Table 1. Development of CPE in primate cell lines inoculated with MPMV. The primate cell lines were inoculated with 0.5 ml of undiluted MPMV in medium RPMI 1640 containing 10 percent fetal calf serum. Positive CPE were characterized by areas of multinucleation. The numbers in parentheses are the number of days after inoculation. —, No CPE observed; ±, tentative identification of CPE; and +, definite CPE.

Cell line	Monkey No.	Occurrence and onset of CPE (days)
	Fetal rhesus	
Brain	4272, 4637	—
Dura mater	4272	—
Bone marrow	4272	—
Testis	4272	—
Stomach	4637	—
Tooth bud	4272	± (30)
Heart	4637	± (6)
Kidney	4637	—
Spleen	4637	—
Adrenal	4637	—
Ovary	4637	—
Liver	4637	—
Eye	4637	—
Retina	4637	—
Spinal cord	4637	—
Mixed embryo	4637	—
Lung	4272, 4637	+ (1)
Foreskin	4272	+ (1)
	Infant rhesus	
Foreskin	4860	+ (1)
	Human	
HEp-2		—
Foreskin*		+ (2)

* From Flow Laboratories, Rockville, Maryland.

hyde, embedded in Epon, and examined by electron microscopy. Analysis of areas of multinucleation in MPMV-infected foreskin cultures demonstrated the presence of large numbers of intracytoplasmic, electron-dense, ring-shaped particles measuring 81 to 85 nm in diameter, resembling those particles described by Chopra and Mason (*6*). Examination of thin sections of the virus pellet revealed structures of similar size and morphology. No virus structures were observed in any of the uninfected cultures of foreskin examined.

Positive CPE were demonstrated for each of 15 separate lots of MPMV assayed on fetal foreskin cells. When the appearance of CPE (multinucleation) was used as an index of infection, the infectivity titer of several lots of virus was determined by inoculating foreskin cultures with decimal dilutions of virus and staining the monolayers with neutral red (0.1 percent) 6 days after inoculation. When individual foci were counted, a linear relation was made between the virus dilution and the number of foci of multinucleated cells. For example, when replicate foreskin cultures were inoculated with dilutions (10^{-2}, 10^{-3}, 10^{-4}) of virus from lot A, the average numbers of foci recorded were 509, 54, and 15, respectively. The titer was expressed as focus-forming units (FFU) per milliliter. This assay was found to give reproducible titers when duplicate titrations were run on a single lot of virus (for example, 1.4×10^8 and 2×10^8 FFU/ml). With one lot of high-titered virus assayed, a second morphologic change was observed which might possibly be indicative of a malignant transformation. At 24 to 48 hours after inoculation, those cultures that received undiluted inoculums were characterized by areas of clustered, rounded cells loosely adhering to the culture surface. This morphologic alteration, commonly correlated with viral transformation, appeared to be associated with a proliferative cellular response. These areas of transformation were similar to those described in transformation studies reported by Baluda (*7*), Manaker and Groupe (*8*), and Rabotti *et al.* (*9*). The cultures that received inoculums containing a 10^{-1} dilution of the seed virus were found to have foci of rounded, highly refractile cells, as well as a large number of areas of multinucleation. At higher dilutions, including a dilution of 10^{-5}, only multinucleation was observed. Over a 16-day period after inoculation no clustering of cells was observed in the higher dilutions as was observed in those cultures inoculated with undiluted material and material diluted 10^{-1}.

The findings presented thus provide a specific CPE that can be associated with the presence of MPMV and allow for a more rapid means of MPMV detection and quantitation than is presently available.

D. L. Fine
J. C. Landon
M. T. Kubicek
Bionetics Research Laboratories, Inc., Division of Litton Industries, Kensington, Maryland 20795

