Experimental Section

All melting points were obtained on a Thomas-Hoover Unimelt, capillary, melting point apparatus and are uncorrected. IR spectral data were obtained on a Perkin-Elmer 257 and NMR spectral data were obtained on a Perkin-Elmer R-24. Spectra were obtained on all compounds and these are compatible with the structural assignments. Microanalyses obtained as indicated by the symbols of the elements are within ±0.4% of the theoretical values.

2-(2-Cyanoethyl)-9α-ethyl-2′-hydroxy-5-methyl-6,7-benzomorphan (1c). Compound 1a (1 g, 4.3 mmol),[8] acrylonitrile (0.3 g, 5.6 mmol), absolute EtOH (10 mL), and Et_3N (5 drops) were stirred overnight at room temperature (23–25 °C), after which TLC showed no 1a. Evaporation to dryness at 20–26 mm and cooling gave a solid which crystallized from EtOAc (5 mL)–ligroin (30–60 °C, 3–4 mL) in prisms: yield 0.96 g, 90%; mp 95-96 °C (gas evolution). Anal. ($C_{18}H_{24}N_2O$) C, H, N.

2-(2-Carbamidoethyl)-9α-ethyl-2′-hydroxy-5-methyl-6,7-benzomorphan (1d). A mixture of 1.0 g (4.33 mmol) of 1a,[8] 0.41 g (5.76 mmol) of acrylamide, 5 drops of Et_3N, and 10 mL of absolute EtOH was stirred at room temperature (4 days) and monitored by TLC [EtOAc–MeOH–concentrated NH_4OH (16:3:1) and $CHCl_3$–MeOH–concentrated NH_4OH (74:24:2), silica gel, iodoplatinate reagent]. The reaction mixture was then filtered and the filtrate concentrated (in vacuo) to a brownish oil. Et_2O was added, and the resulting crystals from overnight standing were recrystallized from EtOAc/Me_2CO (3:1): yield 1.07 g, 90.7% (based on covered starting material); mp 171.5–172.5 °C. Anal. ($C_{18}H_{26}N_2O_2$) C, H, N.

***N*-(2-Cyanoethyl)normeperidine (2b).** A solution of normeperidine (norpethidine,[11] 3.00 g, 10.7 mmol), acrylonitrile (0.81 mL, 12.25 mmol), and anhydrous K_2CO_3 in absolute EtOH (50 mL) was stirred for 6 h at room temperature. The solution was filtered and evaporated in vacuo to yield a brown oil. The oil was dissolved in Et_2O (50 mL), washed with saturated NaCl solution, dried ($MgSO_4$), and evaporated in vacuo to yield an oil from which the hydrobromide salt was made. Recrystallization twice from Me_2CO afforded 0.88 g (29%), mp 207–208 °C. Anal. ($C_{17}H_{23}N_2O_2Br$) C, H, N.

***N*-(2-Cyanoethyl)norketobemidone (3b).** A solution of norketobemidone[10] (3.00 g, 12.9 mmol) and acrylonitrile (1.26 mL, 19.3 mmol) in absolute EtOH (50 mL) was stirred for 2 h at room temperature. The solvent was evaporated in vacuo, yielding a brown tar from which the hydrobromide salt was made. Recrystallization twice from absolute EtOH afforded 3.45 g (73%), mp 130–131 °C. Anal. ($C_{17}H_{23}N_2O_2Br$) C, H, N.

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Active-Site Studies of Neurohypophyseal Hormones: Synthesis and Pharmacological Properties of [5-(N^4,N^4-Dimethylasparagine)]oxytocin[1]

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Synthesis and biological properties of [5-(N^4,N^4-dimethylasparagine)]oxytocin are reported. In this analogue, the hydrogens of the primary carboxamide moiety in the side chain of the asparagine residue in position 5 of the posterior pituitary hormone oxytocin have been replaced by two methyl groups. The protected nonapeptide intermediate was prepared by a stepwise procedure using solution techniques. The analogue possesses 4.60 ± 0.03 units/mg (mean ± SEM) uterotonic activity on the isolated rat uterine horn and 9.14 ± 0.03 units/mg of avian vasodepressor activity. Moreover, it displays an identical intrinsic activity in the in vitro rat uterotonic assay as oxytocin, when tested in the presence of either 0.5 mM Ca^{2+} (standard assay conditions) or at reduced levels of Ca^{2+} (0.3, 0.15, and 0.05 mM). This result is significant in view of the proposed biologically active model of oxytocin, in which the side chain of the 5 position residue was assigned to contain an "active element" responsible for the intrinsic activity of the hormone when bound to the uterine receptor.

