solely responsible for the presence of ALA in the urine and other sources may also contribute to its daily excretion. The data further indicate that true ALA can be measured by automatic amino-acid analyzer. By this method, aminoacetone and glucosamine do not interfere with the quantitative analysis.

Conclusion. Delta-aminolevulinic acid (ALA), a precursor of heme synthesis, was found to be present in normal amounts in the urine of patients without demonstrable erythropoiesis. The presence of true ALA in the urine of these patients was confirmed by a new method using an automatic amino-acid analyzer. By this method, the interfering substances such as glucosamine and amino acetone were eliminated.

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Further Evidence for an Unique Neurohumoral Agent Released from Brain by Morphine Given Intracerebrally (33005)

O. J. PLEKSS AND S. MARGOLIN
Wallace Laboratories, Division of Carter-Wallace, Inc., Cranbury, New Jersey 08512

The constipating effect of morphine in mice, rats, and guinea pigs appears to be mediated through the central nervous system (1–3). Experimental data obtained by Margolin and co-workers (1, 3) indicates that morphine triggers the central nervous system to release a neurohumor carried by the circulating blood to the intestinal receptor sites. This neurohumor, rather than the morphine molecule per se, appears to be directly responsible for the inhibitory effect upon the intestine. This view is supported by the observation that (a) considerably less morphine is required to suppress intestinal propulsion when morphine is injected intracerebrally than when it is given intravenously; and (b) constipation can be produced in mice by injecting them with the blood perfusate obtained from isolated rabbit heads injected intracerebrally with relatively minute quantities of morphine (4). In addition to our studies, Green (2) found that the intracisternal injection of morphine into rats produced a greater inhibition of intestinal propulsion than did the subcutaneous administration of morphine. In the present investigation, parabiotic rats were used to study the effect of intracerebrally administered morphine on intestinal propulsion. Since the parabiotic animals are connected only by their circulatory system and not by their nervous system, they should provide additional proof for the existence of a neurohumoral mechanism for this action of morphine.

Methods. Charles River CD strain male albino rats were used. The parabiotic rats were prepared by surgically connecting littermates according to an adaptation of the method of Bunster and Meyer (5). Anesthesia was induced with sodium pentobarbital (40 mg/kg, i.p.). The skin edges were joined with 9-mm autoclips. Approximately 14 days later, the parabiotic rats were used in the tests. Each parabiotic rat weighed 100–120 gm (approximately the weight of the single animals). Eighteen to 20 hours before the experiments, food was removed but drinking water was constantly available. The intracerebral injections were carried out according to the procedure of Margolin (1), using 0.02 ml of

