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Long-Range Substituent Effects in Morphine-Type Agonists and Antagonists: A Possible Explanation for Some Opiate Anomalies

VERA M. KOLB

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Abstract □ Anomalous variations in the pKa values of variously substituted morphine-type agonists and antagonists are interpreted as a reflection of long-range substituent effects operating in these molecules. Based on the operation of long-range effects, a mechanism is proposed by which substitution into the *N*-normorphine portion of morphine-type agonists and antagonists changes the activity of the parent molecule. Thus, a remote substituent would distort the whole molecule *via* a conformational transmission effect and thereby (*a*) change the fit between the opiate and its receptor; (*b*) change the electron density distribution throughout the molecule and, therefore, at the nitrogen; (*c*) modify the directionality of the lone-electron pair on the nitrogen; and (*d*) affect the pKa of the drug. The operation of long-range effects as proposed here could account for some of the anomalous changes in opiate activity effected by substitution into the parent molecule.

Keyphrases □ Morphine-type agonists and antagonists—substituents, long-range effects, structure–activity relationships □ Opiates—substituents, long-range effects, structure–activity relationships □ Opiate receptors—effect of opiate substituents, structure–activity relationships □ Structure–activity relationships—substituted morphine-type agonists and antagonists

The analgesic activity of morphine-type opiate agonists as well as the antimorphine activity of morphine-type opiate antagonists is influenced by substitution into the *N*-normorphine portion of these molecules (1). In that study (1), the activities of several series of closely related compounds were obtained by means of standard whole animal testing[1].

The data (1) did not lead, however, to an understanding of the process by which a substituent changes the opiate activity of the parent molecule. Often, the direction of the change was unexpected, in which cases the observed behavior of the substituted opiate seemed to be "anomalous."

Table I—Substitution Effect on the Antimorphine Activity of a Series of *N*-Allylnormorphines and *N*-Propylnormorphines [a]

Normorphine Moiety	Relative Antimorphine Activity of *N*-Allyl-normorphines	Relative Antimorphine Activity of *N*-Propyl-normorphines
Unsubstituted	1.0	1.0
3,6-Diacetyl	0.5	0.9
7,8-Dihydro	0.7	1.9
6-Desoxy	2.2	1.9
7,8-Dihydro-6-desoxy	1.8	0.2
7,8-Dihydro-6-keto	1.3	2.3
7,8-Dihydro-3,6-diacetyl	0.1	0.1
3-Methoxy-7,8-dihydro-6-desoxy	1.4	0.4

[a] Taken from Ref. 1.

BACKGROUND

Winter *et al.* (1) measured the antimorphine activities of two series of substituted opiate antagonists: *N*-propylnormorphines and *N*-allylnormorphines. Identical substituents were introduced into the same positions of the *N*-normorphine moiety of these two parent antagonists so that the observed differences in activity between these two series did not reflect differences in the transport rate caused by the substituent *per se*. An examination of the data (Table I) reveals no apparent correlation between the structure (or position) of the substituent and the direction of antimorphine activity change caused by the substituent.

These investigators (1) also found that the introduction of a common substituent into the *N*-normorphine moiety of morphine agonists and the corresponding antagonists (*N*-allylnormorphines) changed respective agonist and antagonist activities which were, as often as not, unrelated (Table II). These data are difficult to rationalize, especially if one accepts the hypothesis that the same cavity of the opiate receptor accommodates morphine-type agonists and antagonists, as suggested in the opiate receptor models of Goldstein (3) and Kolb (4) and as implied in some other opiate receptor models (5).

Within the category of the compounds covered in Table II, the following example is intriguing: 3,6-diacetylmorphine (heroin) exhibits 2.4 (6) or 2.5 (7) times the *in vivo* analgesic activity of morphine[2], and 1.45

[1] Analgesic and antimorphine activities were tested by the tail flick response in rats by the method of D'Amour and Smith (2). Drugs were injected subcutaneously.

[2] Ratio of ED_{50} (milligrams per kilogram) relative to morphine from mouse hot-plate experiments.

