when substrate phage DNA's were incubated separately with cell extracts, repurified, and then mixed before packaging and plating, no recombinants were obtained either (<1 percent of the maximum yield). Thus the recombination event must have taken place in the mammalian cell extracts and not the packaging preparations. These controls were particularly important since it has been reported that recombination between Ig heavy chain switch sites (not present in our constructs) can occur during packaging (*11*) even when packaging extracts were made (as ours were) from bacteria whose recombination systems had been inactivated by *red* and *recA* mutations.

Several different murine cell lines were tested for recombination activity (Table 2) including early B- and T-cell lines. A myeloma line was included in which genome rearrangements are presumed to have ceased, as well as a mature T-cell and a fibroblast line. These might be expected to be negative for recombination activity. The cell lines showing the highest levels of activity, 39B9 and 1881, are known to rearrange their Ig genes in culture, whereas the fully differentiated T-cell and myeloma lines in which DNA rearrangements do not occur showed no activity. The fibroblast line did produce low but significant levels of Spi$^-$ recombinant candidates, but they contained large deletions; no homologous or exact D-J recombinants were found.

The fact that homologous recombination activity is most efficient in cell lines that can actively recombine Ig gene segments and negative in those that do not suggests that we may be observing a partial recombination activity with a role in Ig gene expression. The high level of activity in the early T-cell extracts may also be consistent with this idea, since the genes for T-cell receptor polypeptides are assembled from dispersed segments by a process very similar to that for Ig genes (*12*).

Our results show that the in vitro system can be exploited in the study of mammalian recombination with engineered substrates, but suggest caution in correlating in vivo with in vitro effects.