References and Notes

1. H. C. Chopra, *Proc. Amer. Cancer Res.* **11**, 16 (1970).
2. E. M. Jensen, I. Zelljadt, H. C. Chopra, M. M. Mason, *Cancer Res.* **30**, 2388 (1970).
3. H. C. Chopra, I. Zelljadt, E. M. Jensen, M. M. Mason, N. J. Woodside, *J. Nat. Cancer Inst.* **46**, 127 (1971).
4. E. Y. Lasfargues and M. R. Murray, in *Cancer Cells in Culture*, H. Katsuta, Ed. (Univ. of Tokyo Press, Univ. Park Press, Tokyo, Japan, 1968), p. 231.
5. A. J. Hackett, A. Pfiester, P. Arnstein, *Proc. Soc. Exp. Biol. Med.* **135**, 899 (1970).
6. H. C. Chopra and M. M. Mason, *Cancer Res.* **30**, 2081 (1970).
7. M. A. Baluda, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 415 (1962).
8. R. R. Manaker and V. Groupe, *Virology* **2**, 838 (1956).
9. G. F. Rabotti, J. C. Landon, T. W. Pry, L. Beadles, J. Doll, D. P. Fabrizio, A. J. Dalton, *J. Nat. Cancer Inst.* **38**, 821 (1967).
10. We thank Dr. M. Ahmed (Chas. Pfizer & Co., Inc.) for providing MPMV seed, MPMV-infected NC37 cells, and hyperimmune serum. We acknowledge the cooperation of Dr. W. R. Bryan, Dr. R. A. Manaker, Dr. E. W. Kingsbury, Mr. D. G. Bennett, Miss L. B. Malan, and Mrs. L. E. McAlister in the coordination of these studies. Supported under contract NIH 71-2025 within the Special Virus Cancer Program of the National Cancer Institute.

12 August 1971

Disposition of Morphine in Man

Abstract. *The disposition of morphine was investigated by means of radioimmunoassay after a single intravenous dose (10 milligrams per 70 kilograms) was administered to 10 adult normal male subjects who had not received other drugs for 2 weeks preceding the study. A multiphasic decline in serum concentrations of morphine occurred. Detectable blood concentrations of morphine, or of a metabolite, or of both persisted for 48 hours after a single intravenous dose.*

Although morphine is considered one of our oldest and most efficacious drugs, the details of its disposition have not been adequately established and quantified in man. The principal reason for this has been the insensitivity of the methods available for measuring morphine. The recent development of an extremely sensitive procedure for morphine determination by radioimmunoassay (*1*) afforded the opportunity to perform these studies.

Ten normal adult, white male volunteers (aged 21 to 23 years) who had not received any drugs for 2 weeks preceding the study were given morphine sulfate (10 mg/70 kg, intravenously) at 9 a.m. Blood specimens (2 ml each) were drawn at varying intervals thereafter. The serums were separated and kept frozen until they were assayed within 1 week after venipuncture. The radioimmunoassay of morphine was performed by the method of Spector and Parker (*1*). Rabbit serum, the source of the antibody, was diluted 1 : 200; 0.1 ml of the diluted antiserum was incubated at 4°C for 24 hours with 10 μl of the volunteer's serum. To this mixture, [^{3}H]dihydromorphine (4000 count/min) and normal rabbit serum (0.1 ml) were added. The total volume

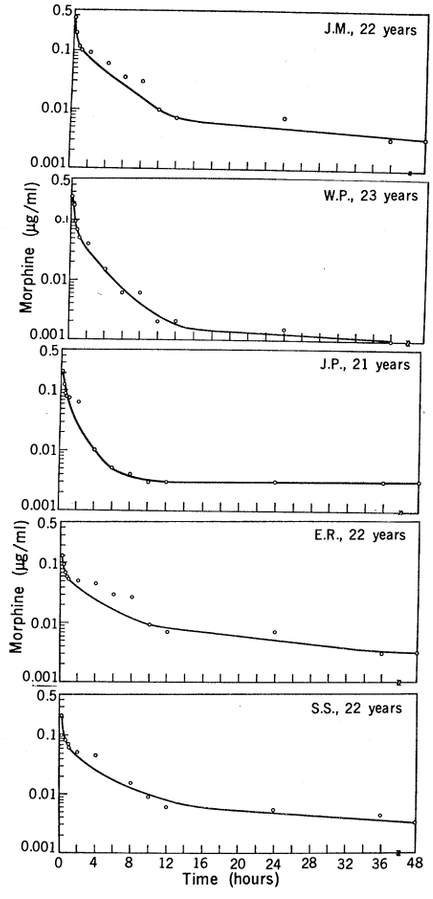

Fig. 1. Disappearance of morphine in serum of five subjects during 48 hours after a single intravenous injection of 10 mg/70 kg.

of the incubation mixture was made up to 0.5 ml with saline buffered with 0.01*M* phosphate buffer, *p*H 7.4. The morphine-antibody complex was precipitated by ammonium sulfate, and the radioactivity in the precipitate was determined with a Packard Tricarb scintillation counter (*1*). This method measures morphine and some of its metabolites; although the method detects the glucuronide and the demethylated metabolites, much higher concentrations of these are required than of the parent drug (*2*). The reproducibility of this method is ± 10 percent (*2*).