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Table II—Typical Change of Analgesic Activity of Morphine Agonists or Antimorphine Activity of the Corresponding Morphine Antagonists as Caused by the Change in the *N*-Normorphine Moiety of These Molecules [a]

Change in *N*-Normorphine Moiety of Morphine or *N*-Allylnormorphine	Analgesic Activity of Morphines		Antimorphine Activity of *N*-Allylnormorphines	
	Increased	Decreased	Increased	Decreased
Muzzling the 3-hydroxyl		++[b]		+[c]
Muzzling the 6-hydroxyl	++			+
Saturation of 7,8-double bond	+			
Removal of 6-hydroxyl	++		+	
Replacement of 6-hydroxyl by ketone	++		+	

[a] Based on quantitative data from Ref. 1. [b] A ++ indicates a large increase or decrease in activity. [c] A + indicates a moderate increase or decrease in activity.

times the *in vitro* receptor binding activity of morphine[3,4] (8). 6-Acetylmorphine is 4.2 times as potent an analgesic as morphine *in vivo*[2] (6). However, the corresponding acetylated antagonists, namely, *N*-allyl-3,6-diacetylnormorphine and *N*-allyl-6-acetylnormorphine, both show a *lower* antimorphine activity than their nonacetylated precursor, as illustrated quantitatively in Table III (1).

The change in the activity[5] of morphine agonists and antagonists by the introduction of a substituent may be associated with the *direct participation* of the substituent in binding to the receptor (9). The data from Table I, however, indicate that this possibility alone cannot account for the differences in activity observed with the same substituents.

The conclusions put forth here about the structure–activity relationships based on the *in vivo* study of Winter *et al.* (1) are meaningful if it is assumed that the concentrations of all of the structurally similar analogs compared are identical at the opiate receptor; the differences in potencies of these analogs, therefore, would be directly related to the respective differences in structure. While this assumption is usually implicit (9), exceptions have been reported. Sometimes even minor structural changes among the analogs significantly affect distribution, metabolism, or the ability of the compound to pass the blood–brain barrier (11), causing considerable differences in the analog concentrations in the brain. Qualification of the above assumption becomes irrelevant if the *in vitro* binding affinities[5] of these analogs to the opiate receptor are compared directly.

Tables IV and V present the binding affinities of a series of opiates to opiate receptors prepared from rat brain homogenate without added sodium chloride. Under these binding conditions, agonists as well as antagonists compete for the same population of identical binding sites (12) at the receptor, and the binding affinities of agonists and antagonists are approximately the same (10). This testing mode provides a meaningful method of observing the substitution effect on the binding affinities of agonists and antagonists and, therefore, of mixed agonist–antagonists[6].

Data from Tables IV and V reveal that the direction of change in the binding affinity resulting from the substitution into the parent molecule is not consistent and, therefore, is unpredictable. Thus, Table IV demonstrates that the binding affinity of oxymorphone exceeds that of morphine while the relationship is *reversed* for their 3-methyl ethers, *i.e.*, oxycodone and codeine. Therefore, the introduction of the same substituent in the same position of oxymorphone and morphine causes opposite effects on their binding affinities.

The inconsistency of this pattern is further illustrated by the examples in Table V. Thus, substitution of the *N*-methyl group with the *N*-allyl group may not change the binding affinity (levorphanol *versus* levallorphan), may improve the binding affinity (morphine *versus* nalorphine), or may lower the binding affinity (oxymorphone *versus* naloxone). As a consequence of this inconsistent pattern, the binding affinities of an *N*-methyl series (*e.g.*, levorphanol, morphine, and oxymorphone equal 1.0, 3.0, and 1.0, respectively) do not necessarily follow the same order as the binding affinities of the corresponding *N*-allyl series (levallorphan, nalorphine, and naloxone equal 1.0, 1.5, and 1.5, respectively).

To rationalize these anomalies exhibited by the *in vivo* as well as *in vitro* systems, one has to gain more insight into the mechanism(s) by which a substituent in the opiate molecule influences its activity. One such mechanism is proposed here.