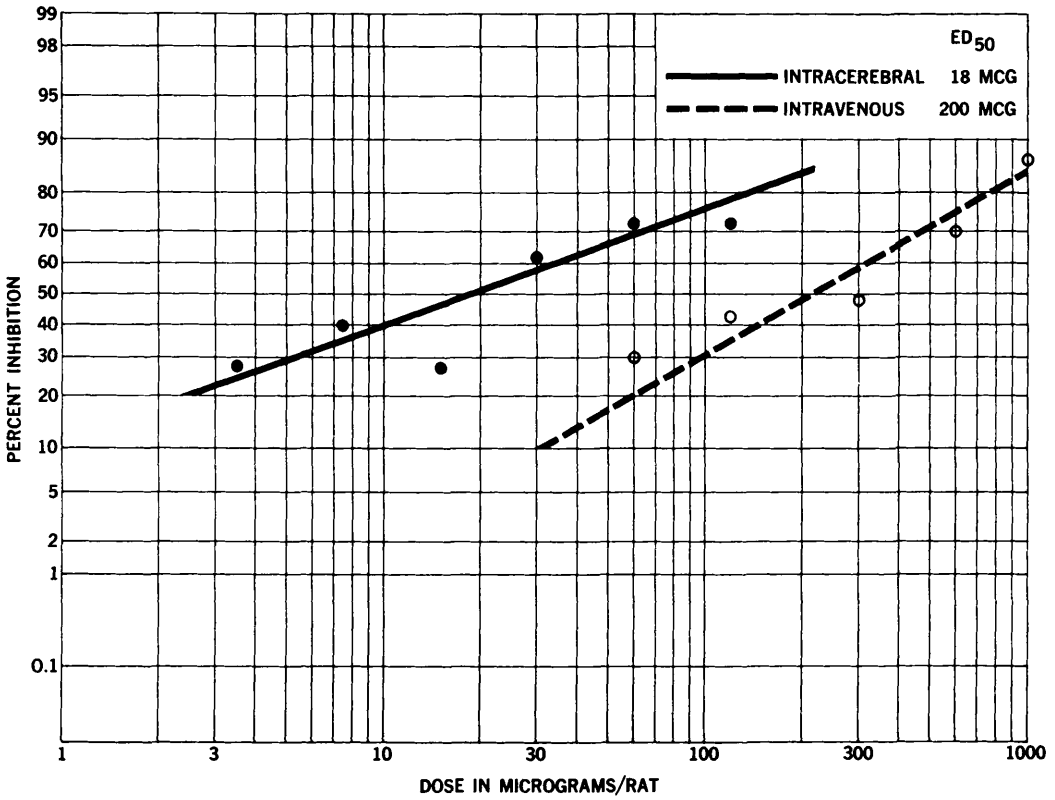

FIG. 1. Inhibition of gastrointestinal propulsive activity in rats following the intracerebral or intravenous injection of morphine sulfate.

either physiological saline, or appropriate concentrations of morphine sulfate dissolved in saline. For the intravenous injection, 0.5 ml of saline or morphine sulfate were given. With parabiotic rats, morphine was injected into one partner (*donor*) while the other partner (*recipient*) received no injection. The charcoal transit through the intestine was measured essentially by the method of Macht and Barba-Gose (6). Thirty min after the injection of morphine or saline, the charcoal marker (1 ml of a 5% suspension of "Norite" in 5% aqueous gum acacia) was given orally to each rat by gastric intubation. Exactly 10 min later, the animals were killed by cervical dislocation. The abdominal cavity was exposed, the stomach and entire small intestine was removed, and the distance (cm) that the charcoal marker traversed was measured. The results were calculated by dividing the distance traveled in the morphine-injected animals by the mean distance traversed in the saline-injected animals and multiplying the resulting ratio by 100. The statistical significance of differences between means were computed by Student's t test. In addition, the morphine doses producing 50% inhibition of charcoal transit through the intestine and the corresponding 95% confidence limits were calculated according to Litchfield and Wilcoxon (7).

Results. After a single intracerebral injection of very small amounts (3.5–60 μg/rat) of morphine sulfate into intact male albino rats, a charcoal marker suspension traveled only a relatively short distance down the small intestine. A 50% reduction of the distance traveled by the charcoal followed the intracerebral injection of 18 (7–45) μg/rat (approximately 150 μg/kg of body wt.). Intravenously, 200 (103–390) μg/rat (approximately 1650 μg/kg of body wt.) was required to cause a 50% reduction in the charcoal movement through the small intestine. Thus,

TABLE I. Distance Traveled by Charcoal Marker Through Small Intestine of Male Albino Rats Following Injection of Morphine Sulfate.

Dose of morphine (μg/rat)	Percentage of small intestine traversed (mean ± SE)		
	Single rat	Parabiotic donor rat	Parabiotic recipient rat
Intracerebral injection			
180	26 ± 6[a] (3)[b]	41 ± 5[a] (4)[c]	61 ±14[a] (4)[c]
160	29 ± 9[a] (6)	48 ± 7[a] (4)	78 ± 8[a] (4)
120	28 ± 5[a] (10)	35 ± 5[a] (9)	80 ± 8[a] (9)
60	28 ± 5[a] (10)	27 ± 5[a] (10)	77 ± 7[a] (10)
30	38 ± 8[a] (10)	33 ± 9[a] (10)	89 ± 3 (10)
15	72 ± 8[a] (6)	—	—
7.5	61 ± 9[a] (6)	—	—
3.5	73 ± 7[a] (6)	—	—
Saline	100 ± 5 (10)	100 ± 7 (10)	100 ± 6 (10)
Intravenous injection			
1000	13 ± 1[a] (5)[b]	26 ± 3[a] (3)[c]	74 ±18 (3)[c]
600	30 ± 4[a] (10)	37 ± 5[a] (10)	89 ± 3 (10)
300	52 ± 5[a] (10)	51 ± 3[a] (10)	89 ± 5 (10)
120	58 ± 7[a] (10)	80 ± 7[a] (10)	99 ± 1 (10)
60	70 ± 5[a] (6)	—	—
Saline	100 ± 4 (10)	100 ± 7 (9)	100 ± 5 (9)

[a] $p < .05$.