V. DARBY
F. BLATTNER
Laboratory of Genetics, University of Wisconsin, Madison 53706

References and Notes

1. A. J. Clark, *Annu. Rev. Genet.* 7, 67 (1973); M. S. Fox, *ibid.* **12**, 47 (1978); G. J. Vance Makin, W. Szybalski, F. R. Blattner, *Genetics* **102**, 299 (1982); F. Stahl, *Genetic Recombination: Thinking About It in Phage and Fungi* (Freeman, San Francisco, 1980).
2. L. S. Symington, L. M. Fogarty, R. Kolodner, *Cell* **35**, 805 (1983).
3. P. Leder, *Sci. Amer.* **246** (No. 5), 102 (1982); H. Sakano, Y. Kurosawa, M. Weigert, S. Tongawa, *Nature (London)* **290**, 562 (1981); Y. Kurosawa and S. Tonegawa, *J. Exp. Med.* **155**, 201 (1982).
4. These results were first presented at the Airlie "Recombination" meeting at Airlie House, Virginia, 31 October to 4 November 1983.
5. J. Zissler, E. Signer, F. Schaefer, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), pp. 445–476; J. Karn, S. Brenner, L. Barnett, G. Cesareni, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5172 (1980).
6. Cells were cultured in RPMI 1640 medium containing 10 percent fetal calf serum and antibiotics. The cells (5×10^7 to 5×10^8) were harvested, washed in serum-free medium, and resuspended in 2 ml of 10 m*M* tris-HCl at *p*H 7.4, 10 m*M* $MgCl_2$, 10 m*M* KCl, and 1 m*M* dithiothreitol. Phenylmethyl sulfonyl fluoride (1 m*M*) was added just before homogenization in a Dounce glass homogenizer. Nuclei were collected by centrifugation at 1000 rev/min in a clinical centrifuge and resuspended in 1 ml of the same buffer. NaCl was added to a concentration of 0.3*M*, and the mixture was incubated on ice for 1 hour. The extract was then centrifuged at 100*g* (Beckman 50Ti rotor), and the supernatant was dialyzed. Portions were frozen in a mixture of CO_2 and ethanol and stored at −80°C.
7. Generally, 10 μg of substrate DNA was incubated with 15 μg of cell extract. The reaction also contained 10 m*M* tris-HCl (*p*H 7.5), 5 m*M* $MgCl_2$, 0.5 m*M* adenosine triphosphate (ATP), and ribonuclease (100 μg/ml) in a total volume of 100 to 150 μl. After 1 hour at 37°C the in vitro reaction was terminated by addition of sodium dodecyl sulfate and proteinase K (to 0.5 percent and 0.25 mg/ml, respectively) and incubated for 30 minutes at 37°C. Phage DNA was purified by several phenol and chloroform extractions, and finally by ethanol precipitation. The phage DNA was then packaged in vitro into capsids, and plated on appropriate host bacteria. In vitro packaging extracts were made from the lysogens BHB2688 and BHB2690 [B. Hohn and K. Murray, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3259 (1977)]. The protocol was a modification of that described by B. A. Zehnbauer and F. R. Blattner [in *Genetic Engineering*, J. K. Setlow and A. Hollaender, Eds. (Plenum, New York, 1982), vol. 4, pp. 249–279].
8. Restriction digests of DNA samples were subjected to electrophoresis on agarose gels and then blotted on to nitrocellulose. Plasmid subclones of D, J, and Spi fragments (in pBR322 derivatives) were labeled with ^{32}P by nick translation and hybridized to the gel blots; hybridizing bands were then detected by autoradiography; the DNA sequences were determined by the method of A. Maxam and W. Gilbert [*Methods Enzymol.* **65**, 499 (1980)].
9. M. S. Meselson and C. M. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 358 (1975).
10. J. W. Szostak, T. L. Orr-Weaver, R. J. Rothstein, *Cell* **33**, 25 (1983).
11. T. Kataoka, S. I. Takeda, T. Honjo, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2666 (1983).
12. G. Siu *et al.*, Cell **37**, 393 (1984).
13. N. Newell, J. E. Richards, P. W. Tucker, F. R. Blattner, *Science* **209**, 1128 (1980).
14. D. L. Rimm, D. Hornes, J. Kuara, F. R. Blattner, *Gene* **12**, 301 (1980).
15. J. R. deWet *et al.*, *J. Virol.* **33**, 401 (1980).
16. References for the cell lines are as follows: PD31 [S. Lewis, N. Rosenberg, F. Alt, D. Baltimore, *Cell* **30**, 807 (1982)]; cell line 1881 [S. C. Riley, E. J. Brock, W. M. Kuehl, *Nature (London)* **289**, 894 (1981)]; cell line 38B9 [T. K. Blackwell and F. W. Alt, *Cell* **37**, 105 (1984)]; cell line 3T3 [G. J. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963)]. Other cell lines were obtained from R. Risser, University of Wisconsin.
17. Supported by NIH grant AI19325, and a grant from the Merck Foundation. We thank Drs. R. Risser, F. W. Alt, and D. Baltimore for cell lines, T. G. Burland for a preparation of phage λcI857 Sam 7 especially low in revertants, D. Waring and B. Bauer for technical support, and D. Daniels for discussions and comments on the manuscript. This is paper 2762 from the Laboratory of Genetics, University of Wisconsin, Madison.

3 August 1984; revised 22 October 1984

Morphine Analgesia Potentiated but Tolerance Not Affected by Active Immunization Against Cholecystokinin

Abstract. *Administration of cholecystokinin was recently found to attenuate opiate analgesia. In the present study, the role of endogenous cholecystokinin in opiate analgesia was examined. Endogenously released cholecystokinin was sequestered by antibodies to cholecystokinin developed in response to an active immunization procedure. Morphine analgesia was potentiated and prolonged in rats immunized against cholecystokinin. The rate of development of morphine tolerance, however, was not affected by the antibodies. Endogenous cholecystokinin appears to function as a short-term modulator of opiate action.*

Accumulating evidence supports the hypothesis that there is a physiological antagonism between cholecystokinin (CCK) and opiates (*1*). The sulfated octapeptide variant of CCK potently and specifically attenuates opiate-mediated forms of analgesia produced by foot shock (*1*), morphine (*1*), and β-endorphin (*2*). Other opiate-dependent processes, including tail pinch–induced feeding (*3*) and suppression of thyrotropin-releasing hormone–induced wet dog shakes by β-endorphin (*4*), are also antagonized by CCK octapeptide. In addition to these experiments with exogenous CCK, indirect evidence supports an involvement of endogenous CCK in nociception. Anatomically, CCK is present in areas of the central nervous system (CNS) known to modulate the intensity of pain perception, such as the periaqueductal gray region and the dorsal horn of the spinal cord (*5*). Also, the finding that levels of CCK in the brain decrease in response to systemically administered morphine (*6*) indicates a functional interaction between CCK and opiate systems. These findings, along with the observation that several other effects of CCK octapeptide are the opposite of those reported for opiates (*1*), suggest that an opiate-antagonistic function of CCK underlies several actions previously ascribed to this peptide.