EVIDENCE FOR LONG-RANGE SUBSTITUENT EFFECTS

While trying to find an explanation for these opiate anomalies, the following question was posed: Might a substituent introduced in an opiate molecule impart long-range effects[7]? That is, might the presence of this substituent distort the *whole* molecule and thereby change the fit between the opiate and its receptor, and might it also change the electron density distribution throughout the molecule and, therefore, at the nitrogen[8]?

This question is valid since such long-range substituent effects have been reported for other large and rigid molecules: steroids (*e.g.*, 17, 23, 24, 31–34), triterpenes (24, 25), spiranes (35), and sparteines (36). Although long-range effects in morphines have not been reported, it is not unreasonable to predict that these effects should operate within the rigid morphine frame also. Long-range inductive effects are transmitted[9] by all available bonds between the substituent and remote sites. The net effect is believed to be the sum of the individual effects (19–22). Electrostatic field effects should be prominent in morphines due to their T-shape, which enables a relatively close steric relation of "T-arms" with "T-body." Conformational transmission effects are to be expected on the basis of the work of Barton and coworkers (23–25) who showed that in large, rigid, polycyclic structures, a local conformational distortion caused by a local strain is transmitted through the entire molecule by a slight flexing of bond angles and torsional angles. In this way, the strain is

Table III—Relative Antimorphine Activities of *N*-Allylnormorphine and Its Acetyl Derivatives [a]

Compound	Relative Antimorphine Activity
N-Allylnormorphine (hydrochloride)	1.0
N-Allyl-3,6-diacetylnormorphine (tartrate)	0.5
N-Allyl-6-acetylnormorphine (sulfate)	0.3

[a] Taken from Ref. 1.

Table IV—Receptor Binding Affinity (EC_{50} of Stereospecific ^{3}H-Naloxone Binding to Rat Brain Homogenates; No Sodium Chloride)

Compound	EC_{50}, nm	Reference
Morphine	3.0	12
Oxymorphone	1.0	12
Codeine	20,000	13
Oxycodone	30,000	13

[3] Value of K_e (morphine)/K_e (heroin) from data in Ref. 8, where K_e = equilibrium dissociation constant (9).

[4] Tested in guinea pig ileum.

[5] The activity is directly related to the binding affinity with the opiate receptor (10).

[6] In contrast, when binding studies are conducted with receptors prepared from rat brain homogenate to which sodium chloride is added, the binding of antagonists is enhanced while that of agonists is dramatically diminished (12). Sodium has been reported to alter: (*a*) the number of binding sites for an agonist and antagonist, increasing the number for the latter but decreasing the number for the former (12; however, *cf.* 14); (*b*) the binding affinities of an agonist and an antagonist, enhancing that of the latter but decreasing that of the former (15, 16); and (*c*) the rates of dissociation and association of opiate agonists and antagonists, accelerating the dissociation rate of the former while accelerating the association rate of the latter (*e.g.*, 15, 16).

[7] Long-range effects are the effects of remote structural features on reactions in organic molecules (17). A substituent in a rigid molecule can influence reactivity at remote centers in three distinct ways: inductive effects (17–22), electrostatic field effects (17), and conformational transmission effects (17, 23–25).

