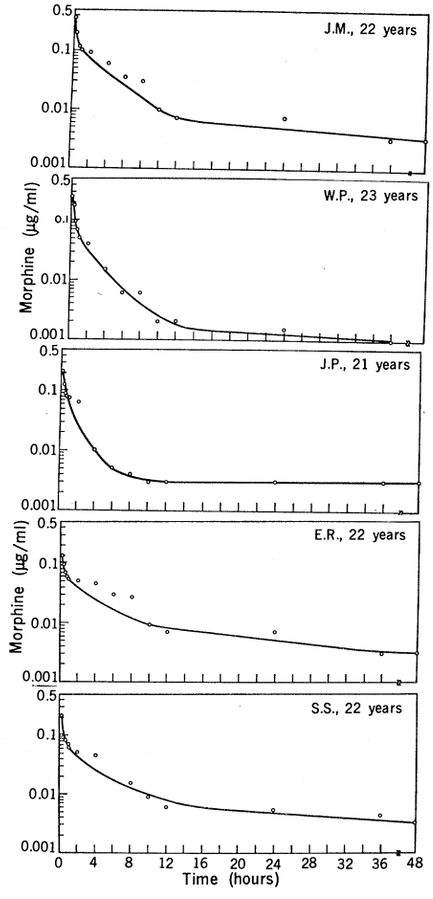

Fig. 1. Disappearance of morphine in serum of five subjects during 48 hours after a single intravenous injection of 10 mg/70 kg.

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*).

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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-

Table. 1 Rate of secretion of intestinal juice in response to various peptides. S.E., standard error.

Peptide	Dose (μg/min)	Infusion (min)	Test (No.)	Secretory volume* (μl/min)			
				Jejunum		Ileum	
				Mean	S.E.	Mean	S.E.
None (saline control)		30	6	−27	47	18	15
Vasoactive intestinal peptide	13	15	2	510		153	
Gastric inhibitory peptide	9	15	2; 1†	400		270	
Glucagon	10	30	6	211	57	275	51
Pentagastrin	5.3	30	11	224	56	189	23
Octapeptide of cholecystokinin	0.3	30	3	46	20	−11	27
Secretin	10	30	3	−64	19	−39	11

* Secretory volume is expressed as rate during infusion minus basal rate. † An equal number of tests was done on jejunum and ileum, except with gastric inhibitory peptide in which there were two jejunal and one ileal.

terior through Gregory cannulas (*4*). A Thomas cannula (*5*) in the stomach was kept open to keep gastric contents from entering the duodenum and releasing endogenous hormones. To facilitate collection of juice, humidified air was pumped continuously at 15 ml/min into the upper cannula, and collections were made from the lower cannula. Secretion was measured for 2 hours without stimulation and during 15 or 30 minutes of continuous intravenous infusion of the peptide. Basal secretion in fasted animals averaged 135 μl/min in jejunum and 38 μl/min in ileum.

Six peptides were tested (*6*): VIP, GIP, glucagon, pentagastrin (*7*), COOH-terminal octapeptide of cholecystokinin (*8*), and secretin. The first four of these peptides stimulated secretion from both jejunum and ileum, and the last two did not stimulate (Table 1). Secretion began within 5 minutes after infusion was started and continued throughout infusion. The electrolyte concentrations (*9*) in the juice (Table 2) were characteristic of the level of intestine; bicarbonate concentration was low in the jejunum and high in the ileum and changed little during stimulation by peptides. Electrolyte data are presented for only glucagon; those for the other peptides were similar. The secretory response to the peptides thus consisted of an increased volume flow rate with little change in electrolyte concentrations.

The doses selected for testing were intended to be comparable to that of glucagon, which we had shown earlier was a stimulant (*10*). However, in the case of the octapeptide of cholecystokinin, the dose we used was the largest tolerated by the animal without producing vomiting. Pentagastrin in doses as low as that of the octapeptide of cholecystokinin (0.3 μg/min) still stimulated gut secretion; thus, although both contain the COOH-terminal tetrapeptide amide that is the minimal fragment of gastrin required for biological activity (*7*), only the former stimulated gut secretion. For all other biological actions previously tested, the octapeptide of cholecystokinin is more potent than pentagastrin (*11*) and the dose of the octapeptide of cholecystokinin used in our study is supramaximal for all of these other actions.