The 5-position asparaginyl residue plays a key role in the preferred solution conformation of oxytocin[2] and in the proposed "biologically active" model of the hormone (Figure 1) at the uterine receptor.[3-5] Both in Me_2SO and aqueous medium[6] the peptide NH of the asparagine residue helps to stabilize the β turn involving the sequence

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Table I. Comparison of the Biological Activities of Oxytocin and Its 5-Position Analogues[a]

no.	compd	in vitro rat uterotonic	avian vasodepressor	rat pressor	rat antidiuretic	intrinsic act., %
I	oxytocin	546[b]	507[c]	3.1[c]	2.7[c]	100[d]
II	[5-alanine]oxytocin	~0.05[e] 0.2-0.3[f]	0.1[e] 0.2-0.3[f]	<0.01[e] <0.01[f]	<0.01[e] 0.002[f]	Id[g]
III	[5-glutamine]oxytocin[h]	1	0.8	0.01		
IV	[5-serine]oxytocin[e]	0.7	0.2	<0.1	<0.01	
V	[5-valine]oxytocin[i]	~0.3	~0.1	~0.01	<0.001	Id[j]
VI	[5-ornithine]oxytocin[k]	~0.24	~0.07	<0.04	~0.002	
VII	[5-(1,4-diaminobutyric acid)]oxytocin[l]	0.03	0.03	<0.01	<0.001	Id[j]
VIII	[5-(aspartic acid)]oxytocin[m]	20	41		0.14	100
IX	[5-(N^4,N^4-dimethylasparagine)]oxytocin	4.6	9			100

[a] Biological activities are expressed as USP units per mg. [b] See ref 21. [c] See ref 41. [d] See ref 42 and 43. [e] See ref 8. [f] See ref 10. [g] Indeterminable. See ref 43. [h] See ref 9. [i] See ref 11. [j] Indeterminable. Walter and Dubois, unpublished. [k] See ref 12. [l] See ref 13. [m] See ref 15.

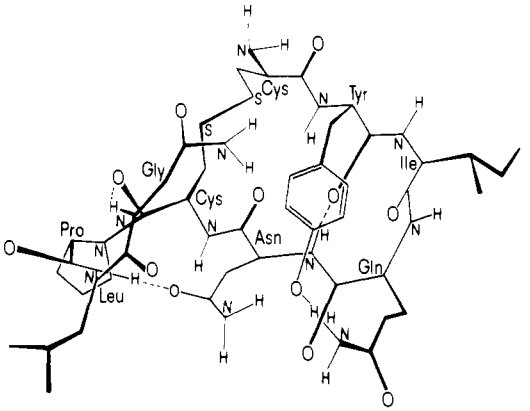

Figure 1. Schematic representation of the oxytocin conformation thought to be optimal for the interaction of the hormone with the uterine smooth muscle receptor. Residues in position 3, 4, 7, and 8, which are thought to contain "binding elements",[7] are located at the corner position of the two β turns proposed for the hormone. The "active elements",[7] are the hydroxy group of Tyr in position 2 and the carboxamide group of Asn in position 5.

-Tyr-Ile-Gln-Asn- by virtue of hydrogen-bond formation to the carbonyl oxygen of the tyrosine residue. In the biologically active model, the tyrosyl side chain overlays the 20-membered covalent ring of oxytocin and thereby forms, together with the carboxamide groups of the side chains of Gln in position 4 and Asn in position 5 as well as the C-terminal glycinamide in position 9, the active site of oxytocin.[3] Thus, the asparagine residue has the dual function to stabilize the three-dimensional conformation and to contribute an "active element".[7]