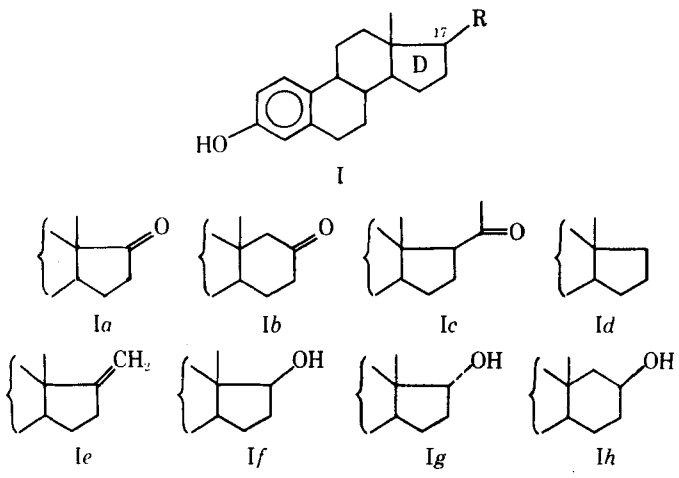
[8] It is generally agreed that an absolutely necessary feature of an opiate molecule is its basic nitrogen (26). It is believed that the opiate nitrogen interacts with the opiate receptor either *via* its lone electron pair (4, 27) or in its protonated form (*e.g.*, 28–30). In either case, the electron density on the nitrogen would appear to be relevant to the opiate activity.

[9] Subject to the normal exponential decrease of the effect with distance (18).

Table V—Receptor Binding Affinity [a] (EC_{50} of Stereospecific ^{3}H-Naloxone Binding to Rat Brain Homogenates; No Sodium Chloride)

Compound	EC_{50}, nm
Levorphanol	1.0
Levallorphan	1.0
Morphine	3.0
Nalorphine	1.5
Oxymorphone	1.0
Naloxone	1.5

[a] Taken from Ref. 12.



shared by the *whole structure*, and the effect of the resulting distortions is transmitted to a remote site, thereby influencing the reaction rate. The mechanism of conformational distortion transmission within a polycyclic framework also was studied (35, 37).

In this context, the following example of the operation of the long-range effects in steroidal phenols (estrone derivatives) is striking. Legrand *et al.* (32) observed that the pKa values of steroidal phenols (I*a*–I*h*) changed with the nature of the substituent in the 17-position, which is *nine* carbons away from the phenolic OH. They attributed these results to the operation of long-range effects (Table VI).

It is reasonable to believe, therefore, that similar long-range effects in morphine systems (II–VII) would be manifested by changes in pKa values with alteration of remote substituents. Indeed, the anomalous variations in the pKa values of a series of substituted morphines (38) provide convincing support for the operation of long-range effects here too. Thus, data in Table VII show that the substituent on nitrogen influences not only the acidity of the amino nitrogen but also that of the phenolic hydroxyl seven atoms away (*cf.*, II and III and V–VII). Conversely, the substituent on the phenolic oxygen influences the pKa of the amino nitrogen (*cf.*, II and IV).

DISCUSSION

Extrapolation of these data suggests the generalization that the introduction of a substituent in a morphine molecule is likely to affect its pKa. The pKa of a morphine influences its transport rate (38) which, in turn, influences the *in vivo* activity. The *in vitro* binding affinity, being a thermodynamic value (39), also is influenced by the pKa value of the compound in question[10]. It is quite possible, however, that the actual binding of the morphines to the receptor in the process that elicits the biological response is kinetically controlled[11]; if so, the intrinsic drug activity is not necessarily influenced by pKa (thermodynamic) values.

Introduction of a substituent in a rigid molecule like morphine is likely to cause distortions of bond angles and torsional angles locally as well as throughout the molecule. For example, X-ray studies of morphine (41) and codeine (42) revealed a difference of about 3° in their T-angles. These distortions could cause a change in the fit between the opiate and its receptor that may be large enough to influence the opiate activity. These distortions may also cause a change in the directionality of the lone-electron pair on the nitrogen, and such a change would influence the

