervals varied from 1 to 120 hr. After the allotted time, media were removed and the viral plaque assay performed.

Viral Plaque Assay. Mouse hepatitis virus (MHV), strain MHV-JHM (ATCC VR-765), was obtained from American Type Culture Collection (ATCC, Rockville, MD). Macrophages were incubated with various concentrations of cocaine. Following incubation, the supernatants were discarded. Fifty microliters of MHV diluted in DMEM containing 2% FBS, which induced a plaque number of approximately 40/well, were added. After incubation of Mø for 1 hr at 37°C, 100 μl of overlay consisting of DMEM, 1% methyl cellulose containing 2% FBS, were added. The plate was incubated for 18–24 hr. The supernatants were discarded and the monolayer stained with 1% Crystal Violet (Sigma) in 80% methanol. The extent of viral plaque formation was then quantified.

Transfer of Antiviral Activity. Two plates of macrophages were set up with confluent monolayers as described above. In the first plate (Plate A), cocaine was added and incubated for 48 hr. The supernatants from the second plate (Plate B) were discarded and replaced with supernatants from Plate A. This transfer was done either as a 1:1 well-to-well exchange or by pooling supernatants from a number of wells from Plate A. Plate B was incubated for 24 hr with the supernatants from Plate A, which was followed by a viral plaque assay as described above.

Effects of Antisera. When the effects of IFN were determined directly on cocaine-exposed cells, the supernatants from the cultured macrophages were discarded, and 20 μl of a 1:6 dilution of anti-IFN in DMEM were added. After 30 min of incubation, the viral plaque assay was performed as described previously except the anti-IFN was not discarded before virus was added. Virus was adjusted so that all wells received the same concentration. Two different designs were utilized when antisera were added to supernatants which were transferred to fresh cultures. Plates A and B were plated out as described. One hundred microliters of supernatants from Plate A were transferred to Plate B. In the first design, 20 μl of anti-IFN diluted to a concentration of 1:20 using DMEM containing 2% FBS were added to the supernatants of Plate B. In the second design, the supernatants from Plate A were pooled and incubated with anti-IFN for 1 hr before addition to the Mø in Plate B. The plates were incubated for 24 hr, and then viral plaque assays were performed as described above.

Effects of Calcium Ionophore. Ionomycin was dissolved in dimethyl sulfoxide at a concentration of 1.25×10^{-4} *M,* which was subsequently diluted with DMEM containing 2% FBS to a final concentration

of 1.25–2.5 × 10^{-6} *M*. Cocaine-treated culture fluids contained 100 μg/ml, and 100 μl of the mixtures were added to each well. Control cultures received either ionomycin or cocaine only. After 48 hr of incubation, cultures were infected, and the virus plaques were allowed to form as described above.

Statistical Analysis of Data. All of the above experiments were repeated at least three times in order to insure reproducibility. A Student's *t* test was used to determine the significance between various treatments. A one-way analysis of variance and Newman-Keuls' multiple range testing was used to determine significance among multiple groups. All values are reported as mean ± SEM. Differences were considered significant at $P < 0.05$. In general, significance is reported between control and experimental values unless otherwise noted.

Results

Lipopolysaccharide Contamination. Because of the sensitivity of Mø to minute traces of lipopolysaccharide (LPS), it was essential to rule out contamination of cocaine by LPS. Using the Limulus amoebocyte lysis test (LAL), the amount of LPS was <0.5 ng/mg cocaine, which was insufficient to account for the amount of antiviral activity detected considering the dilutions of cocaine employed were ≤0.1 mg/ml.

Effects of Cocaine Concentration on Virus Production. Initial studies were done in order to determine if cocaine would affect virus production. It can be seen in Figure 1 that, when Mø were incubated with cocaine 48 hr prior to infection with MHV, a significant reduction in viral plaque size was evident even at the lowest concentration used (1 μg/ml). To determine if cocaine had a dose-dependent effect on virus production *in vitro*, cocaine levels of 1, 3, 11, 33, and 100 μg/ml were incubated with Mø for 48 hr prior to infection with MHV. An inhibition of virus production was observed by a decrease in both plaque number and size. A reduction in plaque number can be seen in Figure 2, with a leveling off of antiviral activity at 33 μg cocaine/ml. As little as 3.5 μg/ml cocaine significantly ($P < 0.001$) reduced viral plaque numbers. After noting this antiviral effect, an experiment was done to determine the kinetics of antiviral activity. While the cocaine concentration was kept constant at 100 μg/ml, the incubation time was varied from 12 to 120 hr. Increases in antiviral activity were seen as early as 12 hr but differences were not significant until 24 hr. Figure 3 illustrates the time course between 48 and 120 hr. Antiviral activity continued to increase for as long as 120 hr. However, in other experiments a leveling off of antiviral activity was noted by 72 or 96 hr.