The results in five of the ten subjects who received 10 mg of morphine sulfate per 70 kg of body weight are shown in Fig. 1. The data reveal a very rapid initial decline of morphine in blood during the first 5 to 10 minutes after injection. The half-life of this very rapid initial phase of equilibration was not calculated because of the difficulty of obtaining accurate values. In the other five subjects, who were studied for only 24 hours, morphine concentrations were not determined as frequently nor for as long as for the five subjects shown in Fig. 1. However, the shape of the morphine decay curve was similar in both groups. Morphine in the serum exhibited a multiphasic decline and was plotted by the method of least squares. In the first 6 hours, there is a precipitous fall in morphine concentration with a half-life of from 1.9 to 3.1 hours. Afterward, the disappearance of the alkaloid is very slow, with a half-life of from 10 to 44 hours. Figure 1 shows that, after a single moderate intravenous dose of morphine, significant blood concentrations persist for at least 48 hours. Of the five volunteers studied for 48 hours, only one lacked detectable quantities of morphine at that time. Previous work established that most of the opium alkaloid is excreted in the urine during the first 24 hours after administration, that detectable levels remains in urine for 36 hours (*3*).

Although these experiments do not reveal the factors responsible for the production of this complex disappearance curve, the rapid phase may represent distribution of the alkaloid between blood and tissue, followed by metabolism and excretion. The prolonged presence of morphine in blood may represent continued metabolism, release of the drug as well as its metabolites from tissues, enterohepatic recirculation (*4*), persistence of a metabolite, or various combinations of these. The small amounts of morphine detected by the radioimmunoassay 48 hours after intravenous administration of the drug may be albumin bound (*5*). Possibly in certain individuals who exhibit allergic reactions to morphine, that portion of the opium alkaloid bound to albumin for long periods may act as an antigen.

Our studies suggest that repeated doses of morphine may be required to maintain analgesia in patients because of the rapid disappearance of the drug. Nevertheless, the observed prolonged concentrations of morphine in the blood may have clinical implications. Toxicity due to drug accumulation in blood may result from repeated administration of morphine, even at what previously was considered widely spaced intervals of 12, 24, or 36 hours. Since this possibility is more likely to occur in the individual with slow metabolism or in patients with various disease states who may be receiving several additional drugs simultaneously, the need for individualization of drug administration is reemphasized (*6*).

SYDNEY SPECTOR
Department of Physiological Chemistry, Roche Institute of Molecular Biology, Nutley, New Jersey 07110
ELLIOTT S. VESELL
Department of Pharmacology, Milton S. Hershey Medical Center, Pennsylvania State University, College of Medicine, Hershey 17033

References

1. S. Spector and C. W. Parker, *Science* **168**, 1347 (1970).
2. S. Spector, *J. Pharmacol. Exp. Ther.* **178**, 253 (1971).
3. H. W. Elliott, N. Nomof, K. Parker, M. L. Dewey, E. L. Way, *Clin. Pharmacol. Ther.* **5**, 405 (1964).
4. J. H. Jaffe, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, New York, ed. 4, 1970), p. 249.
5. G. Nadeau and G. Sobolewski, *Nature* **186**, 169 (1960).
6. E. S. Vesell and J. G. Page, *Science* **159**, 1479 (1968); *ibid.* **161**, 72 (1968); *J. Clin. Invest.* **47**, 2657 (1968); *ibid*, **48**, 2202 (1969).

17 June 1971; revised 23 August 1971. ■

Intestinal Secretion: Stimulation by Peptides

Abstract. *Two peptides isolated from intestinal mucosa, vasoactive intestinal peptide, and gastric inhibitory peptide, stimulate small intestinal secretion in conscious dogs. Glucagon and pentagastrin also stimulate, but secretin and the octapetide of cholecystokinin do not. The stimulants may participate in regulation of intestinal secretion in health and in diseases with excessive secretion.*

In 1938 Nasset (*1*) reported that certain extracts of intestinal mucosa stimulate intestinal secretion. He named the active principle enterocrinin. It has not been isolated and chemically identified. We now report that two peptides isolated from mucosa of the upper small intestine of hogs, vasoactive intestinal peptide (VIP) (*2*) and gastric inhibitory peptide (GIP) (*3*), stimulate small gut secretion. Of the three well-known and chemically identified gastrointestinal hormones—gastrin, cholecystokinin, and secretin—only gastrin (pentagastrin) stimulates intestinal secretion. Glucagon, a pancreatic islet hormone, also stimulates.

Dogs (16 to 25 kg) were surgically prepared at least 2 weeks before study with a 30-cm Thiry-Vella loop of upper jejunum or lower ileum. Both ends of the loops were connected to the ex-