There are 15 identities of amino acid sequence shared by glucagon and GIP (*3*). Nine of these 15 identities also occur in secretin. Perhaps one or more of the areas not shared by secretin [9-Asp, 10-Tyr, 21-Asp, 22-Phe, 23-Val, 25-Gly] (*12*) are essential for stimulation of gut secretion. It will be of interest to learn the sequence of VIP in these positions. The failure of secretin to stimulate gut secretion was confirmed in studies in which electrolyte solutions were perfused through intestinal loops (*10*).

The effect of these peptides on gut secretion is similar to that of cyclic adenosine 3′,5′-monophosphate (*13*), this being the likely final common path for all known stimulants including cholera toxin and prostaglandins. Secondary hormonal, neurogenic, or vascular changes produced by the infused peptide which interact more directly with the intestinal epithelial cell have not been excluded.

Table 2. Electrolyte concentrations of intestinal juice before and during glucagon infusion. S.E., standard error.

Ion	Electrolyte concentration (m*M*)			
	Basal		During glucagon	
	Mean	S.E.	Mean	S.E.
	Jejunum			
Na	145	4.8	144	3.6
K	11.7	1.7	7.5	0.5
Cl	146	1.4	151	0.6
HCO_3	14	3.0	14	2.8
	Ileum			
Na	147	2.6	150	1.9
K	8.1	0.6	5.8	0.5
Cl	84	3.2	86	3.1
HCO_3	67	5.5	80	2.1

Further study is needed to determine whether these peptides play a role in regulation of intestinal secretion. Pentagastrin stimulated gut secretion in doses submaximal for gastric acid secretion, suggesting that gastrin may be a stimulant under normal conditions. A comparable evaluation of the possible role of the other stimulatory peptides cannot be made on the evidence now available. Excessive secretion from the gut is a well-known pathological phenomenon, best illustrated by cholera. The highest rate of fluid secretion we saw in response to peptides was similar to that reported in response to cholera toxin (*14*). As in cholera, the protein content of secretions stimulated by glucagon, pentagastrin, or a combination of these peptides is low (*15*). This indicates that mucosal integrity has been maintained and in unlike the secretion resulting from increased interstitial hydrostatic pressure. An interesting speculative possibility is that cholera toxin acts at least in part by releasing one of the stimulatory gut peptides. This would explain the observation that cholera toxin applied to one loop of gut caused secretion from another loop not so exposed (*16*). Some pancreatic islet tumors produce a substance that causes watery diarrhea and depresses gastric acid secretion (*17*). Glucagon, VIP, and GIP both stimulate gut secretion and inhibit gastric acid secretion (*18*) so the possibility of their involvement in this disease should be considered.

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7. This synthetic peptide contains the COOH-terminal tetrapeptide amide of gastrin that is the minimal fragment for biological activity [J. S. Morley, H. J. Tracy, R. A. Gregory, *Nature* **207**, 1356 (1965)].

8. This synthetic peptide contains the COOH-terminal heptapeptide amide of cholecystokinin that is the minimum fragment for biological activity (it also contains the minimum fragment of gastrin) [M. A. Ondetti, B. Rubin, S. L. Engel, J. Pluscec, J. T. Sheehan, *Amer. J. Digest. Dis.* **15**, 149 (1970)].
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19. We thank J. Washington for technical assistance. Supported by Veterans Administration research funds and grant GB7285X from NSF. G.O.B. is an international postdoctoral fellow of NIH (FO 5 TW 1616).