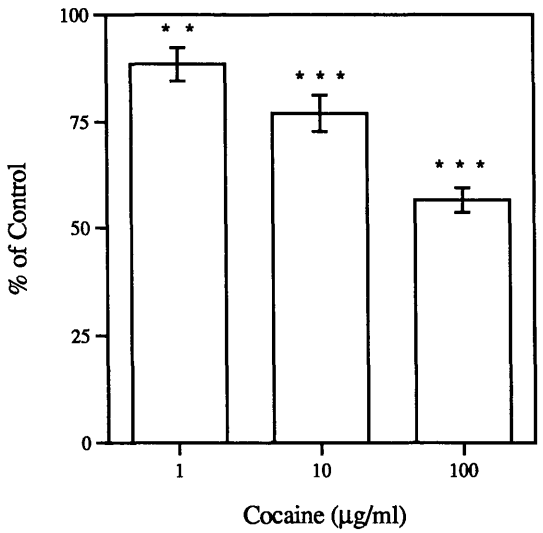

Figure 1. Monolayers of murine thioglycollate-induced macrophages were exposed to cocaine concentrations of 1–100 μg/ml followed by incubation for 48 hr. After removal of media, these cells were infected with mouse hepatitis virus, an overlay added, and the monolayers stained with crystal violet in 80% methanol after the appropriate incubation. The diameters of the plaques from treated cells were measured and compared with controls (156 ± 4.5 μm). $^{**}P < 0.01$; $^{***}P < 0.001$.

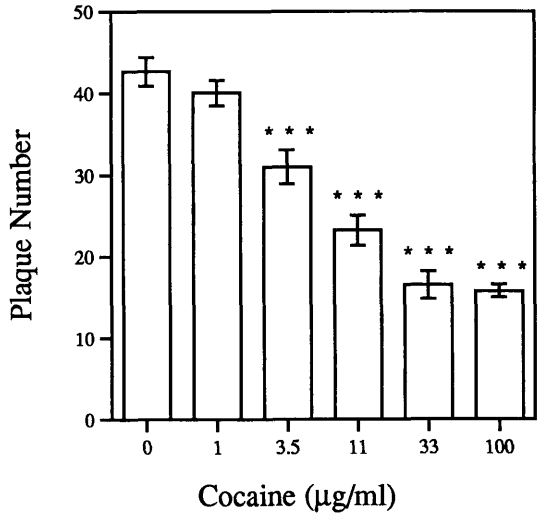

Figure 2. Monolayers of murine thioglycollate-induced macrophages were incubated 48 hr with different concentrations of cocaine dissolved in DMEM containing 2% FBS. After removal of media, these cells were infected with mouse hepatitis virus, an overlay added, and the number of viral plaques quantitated. $^{***}P < 0.001$.

In the above studies, cocaine was left on the Mø monolayers during the entire incubation period. In another series of experiments, cocaine was removed from the Mø and the media replaced. When cocaine was removed from the Mø monolayers between 3 and 6 hr, and replaced with fresh media, no antiviral activity was found after 24 and 48 hr, in contrast to when cocaine was left on the cells.