If endogenous CCK does indeed function to inhibit opiate-dependent mechanisms, then blockade of CCK's action (*7*) should potentiate or prolong the effects

of opiates. We therefore examined the effect of active immunization against CCK (*8*) on the magnitude and duration of the analgesic response to systemically administered morphine.

Adult male Sprague-Dawley rats were immunized against CCK octapeptide (group 1; $n = 8$) or bovine serum albumin (BSA) (group 2; $n = 9$) by subcutaneous administration at 0, 2, 4, and 8 weeks of an emulsion containing Freund's adjuvant and BSA alone or CCK conjugated to BSA (*9*). At 12 weeks the mean capacity of serum from group 1 to specifically bind [^{125}I]CCK octapeptide was 50 pg/ml (*10*). At this time the animals were tested for responsiveness to morphine.

Analgesia was assessed by the tail-flick test (*11*). Tail-flick latencies were measured before and at six 30-minute intervals after subcutaneous administration of morphine sulfate (10 mg/kg) (*12*). Although no statistically significant differences between groups in baseline pain responsiveness were identified, group 1 showed a significantly greater analgesic response to morphine and remained analgesic longer than did group 2 [$F(1, 79) = 4.68$, $P < 0.03$) (Fig. 1).

In addition to modulating the short-term analgesic response to a single morphine injection, endogenous CCK may also exert a prolonged inhibitory influence on opiate analgesia. If this is the case, then sequestering endogenously released CCK should retard or abolish development of morphine tolerance. To examine this possibility, we gave the CCK-immunized rats morphine sulfate (10 mg/kg subcutaneously) at 12-hour intervals for 60 hours. Tail-flick latencies were measured 60 and 90 minutes after additional morphine injections given 24, 36, 48, and 60 hours after the initial morphine injection. Group 1 (CCK-immunized) rats showed higher levels of analgesia than group 2 animals at all times tested [$F(1, 130) = 5.49$; $P < 0.02$] (Fig. 2). However, the rate of decline in the ability of morphine to produce analgesia (development of tolerance) did not differ between groups (Fig. 2).

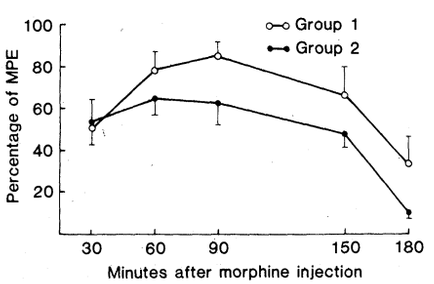

Fig. 1. Increase in the magnitude and duration of morphine analgesia caused by active immunization against CCK (group 1). Morphine sulfate (10 mg/kg) was administered subcutaneously at time zero. Tail-flick latencies (*12*) were measured at 30-minute intervals for 180 minutes. Analgesia, expressed as a percentage of the maximum possible elevation (MPE) (*12*), was significantly higher in group 1 than in BSA-immunized animals (group 2) ($P < 0.03$). For instance, group 2 showed a peak analgesic response at 30 minutes; the analgesia of group 1, however, continued to increase, not reaching its peak until 90 minutes (the value at 30 minutes differs significantly from the value at 90 minutes, $P < 0.007$).

That active immunization against CCK octapeptide increased the magnitude and the duration of morphine analgesia suggests that endogenous CCK is released in response to activity in opiate-dependent systems (*13*) and functions to inhibit opiate action. Such negative feedback would serve to return nociceptive thresholds to normal and thereby maintain optimum levels of responsiveness.

Formation of antibodies to CCK did not affect baseline pain responsiveness or the rate at which tolerance to the analgesic effects of morphine developed. Therefore it does not appear that CCK has a tonic influence on nociceptive mechanisms. These findings suggest that at least two components are involved in determining sensitivity to morphine: (i) short-term modulation by CCK of the response to single injections of morphine and (ii) a separate modulating principle that is not CCK-mediated and that pertains to the development of tolerance under conditions of repeated morphine exposure.