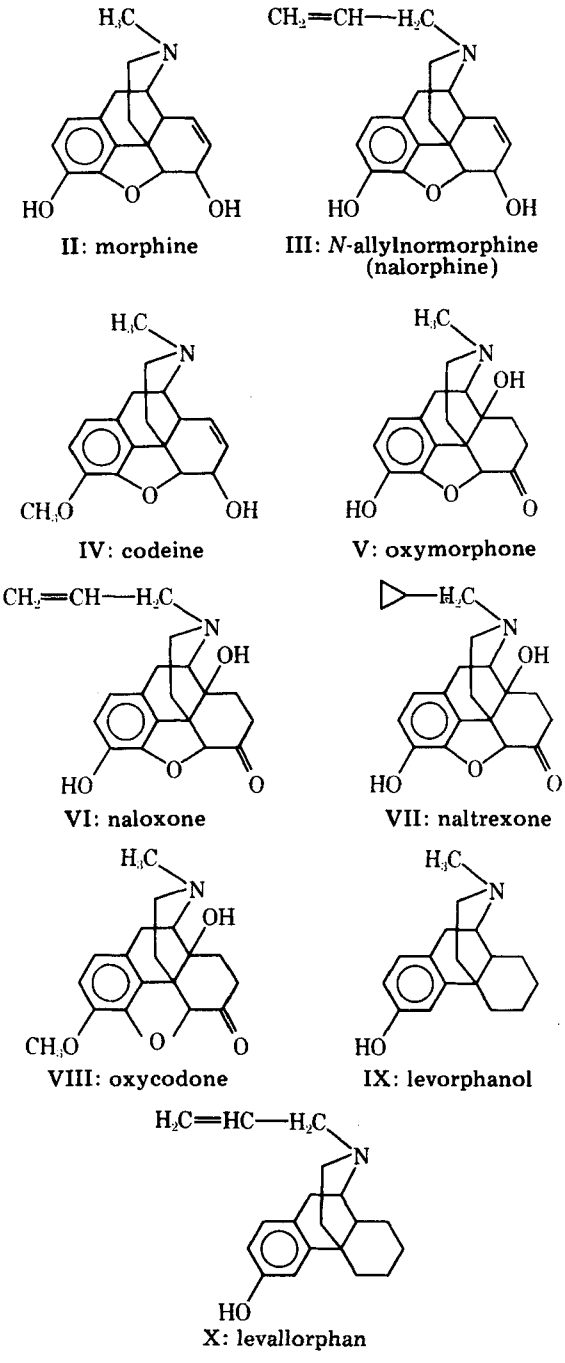


Table VI—Dependence of pKa Values of Steroidal Phenols on the Substituent in the 17-Position [a]

Compound	ΔpKa (in CH_3OH–CH_3ONa) [b]
Estrone (I*a*)	0.10
D-Homoestrone (I*b*)	0.09
Progesterone (I*c*)	0.07
3-Hydroxy-1,3,5(10)-estratriene (I*d*)	0.01
3-Hydroxy-17-methylene-1,3,5(10)estratriene (I*e*)	0.02
17β-Estradiol (standard) (I*f*)	0.00
17α-Estradiol (I*g*)	0.02
D-Homoestradiol (I*h*)	0.00

[a] Taken from Ref. 32. [b] These pKa values (pKa 17β-estradiol − pKa compound) were obtained by statistical interpretation of the results from several pKa measurements.

[10] This reasoning is valid whether the free (4, 27) *or* protonated (*e.g.*, 28–30) amine function is the *active species* at the receptor.

[11] For example, one could easily visualize "clastic binding" (40), which involves an electron-transfer reaction, as kinetically controlled.

Table VII—pKa Values of Some Morphine-Type Opiate Agonists and Antagonists [a]

Compound	Temperature	pKa[b] (in Water) pKa1 (Proton on Nitrogen)	pKa2 (Phenolic Hydrogen)
Morphine[c]	20°	8.02	9.76
	37°	7.93	9.63
N-Allylnormorphine[d]	20°	7.73	9.36
	37°	7.59	9.28
Codeine[e]	20°	8.18	—
	37°	8.10	—
Oxymorphone[d]	20°	8.25	9.71
	37°	8.17	9.54
Naloxone[d]	20°	7.94	9.44
	37°	7.82	9.25
Naltrexone[d]	20°	8.38	9.93
	37°	8.13	9.51

[a] Taken from Ref. 38. [b] The pKa values were measured by a microelectrometric titration method. Each pKa value represents the average of at least five determinations, and the maximum deviation from the given value is ±0.02. [c] Sulfate. [d] Hydrochloride. [e] Phosphate.

opiate activity according to Belleau *et al.* (27) and Kolb (4). Belleau *et al.* (27) pointed out that (referring to the activities of *N*-methylmorphinans *versus* *N*-methyl-D-normorphinans): "It appears probable then that conformational transmission of subtle distortions effects in the lone pair *orientation* of morphinans and related analogs may account at least in part for structurally induced variations in their pharmacological properties."