Transferring of Antiviral Activity. In order to determine if antiviral activity was transferable to Mø that were not directly exposed to cocaine, a transfer of supernatants from cocaine-exposed Mø to nonexposed Mø was done followed by infection with MHV. In this experiment, transferred supernatants produced similar antivi-

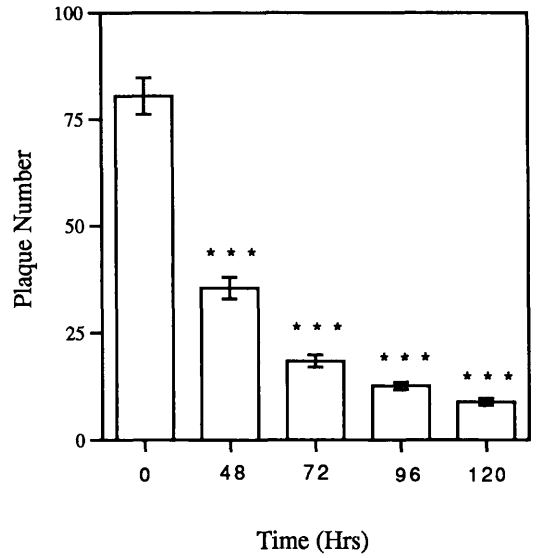

Figure 3. Monolayers of murine thioglycollate-induced macrophages were incubated for various intervals with 100 μg/ml of cocaine dissolved in DMEM containing 2% FBS. After removal of media these cells were infected with mouse hepatitis virus, an overlay added, and the number of viral plaques quantitated. ***$P < 0.001$.

ral effects in Mø cultures as seen in cocaine-exposed cultures. It can be seen in Figure 4 that supernatants from cultures exposed to different amounts of cocaine were antiviral in a dose-dependent manner. Although differences were noted in supernatants from cultures exposed to 3 μg cocaine/ml, only those exposed to 11 μg/ml or more were significantly different ($P < 0.05$) from the controls.

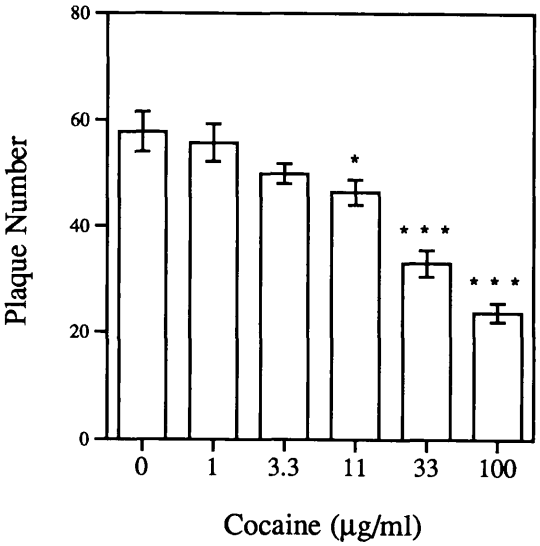

Figure 4. Monolayers of murine thioglycollate-induced macrophages were exposed to either control media or to cocaine for 48 hr. Supernatants from these cultures were removed and transferred to fresh cultures. After 24 hr, the supernatants were removed and these cells were infected with mouse hepatitis virus, an overlay added, and the number of viral plaques quantitated. *$P < 0.05$; ***$P < 0.001$.

In order to determine how much of the antiviral effect was caused by cocaine transferred in supernatants, rather than by the presence of a newly synthesized antiviral substance, a number of different experimental designs were employed. The first design was set up to determine if cocaine needed to be present continuously for an antiviral effect to be exhibited. Two groups of Mø, Groups A and B, were exposed to cocaine for 48 hr. After 48 hr, supernatants from Group A were discarded, replaced with DMEM not containing cocaine, and allowed to incubate an additional 24 hr prior to infection by virus. Group A showed a 70% reduction in virus production, which was only slightly less than the viral reduction noted using Group B (80%) where the cocaine was "present continuously" (Fig. 5A). Similar effects were also noted using media from Mø exposed to cocaine for either 24 or 72 hr followed by media removal and 24 hr incubation.

In the second design, two sets of Mø were set up to determine if the antiviral action of cocaine could be transferred in the culture media of cells not exposed to cocaine continuously. Both sets of Mø were incubated with cocaine for 48 hr. The supernatants in Group A were then removed and replaced with fresh DMEM not containing cocaine, and allowed to incubate for an additional 24 hr. Mø in Group B had continuous exposure to cocaine for the entire 72-hr period. After the 72-hr incubation period, the supernatants from both Group A and B were removed and added to two separate plates of Mø. These plates were then incubated for 24 hr prior to infection with MHV. With the Mø exposed to the supernatants from Group A, there was approximately a 25% reduction of viral plaque formation compared with the control. Supernatants from Group B resulted in a 55% reduction of viral plaques (Fig. 5B). Essentially the same effects were also noted in experiments using supernatants from either 24- or 72-hr exposures with a 24-hr wash prior to transfer of supernatants.