As can be seen from Table I, any chemical modification introduced into the residue in position 5 of oxytocin prior to 1978 resulted in analogues with negligible biological activities.[8-13] Particularly relevant is also that the intrinsic activity[14] of these analogues in the in vitro uterotonic assay, to the extent that the peptides had been tested, was too low to be determined (Table I, peptides II, V, and VII). This series also included [5-(1,4-diaminobutyric acid)]-oxytocin, an analogue where the carbonyl moiety of the primary carboxamide group of the asparagine side chain of oxytocin had been replaced by a methylene group.[13] [5-(Aspartic acid)]oxytocin, which was studied recently and in which the hydrophilic $-NH_2$ portion of the primary carboxamide was replaced by the hydrophilic $-OH$, was shown to be the first 5-position analogue to elicit significant in vitro rat uterotonic activity (20.3 ± 0.8 units/mg), as well as being able to elicit the same maximum contractile response of this tissue as oxytocin.[15] The results of these two compounds may suggest that the hydrogens of the N^4-amine portion of the asparaginyl side chain are not as essential for the endowment of oxytocin with its oxytocin-like activities as the carbonyl moiety. To further explore this hypothesis, we report here the synthesis and some biological activities of [5-(N^4,N^4-dimethylasparagine)]oxytocin, in which the hydrogens of the N^4-amide of the asparagine are replaced with methyl groups.

The synthesis of [5-(N^4,N^4-dimethylasparagine)]oxytocin was performed by stepwise[16] solution techniques from Boc-Cys(Bzl)-Pro-Leu-Gly-NH_2.[17] Boc-Asp[$N(CH_3)_2$][1] was preactivated[18] with DCC and 1-hydroxybenzotriazole and coupled to the partially deprotected tetrapeptide, and, thereafter, elongation of the peptide chain was achieved with active esters. The protecting groups were removed, the disulfide was formed, and the product was purified by methods previously described.[19]

In the in vitro rat uterotonic assay[20] [5-(N^4,N^4-dimethylasparagine)]oxytocin possesses a potency of 4.60 ± 0.5 (mean ± SEM) units/mg in the absence of added Mg^{2+}; the comparable value for oxytocin is 546 ± 18 units/mg.[21] Dose–response studies on the isolated rat uterus with [5-(N^4,N^4-dimethylasparagine)]oxytocin and oxytocin using the individual injection technique,[22] with either 1.0 mM added Mg^{2+} or without the added Mg^{2+} in the bathing medium and in the presence of either 0.5 mM Ca^{2+} (standard assay conditions) or reduced Ca^{2+} levels (0.3, 0.15, and 0.05 mM), revealed identical intrinsic activities[14,22] of the analogue compared with oxytocin. In addition, [5-(N^4,N^4-dimethylasparagine)]oxytocin exhibits 9.14 ± 0.03 units/mg avian vasodepressor activity.[23] In the antidiuretic assay,[24] doses of different amounts elicited the same responses, and responses parallel to standard could not be obtained in the pressor assay.[25]

The identical intrinsic uterotonic activities of [5-(aspartic acid)]oxytocin and [5-(N^4,N^4-dimethylasparagine)]oxytocin with that of oxytocin may point to the significance of the carbonyl in position 4 (the γ position of the side chain) of the residue in position 5. The mere presense of a carbonyl in the side chain, as in [5-glutamine]oxytocin,[8] is insufficient by itself; the position of the carbonyl in the side chain appears to be a crucial factor.

In order to check this hypothesis, the study of [5-(2-amino-4-oxopentanoic acid)]oxytocin, which would retain the carbonyl in the γ position of the side chain of the residue in position 5, is suggested. Moreover, since the Asn in position 5 is proposed to be an active element for the elicitation of the antidiuretic response of vasopressins,[26] the study of [5-(N^4,N^4-dimethylasparagine)]vasopressin analogues should prove to be instructive.

Experimental Section

All melting points were determined in open capillary tubes and are reported uncorrected. Thin-layer chromatograms were done on silica gel plates with sample loads of 10 to 50 μg. The following

solvent systems were used and allowed to ascend for 10–12 cm: A, 1-butanol–acetic acid–water (4:1:1, v/v/v); B, 1-butanol–pyridine–acetic acid acid–water (15:10:3:6); C, ethyl acetate–pyridine–acetic acid–water (5:5:1:3); D, 1-butanol–acetic acid–water (4:1:5, upper phase). The compounds were revealed by reaction with ninhydrin or chlorine followed by 1% starch–potassium iodide or tolidine[27] solutions. For amino acid analysis,[28] aliquots of the compounds were hydrolyzed with constant-boiling hydrochloric acid in evacuated vials for 22 h and the hydrolysate was analyzed as outlined by Moore[29] on a Durrum D-500 amino acid analyzer. Cystine was determined as cysteic acid.[30] Optical rotations were measured with a Carl Zeiss precision polarimeter (0.005°). Elemental analyses were done by the Microanalytical Laboratory of the National Hellenic Research Foundation, and data fall within ±0.4% of theory. High pressure LC of the purified products were done accordingly: sample size, 100 μL of a 1 mg/mL solution; column, Whatman Partisil PXS 10/25 ODS, 4.6 × 250 mm; temperature, ambient; pressure, 17.6–35 kg/cm^2; gradient, 15–50% acetone (aqueous) in 1 h; flow rate, 23.2 mL/h.