A substituent introduced in the morphine molecule also would influence the electron density distribution throughout the molecule; the nitrogen atom would certainly be affected. The electron density on the nitrogen seems to be relevant to opiate activity (see footnote 8), especially if one accepts the elastic binding hypothesis proposed by Belleau and Morgan (40), which invokes a stereospecific electron transfer from the lone electron pair on the opiate nitrogen to the receptor. Electron transfer processes are known to depend greatly on the electron density of the electron donors (43).

Evaluation of long-range effects of substituents is complicated by the fact that these effects are not necessarily additive[12]. Net substituent effects within steroids were studied quantitatively by Barton *et al.* (24) who actually measured the "group-rate factors" for a series of substituents[12]. Unfortunately, such quantitative data are not yet available for morphines.

All of these possible processes by which a substituent in the morphines influences the opiate activity *via* long-range effects are variations of the proposed general "long-range effect" mechanism.

Finally, the following comparison between the morphines and the estrones (and some other steroidal hormones) can be made: both classes of compounds are large, rigid, biologically active molecules that interact with their respective receptors by a lock-and-key mechanism (44, 45), and both classes of compounds exhibit long-range effects. The importance of these effects to the biological activity of steroids is already recognized (34). An intriguing question arises: Is the long-range effect mechanism a general mechanism by which a fine tuning of the biological activity is achieved in all rigid molecules that act at the receptor? An answer may be forthcoming.

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[12] Barton *et al.* (24) showed that the observed rate of condensation of 5α-steroidal 3-ketones with benzaldehyde is: $R_s = R_0(f_1)(f_2)(f_3)\ldots(f_n)$, where R_s is the rate of the substituted steroid, R_0 is the rate of the parent (unsubstituted) steroid, and f_n is the group rate factor for the substituent concerned.

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Modification of Metoclopramide GLC Assay: Application to Human Biological Specimens

Y. K. TAM *, J. E. AXELSON *x, and R. ONGLEY ‡

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Abstract □ A modified electron-capture GLC assay for metoclopramide in human biological specimens is reported. This assay involves the incorporation of a back-extraction method to remove endogenous contaminants. Its applicability was demonstrated by studying the time course of metoclopramide in plasma and urine from a human subject. The lowest quantifiable metoclopramide concentration in plasma was 7 ng/ml, provided 0.5 ml of plasma was used.

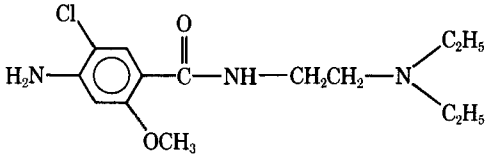
Keyphrases □ Metoclopramide—analysis, electron-capture GLC, human plasma and urine □ Antiemetic agents—metoclopramide, electron-capture GLC analysis, human plasma and urine □ GLC, electron capture—analysis, metoclopramide, human plasma and urine

A highly sensitive electron-capture GLC assay was developed recently for the determination of metoclopramide (I), mol. wt. 299.81, in biological specimens from the rat (1). However, when the same procedures were used to analyze human plasma, quantitation of I was impossible due to interferences from endogenous substances (Fig. 1*a*). A slight modification of this method, involving a "cleanup step," successfully removed endogenous substances from the human plasma samples. The purpose of this report is to describe this improved assay.

EXPERIMENTAL

Materials—Metoclopramide monohydrochloride monohydrate[1] (II) (mol. wt. 336.31), benzene[2], 4% ammonium hydroxide[3], 1 *N* NaOH[4], 5 *N* NaOH[4], 1 *N* HCl[5], heptafluorobutyric anhydride[6] (III), and diazepam[7] were used.