Effect of Specific Antisera and Ionomycin on Virus Production. In order to identify the antiviral agent(s) involved in cocaine inhibition of MHV, four different antisera were tested. Anti-TGFβ and anti-TNF-α had no effect on the antiviral activity in supernatants from cocaine-exposed Mø (data not shown). The addition of either anti-IFN-α+β or anti-IFN-β, however, had an effect on both plaque number and size. The presence of one or the other anti-IFN caused an increase in plaque size over that of either the virus control plaques or those exposed to cocaine (data not shown). Anti-IFN caused a significant increase in plaque number ($P < 0.01$) compared with wells treated with cocaine only when added to the transfer supernatants on a well-to-well basis, as illustrated in Figure 6. The increase in plaque size as well as number was seen when either anti-IFN-α+β or anti-IFN-β was incubated with

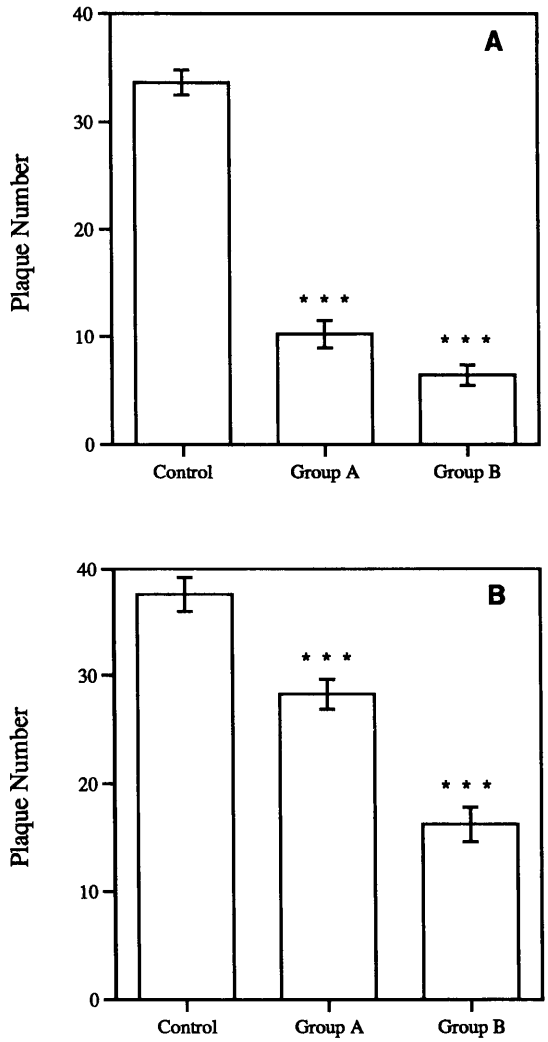

Figure 5. (A) Monolayers of murine thioglycollate-induced macrophages were exposed to either control media or to 100 μg/ml cocaine for 48 hr. Supernatants from Group A were discarded and replaced with fresh media for an additional 24 hr. Supernatants from Group B were left undisturbed. Both groups were then infected with mouse hepatitis virus, an overlay added, incubated, and the number of viral plaques quantitated. (B) Cultures were treated exactly as in Panel A except that the media from both groups were transferred separately after the 72-hr incubation to fresh Mø, incubated for an additional 24 hr, and infected with mouse hepatitis virus, an overlay added, and the number of viral plaques quantitated. $^{***}P < 0.001$.