N^2-[(*tert*-Butyloxy)carbonyl]-N^4,N^4-dimethylasparagine (Boc-Asp[N(CH$_3$)$_2$]-OH; 1). Palladium oxide (0.5 g) was added to a solution of N^2-[(*tert*-butyloxy)carbonyl]-N^4,N^4-dimethylasparagine benzyl ester[31] (3.5 g, 10 mmol) in 90% 2-propanol, and catalytic hydrogenation was carried out. After 6 h, the reaction mixture was filtered and the filtrate evaporated under reduced pressure. The oily residue obtained (2.5 g) failed to solidify after several trials. TLC in system A revealed one spot with an R_f of 0.85 against R_f 0.9 for the corresponding benzyl ester: NMR (CDCl$_3$) δ 1.45 [s, 9 H (CH$_3$)$_3$C–], 3 [d, 6 H, –N(CH$_3$)$_2$], 5.9 (br d, 1 H, –OCONH), 9.9 (br s, 1 H, –COOH).

For analytical purposes, a portion of the oil (0.8 g, 3.1 mmol) was dissolved in 50 mL of dry ether, and dicyclohexylamine (0.71 mL) was added. After cooling for 24 h, the desired product crystallized. It was filtered and washed well with dry ether, mp 145–147 °C. After recrystallization from ethyl acetate, it yielded 0.8 g (60%): mp 146–149 °C; [α]$^{20}_D$ +19° (*c* 1, ethanol). Anal. (C$_{22}$H$_{43}$N$_3$O$_5$) C, H, N.

Boc-Asp[N(CH$_3$)$_2$]-Cys(Bzl)-Pro-Leu-Gly-NH$_2$ (2). A sample of Boc-Cys(Bzl)-Pro-Leu-Gly-NH$_2$[17] (1.5 g, 2.26 mmol) was dissolved in CF$_3$CO$_2$H (4 mL); after 45 min the solvent was removed under reduced pressure; the residue was triturated with ether, filtered, washed with ether and dissolved in DMF (5 mL); and the pH of the solution was adjusted to 7.5 with *N*-methylmorpholine. An aliquot of 1 (1 g, 3.9 mmol) was dissolved in DMF (5 mL), cooled at 0 °C, preactivated[18] with 1-hydroxybenzotriazole (0.72 g, 5.3 mmol) and DCC (0.82 g, 3.9 mmol) for 1 h, and added to the partially protected tetrapeptide. The reaction mixture remained for 1 h at 0 °C and then for 48 h at room temperature. Progress of the reaction was followed by a combination of TLC and the Kaiser test[32] (used throughout the elongation of the peptide chain). The precipitated DCU was filtered, and the solvent was evaporated in vacuo. The remaining oily residue was solidified by the addition of ether, isolated by filtration, washed with 5% NaHCO$_3$ and water, and dried over P$_2$O$_5$. The product was washed with ethyl acetate–ether (1:1) under reflux and finally was purified from DMF–H$_2$O (1:5 v/v): yield 1.1 g (58%); mp 143–145 °C; [α]$^{20}_D$ –66.5 (*c* 1, DMF); TLC R_f (solvent system A) 0.62. Anal. (C$_{34}$H$_{53}$N$_7$O$_8$S) C, H, N.

Boc-Gln-Asp[N(CH$_3$)$_2$]-Cys(Bzl)-Pro-Leu-Gly-NH$_2$ (3). A portion of **2** (0.9 g, 1.25 mmol) was deprotected, neutralized, and dissolved (DMF, 5 mL) as before and allowed to react with Boc-Gln-ONp[33] (0.5 g, 1.35 mmol). After 2 days at room temperature, the solvent was removed under reduced pressure and the remaining oily residue was solidified by the addition of ethyl acetate–ether (1:1). The product was isolated by filtration, washed several times with 5% NaHCO$_3$ and water, and dried over P$_2$O$_5$. It was then recrystallized from ethyl acetate: yield 0.73 g (70%); mp 163–164 °C; [α]$^{20}_D$ –52.6° (*c* 1, DMF); TLC R_f (solvent system A) 0.65. Anal. (C$_{39}$H$_{61}$N$_9$O$_{10}$S) C, H, N.