[b] No. of rats given in parentheses.

[c] No. of parabiotic pairs given in parentheses.

in the single rat morphine sulfate was 11 times more effective given intracerebrally than intravenously (Fig. 1).

When the action upon the propulsive activity of the intestine was studied in male albino parabiotic rats, the degree of inhibition of the traverse of the small intestine by the charcoal marker in the *donor* rat approximated that observed in the single normal rat described above. By intracerebral injection, a 50% reduction in intestinal charcoal marker transit in the *donor* rat was obtained with 18.5 (6–56) μg/rat (approximately 150 μg/kg of body wt.). By the intravenous route of parabiotic rats, a 50% reduction in gastrointestinal transit of charcoal marker occurred in the *donor* rat with 365 (183–730) μg/rat (approximately 3000 μg/kg of body wt.), or 20 times the dose required by the intracerebral route.

The successful detection of the transfer of a neurohumoral agent from the *donor* rat to the *recipient* was evidenced by the data displayed in Table I and Fig. 2. When morphine sulfate, 60–180 μg/rat, was given to the *donors* intracerebrally, a marked, statistically significant suppression of intestinal propulsive activity was also noted in the *recipients*. The ED_{50} for the *recipients* was estimated to be 320 (185–865) μg/rat, whereas the ED_{50} for the *donors* was 18.5 (6–56) μg/rat.

In contrast to the results obtained by the intracerebral route, a much larger dose, 365 (183–730) μg/rat of morphine, was required by the intravenous route to produce a 50% reduction in the transit of charcoal through the small intestine of the *donor*. It is noteworthy that no significant inhibition was observed in the *recipients* when the *donors* were given morphine intravenously in doses up to 1000 μg/rat. It may be estimated from the dose–response curve that by the intravenous route 2000 (777–5200) μg/*donor* rat would be required to produce a 50% inhibition in the *recipient*. Thus, about seven times more morphine would be required by the intravenous route than by the intracerebral route to produce the same degree of inhibition on the gastrointestinal tract of the *recipient* rats.

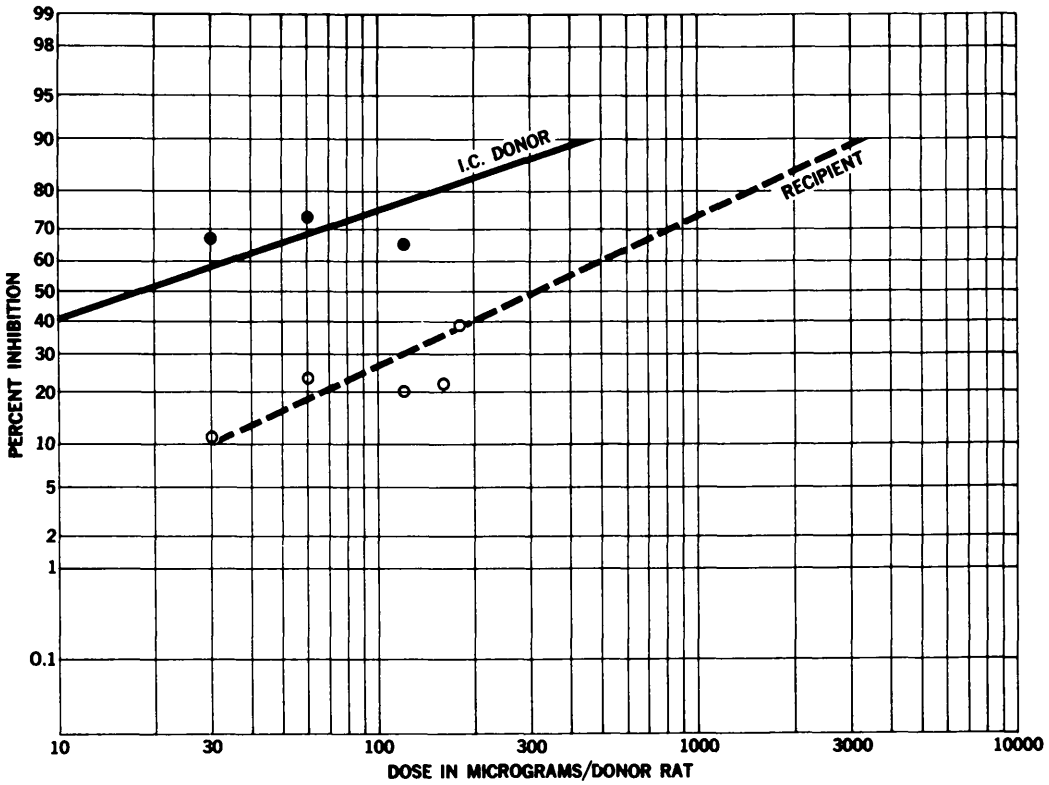

FIG. 2. Inibition of gastrointestinal propulsive activity in parabiotic rats following an intracerebral injection of morphine sulfate.