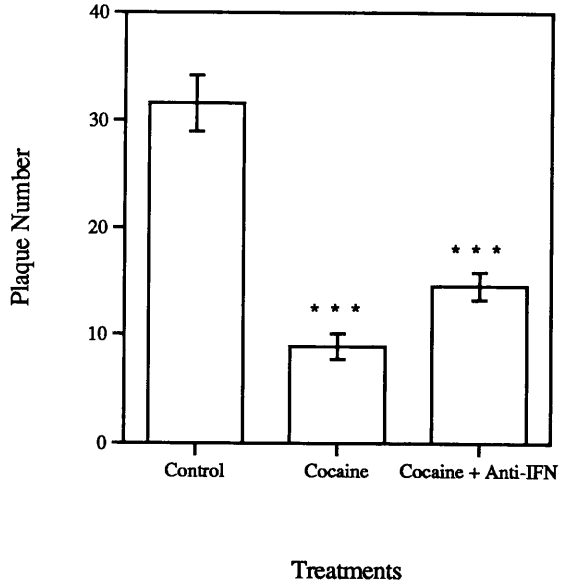

Figure 6. Supernatants from macrophage cultures incubated with 100 μg of cocaine for 48 hr were transferred to fresh macrophage cultures and incubated with or without antisera to β-interferon. After 24 hr, the media were removed, and these cells were infected with mouse hepatitis virus, an overlay added, and the number of viral plaques quantitated. The difference between cells exposed to cocaine only and those exposed to cocaine and anti-IFN was considered significant ($P < 0.01$). $^{***}P < 0.001$.

pooled supernatants from cocaine-exposed Mø. The size difference was frequently, but not always, significantly different from the control Mø without antisera.

In order to determine if the effect of cocaine on virus production depended on intracellular calcium levels, ionomycin was added to cocaine-treated cultures. The addition of ionomycin to cultures and its subsequent removal resulted in an increase in the number of viral plaques similar to that of the controls without cocaine. It can be seen in Figure 7 that ionomycin when added to cocaine-exposed Mø completely reversed the inhibitory effects of cocaine.

Discussion

The metabolism of cocaine by Mø *in vitro* has not been studied. Preliminary studies by the present investigators indicated that cocaine was still present in the culture supernatants by 72 hr, but at considerably lower levels. Benzoylecgonine, a major metabolite of cocaine, was found at high levels. However, these levels were similar to those occurring in media in the absence of Mø. This suggests that the conversion of cocaine to benzoylecgonine in the cultured supernatants was due to spontaneous hydrolysis and not to Mø metabolism.

Peterson *et al.* have reported that cocaine enhances viral replication (10). The present studies demonstrate an inhibition of virus production. A number of obvious differences exist between our studies and theirs, including the host species involved as well as the nature of the viral agent. The Mø used in the present study were induced by thioglycollate, which is a classical inducer of inflammatory Mø. These Mø would be more likely to inhibit a viral infection than the normal human mononuclear cells used by the other investigators. Another major difference is that production of infectious virus, as determined by plaque assay, was measured in the present studies rather than an increase in viral proteins such as p24. Finally, the properties of the antiviral substance described in the present study suggest those of an inducible cytokine that was transferable. The fact that neither anti-TGFβ nor anti-TNF reversed the anti-

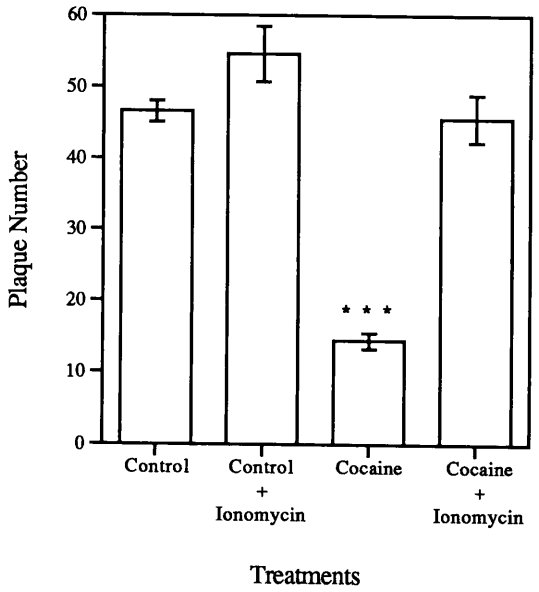

Figure 7. Monolayers of murine thioglycollate-induced macrophages were incubated for 48 hr with 100 μg/ml of cocaine and with or without 1.25×10^{-6} *M* ionomycin dissolved in DMEM containing 2% FBS. After removal of the media, these cultures were infected with mouse hepatitis virus, an overlay added, and the number of viral plaques quantitated. ***$P < 0.001$.

viral effect appears to rule out a similar mechanism reported by Peterson *et al.* (11, 12). It should, however, be emphasized that these investigators utilized considerably lower levels of cocaine (10^{-6}–10^{-9} *M*) than we did, which could possibly dictate not only the amount but also the type of cytokine produced. Further studies are needed to clarify this point.