Watkins *et al.* (*14*) recently reported that the putative CCK antagonist proglumide reverses morphine tolerance, since proglumide enhanced the analgesic response to a single dose of morphine sulfate administered to morphine-tolerant rats. We question this interpretation, and maintain that a different research design (comparing control and experimental animals at serial time points as tolerance is developing) is necessary to establish whether an agent truly alters the development of tolerance. Consistent with our findings, Yang *et al.* (*15*) reported that antibody to CCK or proglumide potentiates morphine analgesia but that neither agent affects the development of morphine tolerance. They also reported that proglumide reverses the antagonistic action of Phe-Met-Arg-Phe-amide on morphine analgesia, thus adding to uncertainties (*7, 8*) about the specificity and mechanism of action of proglumide.

It is unlikely that our findings are attributable to a direct effect of CCK antibodies on CNS sites, since γ-globulins do not readily pass the blood-brain–cerebrospinal fluid barriers, as evidenced by the fact that samples of cerebrospinal fluid from group 1 did not exhibit any specific binding of [^{125}I]CCK octapeptide (*10*). Therefore, the potentiation of morphine analgesia reported here is best accounted for by a sequestering of peripheral CCK, thus reducing the amount of CCK available for interaction with peripheral or central CCK receptors. It is not known whether peripheral CCK crosses the blood-brain barrier; however, the idea that peripherally circulating CCK might indeed have access to CNS receptor sites is supported by evidence that intraperitoneal administration of CCK potently attenuates morphine-induced suppression of the spinally mediated tail-flick reflex (*1*).

In the clinical treatment of pain, concomitant administration of morphine and a specific CCK antagonist may allow a reduction in the initial dose of morphine required and a decrease in the frequency of its administration. Thus, while block-

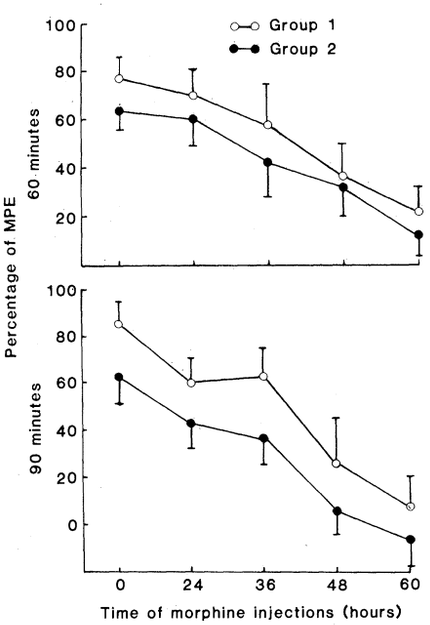

Fig. 2. Failure of active immunization against CCK to affect the development of morphine tolerance. Immunized rats received morphine sulfate (10 mg/kg) at 12-hour intervals for 3 days. Tail-flick latencies were recorded 60 minutes (top) and 90 minutes (bottom) after administration of morphine 24, 36, 48, and 60 hours after the initial injection (see Fig. 1). While group 1 continued to display higher levels of analgesia than did controls ($P < 0.02$, analysis of variance), the rate of decline in the analgesic efficacy of morphine did not differ between groups. For example, comparing levels of analgesia recorded 60 minutes after the initial morphine injection (hour 0) to values recorded 60 minutes after the sixth injection (60 hours), the percent change in the analgesic response was 57.2 in group 1 and 52.5 in group 2 (top graph).

ade of CCK's action will not retard the development of morphine tolerance, the problem of tolerance may be mitigated by a reduction in the total amount of morphine needed to alleviate pain.