No significant differences in the intestinal passage of charcoal were noted between single and parabiotic rats when saline was injected intracerebrally or intravenously. In addition, no differences were noted between single and *donor* rats when morphine was administered intracerebrally or intravenously (Table I). Therefore, the surgical manipulation in forming the pair did not alter the reaction of the rats to morphine or saline.

Discussion. The demonstration of an inhibitory effect upon gastrointestinal propulsion in the *recipient* rats following intracerebral injection of minute amounts of morphine sulfate into the *donors* indicated that a neurohumoral substance was being transferred from the circulation of the *donor* rat into that of the *recipient*. That this was a neurohumoral substance discharged from the central nervous system was indicated by the rather large amount of morphine required to produce an analogous effect when given intravenously to the *donor*. These findings with the parabiotic rats were consistent with the observations in mice (3) that intracerebrally injected morphine continued to act upon the gastrointestinal tract after surgical interruption of sympathetic and parasympathetic outflows, or after spinal cord transection at C_4 plus subdiaphragmatic vagotomy. The data obtained with intact albino mice, suggested a discharge of a neurohumoral substance from the brain tissue by the intracerebral morphine, and that this neurohumor (rather than the morphine per se) was actually arresting gastrointestinal propulsion.

The possibility of an inhibitory action of morphine upon intestinal propulsion, mediated centrally, was suggested by Krueger (8) and Vaughn-Williams (9), and appears to be supported by our findings. Although the experiments of Plant and Miller (10), in which extensive surgical denervation of the intestinal tract was performed prior to the

intravenous injection of large amounts of morphine, were interpreted as a demonstration of a direct action of the morphine upon the gut, they failed to consider the possibility that the morphine injected intravenously was, in fact, acting indirectly through the central nervous system via a neurohumoral discharge. They did not carry out the intra-arterial craniad injections or intracerebral injections that could have permitted a differentiation. By employing single *intracerebral* injections of morphine in intact unanesthetized mice and rats, and comparing the response obtained, with that measured after a single *intravenous* injection, pharmacological evidence has been obtained for a centrally mediated action of morphine in inhibiting gastrointestinal propulsion. We have reported evidence for the discharge of a neurohumoral agent into the blood perfusate collected from isolated rabbit heads injected intracerebrally with small amounts of morphine (4). Since vasopressin, oxytocin, serotonin, histamine, acetylcholine, adrenalin, or noradrenalin did not simulate the inhibitory effect of morphine upon gut motility, the neurohumor must differ from these. In addition, the effect of the intracerebral morphine was not altered by: aminophylline, papaverine, TEA, atropine sulfate, dibenamine, physostigmine, hexobarbital, mephenesin, strychnine, C. botulin Toxin A, or procaine (3). On the other hand, intracerebral or intravenous pretreatment with nalorphine antagonized the effects of morphine on the small intestine in mice (3). These findings raise the possibility that some of the pharmacological properties attributed to the morphine molecule per se, seemingly are due to an unique neurohumor released by the morphine from central nervous system tissue.

Summary. Intracerebral injection of small amounts (3.5–60 μg/rat) of morphine sulfate in intact rats caused a marked suppression of gastrointestinal propulsive activity. Since 11 times greater amounts of morphine are required intravenously for an equivalent effect, the constipating action of morphine upon the gut primarily occurs via the central nervous system. The intracerebral injection of similarly small amounts of morphine into the *donor* of parabiotically paired rats resulted in suppression of gastrointestinal motility in the *recipient* as well as the *donor*. Since the parabiotic rats are joined only through their circulatory system (no neurological bridge exists), the effect of the intracerebral morphine given to the *donor* upon the gastrointestinal tract of the *recipient* could only be achieved by a neurohumoral agent transferred through the cross-circulating blood. These findings suggest that certain pharmacological properties attributed to the morphine molecule per se, may be due to an unique neurohumor released from brain tissue.

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