The present studies have reported an increase in both plaque size and plaque number after addition of anti-IFN. It is apparent that MHV induced IFN in the present system without the addition of cocaine, since plaque size was increased in the presence of the antisera even in the controls. In order to maximize the effect of anti-IFN, a number of different designs were used, including direct exposure of cocaine-exposed cells to the antisera as well as to the supernatants that were transferred from cocaine-exposed cells to fresh cells. In both experimental designs, both plaque size and number were increased in the presence of anti-IFN. In certain experiments, control viral plaques were slightly larger in the presence of the antisera. In general, there appeared to be more of an effect on plaque size in the direct exposure studies and a greater increase in plaque number in the transfer experiments. This could be explained, in part, by different experimental designs. In direct studies, a higher concentration (1:6) of antisera was added, incubated for 30 min, prior to dilution with the virus and overlay. Whereas, with the transfer supernatants the antisera was diluted with the supernatants prior to addition to fresh cells.

The greater effect of anti–IFN-α+β compared with anti–IFN-β on both plaque size and number suggest that cocaine induced both types of IFN. Since the other rabbit antisera employed did not affect virus production, it appears that IFN levels were involved. These data fit the literature which reported that Mø produce predominantly IFNβ. It should be emphasized that anti-IFN only partially reversed the antiviral effects of cocaine. It was noted previously (1) that cocaine needed to be continuously present in order to function in certain systems. In the present studies when culture fluids containing cocaine were removed from cells prior to 12 hr of exposure, an antiviral effect was not demonstrated. However, when media containing cocaine were removed after 48 hr of exposure and replaced with DMEM not containing cocaine for 24 hr (Figs. 4 and 5), a significant, but reduced, antiviral effect was still found in the fresh supernatant, suggesting that cocaine induced the production of an antiviral substance and that cocaine did not have to be present continuously. This supports the concept that cocaine induces the production of an antiviral substance that is transferable and requires longer than 12 hr to be detectable; however, continual presence of cocaine in culture fluids does appear to enhance antiviral activity. Interestingly, maximal levels of IFN were obtained after 48 hr, suggesting that this may not be a direct effect of cocaine on IFN induction but rather an indirect effect, since maximal IFN-α+β induction *in vitro* is usually in about 6 hr using a classical inducer such as poly I:C.

It was reported that cocaine interferes with calcium mobilization (14). Further studies (15, 16) indicated that both calcium inhibitors and cocaine interfere with induction of reactive oxygen intermediates and reactive nitrogen intermediates by Mø. It has been reported that calcium antagonists inhibit the replication of certain viruses including influenza virus (17) and vesicular stomatitis virus (18). The stimulation of virus production in cocaine-exposed cultures by the calcium ionophore ionomycin indicates a similar effect in the present study. It appears that intracellular calcium levels are involved in the inhibition of virus production. This is further supported by the observation in this study that early removal of cocaine from Mø cultures prevents production of the antiviral effect. This study demonstrates that cocaine induces an antiviral effect in Mø that is mediated in part by interferon and in part by intracellular calcium.

The observed decrease in plaque formation due to cocaine exposure is indicative of at least two possibilities. Cocaine is inhibiting either the adsorption of virus to the Mø or viral replication. Preliminary experiments currently being conducted suggest that cocaine is affecting MHV replication. The transfer of antiviral activity from supernatants that were obtained from Mø exposed to cocaine, washed, and then incubated with media without cocaine for 24 hr supports an effect at the synthesis

phase of replication. That the addition of anti-IFN, a known inhibitor of viral replication, or ionomycin was able to reverse partially the effects of cocaine supports this conclusion further. However, a downregulation of the MHV receptor cannot yet be ruled out.