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Inhibition of Normal Growth by Chronic Administration of Δ-9-Tetrahydrocannabinol

Abstract. *Body weight, food and water intake, and feces weight of 20 albino rats were recorded daily for 70 days. On days 11 to 40, 12 rats received behaviorally effective doses of Δ-9-tetrahydrocannabinol, either orally or intraperitoneally. These rats ate significantly less than placebo-dosed controls during the treatment period, and gained significantly less weight. Food intake recovered in the 30-day posttreatment period, but the former drug group still weighed less than the controls on day 70. In addition, all rats who had received intraperitoneal injections of Δ-9-tetrahydrocannabinol showed evidence of chronic diffuse nonsuppurative peritonitis.*

The isolation of marihuana's major active component, Δ-9-tetrahydrocannabinol (△-9-THC) (*1*), and its subsequent synthesis in relatively pure form (*2*) has provided the opportunity for better controlled quantitative research in the fields of physiology, biochemistry, pharmacology, and psychology (see *3*, *4*). The present study was begun as an attempt to verify under controlled conditions two incidental observations on the effects of Δ-9-THC in laboratory rats. First, rats being given daily intraperitoneal injections of Δ-9-THC very often showed precipitous losses in body weight, despite having free access to both food and water 22 hours of each day. Secondly, six of eight rats involved in a shock-avoidance study died during or shortly after the 2-week period during which they received daily intraperitoneal injections of Δ-9-THC. Postmortem examination suggested that death in all six cases had resulted from complications associated with an irritative or chemical peritonitis. We now report our observations of decreased body weight and food intake of rats given doses of Δ-9-THC daily for 30 days. Rats receiving intraperitoneal injections of the drug developed peritonitis in every case, while no rat receiving oral doses of the drug developed this condition. Both groups, however, consumed less food than controls over 30 days of drug administration and weighed considerably less even after 30 "recovery" days.

The subjects were 20 male albino rats, weighing between 250 and 300 g at the start of the study. All animals were individually housed in metal metabolism cages, with free access to bottled tap water and 45-mg animal feed pellets (P. J. Noyes Co.). The room was lighted for exactly 12 hours daily and temperature ranged between 20° and 24°C. Body weights were recorded daily, along with food and water intake and feces weight. After 10 days of this regimen, subjects were assigned randomly into four groups. Two groups of six animals subsequently received doses of Δ-9-THC each morning for the next 30 days (*5*). These two drug groups differed in both route of administration (intraperitoneal for one, gavage for the other) and dosage (4 mg/kg for the intraperitoneal groups, and 8 mg/kg for the oral group). The remaining eight rats served as placebo controls. Four received daily intraperitoneal injections of vehicle and four received daily vehicle doses via gavage.

Figure 1 shows the effect of daily Δ-9-THC injections on body weight. It is clear that both control groups, which were not significantly different from each other, continued to gain weight throughout the 30 days of placebo administration (mean gain, 53 g). The two drug groups, which also did not differ from each other, showed a notable loss in weight over the first 4 days of Δ-9-THC administration, followed by a very slow recovery up to their initial weights (mean gain, 0.7 g). Analysis of variance on net change in body weight during these 30 days revealed a significant treatment effect ($F = 9.49$; d.f. = 3,16; $P < .005$). Individual comparisons indicated that route of administration was not a significant factor in body weight changes. The drug versus placebo control comparison was significant beyond the .001 level ($F = 27.8$; d.f. = 1,16), however. After 30 additional days, during which neither drug nor placebo was administered, there was still a significant difference in body weight between the former drug and control groups ($t = 4.13$; d.f. = 17; $P < .001$; two-tailed). However, analysis of variance on weight *gain* over this 30 days posttreatment period failed to detect a significant effect of prior drug treatment.

Significant changes in food intake were also observed during the course of the study, and the data suggest that the effects on body weight reported above were due predominantly, if not entirely, to these changes, for the four groups of rats were essentially indistinguishable in terms of water consumed and feces weight. The animals in the two groups receiving Δ-9-THC consumed an average of only 522 g of food, while controls averaged 602 g ($F = 22.64$; d.f. = 1,16; $P < .001$). The two drug groups did not differ significantly from each other on this measure, nor did the controls. In order to assess our hypothesis that this difference in food intake was the primary cause of the observed differences in body weight, an analysis of covariance was performed, with food intake as the covariate or predictor variable. With total food intake controlled for in this way, the significant effect upon body weight disappears ($F = 1.95$; d.f. = 3,16). This close parallel between food intake and body weight is substantiated further by the data from the 30-day posttreatment period. No significant differences between groups was seen in food intake during this period ($F = 2.80$; d.f. = 3,15), just as there were no differences in weight gain. There was still a striking difference in the