PATRICIA L. FARIS*
CAROL L. MCLAUGHLIN
CLIFTON A. BAILE
JOHN W. OLNEY
Departments of Psychiatry, Pathology, and Preventive Medicine, Washington University School of Medicine, St. Louis, Missouri 63110
BARRY R. KOMISARUK
Institute of Animal Behavior, Rutgers University, Newark, New Jersey 07102

References and Notes

1. P. L. Faris, B. R. Komisaruk, L. R. Watkins, D. J. Mayer, *Science* **219**, 310 (1983). The antagonism of analgesia by the sulfated octapeptide variant of CCK is specific with regard to the molecular form of CCK as well as to opiate-dependent analgesia.
2. S. Itoh, G. Katsuura, Y. Maeda, *Eur. J. Pharmacol.* **80**, 421 (1982).
3. J. E. Morley and A. S. Levine, *Science* **209**, 1259 (1980); C. B. Nemeroff *et al.*, *ibid.* **200**, 793 (1978).
4. S. Itoh and G. Katsuura, *Jpn. J. Pharmacol.* **32**, 667 (1982).
5. T. Hökfelt, O. Johansson, A. Ljungdahl, J. M. Lundberg, M. Schutlzberg, *Nature (London)* **284**, 515 (1980); L.-I. Larsson and J. F. Rehfeld, *Science* **213**, 768 (1981).
6. C. B. Lamers *et al.*, *Am. J. Physiol.* **239**, E232 (1980).
7. The CCK receptor antagonists currently available are dibutyryl cyclic guanosine 5′-monophosphate [S. R. Peikin, C. L. Costenbader, J. D. Gardner, *J. Biol. Chem.* **254**, 5321 (1979)], benzocript, and proglumide [W. F. Hahne, R. T. Jensen, G. F. Lemp, J. D. Gardner, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6304 (1981)]. Although these substances antagonize CCK receptors in pancreatic acinar cells, such receptors have been shown to be distinct from those in the brain [R. B. Innis and S. H. Snyder, *ibid.* **77**, 6917 (1980)]. While proglumide has been reported to inhibit the neuroexcitatory effects of CCK octapeptide on A10 dopaminergic neurons [L. A. Chiodo and B. S. Bunney, *Science* **219**, 1449 (1983)] and to potentiate morphine analgesia [J. Tang, J. Chou, M. J. Iadarola, H.-Y. T. Yang, E. Costa, *Soc. Neurosci. Abstr.* **9**, 288 (1983)], its mechanism of action is not known. N. D. Bui and M. Deschodt-Lanckman [*Arch. Int. Physiol. Biochim.* **90**, B5 (1982)] reported that the effect of proglumide on the activity of a CCK octapeptide–degrading brain aminopeptidase is not due to CCK receptor occupancy. Furthermore, it was recently reported that proglumide does not inhibit binding of [^{3}H]pentagastrin or [^{125}I]CCK octapeptide to brain CCK receptors [P. Gaudreau, R. Quirion, S. St.-Pierre, C. B. Pert, *Peptides* **4**, 755 (1984); R. Murphy, paper presented at the International Symposium on Endocoids, Fort Worth, 1984].
8. Because of the uncertainty of the mode of action and the specificity of pancreatic CCK receptor antagonists on other CCK receptors, including vagal CCK receptors, we chose to assess the function of endogenous CCK by sequestering peripherally circulating CCK with antibodies to this peptide. This method of antagonizing CCK action has been successful in studying the satiety-producing effects of CCK [C. A. Baile, C. L. McLaughlin, F. C. Buonomo, M. C. Boy, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42** 392 (1983)]. In addition, we recently found that immunization against β-endorphin strongly antagonizes morphine analgesia. Compared to control values, analgesia in animals so immunized was reduced 24 percent ($P < 0.003$, paired t-test) and 29 percent ($P < 0.004$) 60 and 90 minutes after administration of morphine sulfate (10 mg/kg) (P. L. Faris *et al.*, in preparation). This finding supports the use of autoimmunization as an effective method for assessing the involvement of peripheral peptides in the modulation of nociception.
9. The BSA-immunized group controls for the antibodies developed in the CCK-immunized group against BSA as well as for a general stress effect of the immunization procedure.
10. Samples (10 μl) of serum from the tail vein or cerebrospinal fluid from the cisterna magna were incubated in Veronal buffer (*p*H 8.5) with [^{125}I]CCK octapeptide (10,000 count/min; New England Nuclear) for 48 hours. Bound radioactivity was precipitated with goat antibody to rat immunoglobulin G. Specific binding was calculated by subtracting nonspecific binding (that measured in normal rat serum) from total binding. The normal concentration of circulating CCK in rat is not known. However, resting values for dogs and humans are approximately 64 and 26 pg/ml, respectively [G. M. Fried *et al.*, *Gastroenterology* **85**, 1113 (1983); P. N. Maton, A. C. Selden, V. S. Chadwick, *Regul. Peptides* **4**, 251 (1982)]. Thus it is likely that the binding capacity of serum from our experimental animals (~50 pg/ml) was high enough to sequester a large percentage of the endogenously released CCK. It is also important to note that serum from our CCK-immunized rats could not have bound morphine, since this would have resulted in a decrease in free morphine and hence a decrease in analgesia rather than the observed potentiation.
11. F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.* **72**, 74 (1941). The tail-flick test measures the latency between the onset of a radiant heat source focused on the tail and the spinally mediated tail flexion.
12. If a tail flick did not occur within 6 seconds, the radiant heat was terminated to prevent tissue damage. The degree of analgesia was expressed as a percentage of the maximum possible elevation by applying the following equation: $(EL - BL)/(6 - BL \times 100)$, where EL is experimental tail-flick latency and BL is baseline latency (3.0 to 3.5 seconds).
13. J. Tang, J. Chou, M. J. Iadarola, H.-Y. T. Yang, E. Costa [*Soc. Neurosci. Abstr.* **9**, 288 (1983)] demonstrated that CCK is released into spinal perfusate after perispinal administration of morphine.
14. L. R. Watkins, I. B. Kinscheck, D. J. Mayer, *Science* **224**, 345 (1984).
15. H.-Y. T. Yang *et al.*, paper presented at the International Symposium on Endocoids, Fort Worth, 1984.
16. Supported by research science awards MH-38894 (J.W.O.) from the National Institute of Mental Health, ES-07066 from the National Institute of Environmental Health Sciences, and a grant from Monsanto Co. (C.A.B. and C.L.M.). We thank S. Goodman for secretarial assistance and J. Labruyere for preparation of illustrations.
* To whom correspondence should be addressed at the Department of Psychiatry.