The present study was carried out under rather restricted conditions *in vitro*. Possible extrapolation to *in vivo* conditions is not currently justified. Nevertheless, the induction of cytokines by Mø exposed to cocaine, which includes TGFβ, IL-6, and now IFN indicates the immunomodulatory potential of cocaine. Regulation of intracellular calcium further underscores the broad actions of this substance.

1. Watzl B, Watson RR. Immunomodulation by cocaine-A neuroendocrine mediated response. Life Sci **46:**1319–1329, 1990.
2. Pacifici R, Avico U, Belogi L, Borelli G, Croce C, Di Carlo S, Pichini S, Santiangeli C, Zuccaro P, Tubaro E. Immunological effect of cocaine and host resistance in mice. Int J Immunother **8:**91–99, 1992.
3. Lefkowitz SS, Vaz A, Lefkowitz DL. Cocaine reduces macrophage killing by inhibiting reactive nitrogen intermediates. Int J Immunopharm **15:**717–721, 1993.
4. Vaz A, Lefkowitz SS, Lefkowitz DL. Cocaine alters the respiratory burst and phagocytic activity of murine macrophages. Clin Immunol Immunopath **69:**161–166, 1993.
5. Vaz A, Lefkowitz SS, Lefkowitz DL. Effects of cocaine on the respiratory burst of murine macrophages. In: Friedman H, Klein TW, Spector S, Eds. Drugs of Abuse, Immunity and AIDS. New York: Plenum Publishers, p135–142, 1993.
6. Vaz A, Lefkowitz SS, Castro A, Lefkowitz DL. The effects of cocaine and its metabolites on the production of reactive oxygen and reactive nitrogen intermediates. Life Sci **55:**PL439–PL444, 1994.
7. Chen GJ, Tachibana K, Huang DS, Watson RR. Cocaine modulation in vitro of tumor necrosis factor production by macrophages from retrovirally-infected mice. Life Sci **52:**1641–1647, 1993.
8. Wang Y, Huang DS, Watson RR. In vivo and in vitro cocaine modulation on production of cytokines in C57BL/6 mice. Life Sci **54:**401–411, 1994.
9. Shen HM, Kennedy JL, Ou DW. Inhibition of cytokine release by cocaine. Int J Immunopharm **16:**295–300, 1994.
10. Peterson PK, Gekker G, Chao CC, Schut R, Molitor TW, Balfour HH. Cocaine potentiates HIV-1 replication in human peripheral blood mononuclear cells: Involvement of transforming growth factor–β. J Immunol **146:**81–84, 1991.
11. Peterson PK, Gekker G, Chao CC, Schut R, Verhoef J, Edelman CK, Erice A, Balfour HH. Cocaine amplifies HIV-1 replication in cytomegalovirus-stimulated peripheral blood mononuclear cell cultures. J Immunol **149:**676–680, 1992.
12. Peterson PK, Gekker G, Chao CC, Hu S, Edelman CK, Balfour HH, Verhoef J. Human cytomegalovirus-stimulated peripheral blood mononuclear cells induce HIV-1 replication via a tumor necrosis factor–α–mediated mechanism. J Clin Invest **89:**574–580, 1992.
13. Mattana J, Gibbons N, Singhal PC. Cocaine interacts with macrophages to modulate mesangial cell proliferation. J Pharmacol Exp Ther **271:**311–318, 1994.
14. Matsui K, Friedman H, Klein TW. Molecular mechanisms associated with cocaine-induced modulation of human T lymphocyte proliferation. In: Friedman H, Klein TW, Spector S, Eds. Drugs of Abuse, Immunity and AIDS. New York: Plenum Publishers, p127–134, 1993.
15. Shen HM, Wiederhold MD, Ou DW. The suppression of macrophage secretion by calcium blockers and adenosine. Immunopharm Immunotox **17:**301–309, 1995.
16. Shen HM, Sha LX, Wiederhold MD, Ou DW. Suppression of macrophage reactive intermediates by cocaine. Int J Immunopharm **17:**419–423, 1995.
17. Nugent KM, Shanley JD. Verapamil inhibits influenza A virus replication. Arch Virol **81:**163–170, 1984.
18. Schlesinger MJ, Cahill D. Verapamil and chlorpromazine inhibit the budding of sindbis and vesicular stomatitis viruses from infected chicken embryo fibroblasts. Virology **168:**187–190, 1989.