GLC—A reporting gas–liquid chromatograph[8] equipped with a ^{63}Ni-electron-capture detector (^{63}Ni-15 mCi) and a 1.2-m × 2-mm i.d. glass column, containing 3% OV-225 coated onto 80–100-mesh Chromosorb W, was used to analyze plasma samples. The operating temperatures for routine analysis were: injection port, 250°; oven, 245°; and detector, 350°. The carrier gas (95% argon–5% methane) flow rate was 40 ml/min. For urine analyses, all GLC conditions were identical except that a 0.6-m × 2 mm-i.d. 3% OV-225 column was used and the oven temperature was 230°.



I

Extraction Procedure—*Plasma*—A 0.5-ml sample of human plasma (blank) was spiked with serial concentrations of II (concentration equivalent to 7–93 ng of I/ml of water) (Table I). To each spiked sample, 0.5 ml of 1 *N* NaOH was added. The final volume was made up to 2 ml with water (pH ≃ 12). The aqueous phase was extracted with 8 ml of benzene by shaking with a wrist-action shaker[9] for 15 min. After centrifugation at 3000×*g* for 10 min, 7 ml of the organic phase was removed and back-extracted (extraction time, 15 min) with 2 ml of 1 *N* HCl.

The benzene layer was aspirated, and the sample was washed two times with benzene (4 ml each). The remaining aqueous layer was alkalinized with 0.5 ml of 5 *N* NaOH (pH ≃ 12). Finally, this layer was extracted with 6 ml of benzene (extraction time, 15 min). A 5-ml aliquot of the organic phase was removed and dried under a gentle nitrogen stream at ambient temperature. The residues were reconstituted with 0.5 ml of internal standard solution (50 ng of diazepam/ml of benzene).

Urine—A 0.1–0.5-ml volume of urine containing I was extracted in the same manner as described by Tam and Axelson (1).

Derivative Formation and Quantitative Analysis—A 20-μl volume of III was added to the reconstituted solution. The reaction mixtures were incubated at 55° for 10 min. Excess III was removed by hydrolysis with 0.5 ml of water and neutralization with 0.5 ml of 4% NH_4OH (1). A 5-μl volume of the derivative solution was injected into the reporting gas–liquid chromatograph by an automatic sampler. Quantitative estimation of I in human plasma and urine samples was accomplished by plotting the area ratios of the derivative and the internal standard against a range of solutions of known I concentration.

Human Study—A 20-mg metoclopramide monohydrochloride[10] (IV) dose was administered orally to a fasted normal healthy volunteer during a drug interaction study with griseofulvin. Five-milliliter blood samples were withdrawn at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 24 hr after drug administration. The blood samples were immediately centrifuged, and the plasma was separated. Cumulative urine samples were collected up to 96 hr after drug consumption. Both the plasma and urine samples were stored at −20° until analysis.

Table I—Quantitative Metoclopramide Estimation in Human Plasma

Metoclopramide Concentration, ng/ml	n^a	Area Ratio	*SD*
7	3	0.409	±0.03
15	3	0.812	±0.05
37	3	2.140	±0.25
56	3	2.935	±0.20
93	3	4.768	±0.36

[a] Each *n* is the average of three injections.

[1] Analysis No. 9207, A. H. Robins, Montreal, Canada.
[2] Distilled in glass, Caledon, Georgetown, Ontario, Canada.
[3] Reagent ACS code 1293, Allied Chemical Canada Ltd., Pointe Claire, Quebec, Canada.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Reagent ACS, Allied Chemical Canada Ltd., Pointe Claire, Quebec, Canada.
[6] Pierce Chemical Co., Rockford, Ill.
[7] Lot R-6685, Hoffmann-La Roche, Montreal, Canada.
[8] Hewlett-Packard model 5840.
[9] Patent pending, Burrell Corp., Pittsburgh, Pa.
[10] Maxeran tablets (10 mg), Nordic, Laval, Quebec, Canada.

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