3 February 1984; accepted 23 August 1984

Incidence of Low Birth Weight Among Love Canal Residents

Abstract. *The incidence of low birth weight among white live-born infants from 1940 through 1978 was studied in various sections of the Love Canal. A statistically significant excess was found in the historic swale area from 1940 through 1953, the period when various chemicals were dumped in this disposal site. Potential confounding factors such as medical-therapeutic histories, smoking, education, maternal age, birth order, length of gestation, and urban-rural difference did not appear to account for this observation. Low birth weight rates were comparable to those of upstate New York from 1954 through 1978, the period when there was no deposition of chemical wastes.*

Concern about adverse health effects that might be associated with hazardous chemicals dumped at sites such as the Love Canal has been growing. Over 200 chemicals have been found in the Love Canal dump site (*1*) and many, such as benzene (*2*) and lindane (*3*), have been shown to have toxic effects on man in industrial settings. The spectrum of human hazards that might be associated with other compounds isolated in the canal, such as certain isomers of dioxin (*4*), is not known.

Two major difficulties encountered in designing epidemiologic studies of chronic diseases in multichemical settings are the uncertainty in selecting appropriate end points and the long induction period between exposure and clinical diagnosis. Certain adverse reproductive events, however—low birth weight is an example—are objectively identifiable in a relatively short period of time. We analyzed data on the incidence of low birth weight among infants born in the Love Canal area from 1940 through 1978. This time span includes periods of active dumping at the site (1940 through 1953) and no formal dumping (1954 through 1978).

The study population included all people residing in single-family houses located in a series of parallel streets (97th Street through 103rd Street), bounded on the north and south respectively by two avenues (Colvin and Frontier) (*5*). Backyards of 99 houses on 97th and 99th streets directly abut the canal.

Because there are no historical environmental data on houses in the study area, we had to infer which subsets of the study population might have maximum exposure to chemicals. We reasoned that one group might be the families who lived on 97th and 99th streets directly adjacent to the chemical dump site. The accumulation of rain and ground water, facilitated by either natural or man-made activity, could have raised the level of chemical wastes to the topsoil layer, thus facilitating lateral migration. This slowly overflowing bathtub effect could result in the transport of waste products to adjacent backyards and basements.

Another possibility was that certain chemicals in the dump site might have spread preferentially to houses located on the natural drainage pathways in the area. Before the development of housing in this area, a number of natural shallow depressions traversed the area, some of