Boc-Ile-Gln-Asp[N(CH$_3$)$_2$]-Cys(Bzl)-Pro-Leu-Gly-NH$_2$ (4). A portion of **3** (0.7 g, 0.83 mmol) was initially treated as above and allowed to react with Boc-Ile-OSu[34] (0.55 g, 1.65 mmol) in DMF (5 mL). After 3 days at room temperature, the solvent was removed under reduced pressure, and the oily residue was solidified by the addition of ether, filtered, washed several times with 5% NaHCO$_3$ and water, and dried (P$_2$O$_5$). It was then washed with ethyl acetate–ethanol (3:1) under reflux and finally recrystallized from ethanol: yield 0.43 g (55%); mp 167–169 °C; [α]$^{20}_D$ –46° (*c* 1, DMF); TLC R_f (solvent system A) 0.7. Anal. (C$_{45}$H$_{72}$N$_{10}$O$_{12}$S) C, H, N.

Boc-Tyr(Bzl)-Ile-Gln-Asp[N(CH$_3$)$_2$]-Cys(Bzl)-Pro-Leu-Gly-NH$_2$ (5). An aliquot of **4** (0.4 g, 0.42 mmol) was deprotected, neutralized, dissolved (DMF, 5 mL), and allowed to react with Boc-Tyr(Bzl)-OSu[34] (0.3 g, 0.64 mmol). After 3 days at room temperature, the solvent was removed under reduced pressure and the oily residue triturated with cold water (40 mL) to precipitate a solid. The product was filtered, washed several times with 5% NaHCO$_3$ and water, and dried (P$_2$O$_5$). It was then washed with ethyl acetate under reflux and finally recrystallized from ethanol: yield 0.38 g (78%); mp 185–187 °C; [α]$^{20}_D$ –41° (*c* 1, DMF); TLC R_f (solvent system A) 0.7. Anal. (C$_{61}$H$_{87}$N$_{11}$O$_{13}$S) C, H, N.

Z-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn[N(CH$_3$)$_2$]-Cys(Bzl)-Pro-Leu-Gly-NH$_2$ (6). A sample of **5** (0.37 g, 0.31 mmol) was deprotected and treated as for **2** using Z-Cys(Bzl)-OSu[34,35] (0.26 g, 0.45 mmol) as acylating agent. After 3 days, the reaction was worked up as for **5** with the final purification afforded by dissolution in DMF (2 mL) and precipitation by the addition of ethyl acetate (10 mL): yield 0.25 g (59%); mp 207–211 °C. After reprecipitation from the minimal amount of hot ethanol, it had a mp of 213–215 °C; [α]$^{20}_D$ –61° (*c* 1, acetic acid); TLC R_f (solvent system A) 0.74, R_f (solvent system D) 0.70. Anal. (C$_{74}$H$_{96}$N$_{12}$O$_{14}$S$_2$) C, H, N. Amino acid analysis: Asp, 1.01; Glu, 1.04; Pro, 1.01; Gly, 1.00; Ile, 0.98; Leu, 1.03; Tyr, 0.91; Cys(Bzl), 1.92; NH$_3$, 1.88.

[5-(N^4,N^4-Dimethylasparagine)]oxytocin (7). A sample of **6** (107.4 mg, 74.5 μmol) was deprotected by sodium in liquid ammonia.[36] After evaporation of the ammonia by a nitrogen stream, the residue was dissolved in deaerated 50% aqueous methanol, and the disulfide formed by oxidation with 1 equiv of 1,2-diiodoethane.[37] Following the desalting of the product by gel filtration on Sephadex G-15 (117 × 2.5 cm) with 50% acetic acid (elution volume 145 mL),[38] final purification was attained by partition chromatography on Sephadex G-25[39] with the solvent system 1-butanol–3.5% acetic acid containing 1.5% pyridine (1:1 v/v, aqueous phase), R_f 0.40, followed by gel filtration on Sephadex G-25 with 0.2 M acetic acid: yield following lyophilization 51.4 mg; TLC showed a single spot with R_f (solvent system A) 0.27, R_f (solvent system B) 0.59, R_f (solvent system C) 0.64, R_f (solvent system D) 0.25; [α]$^{26}_D$ –22° (*c* 0.54, 1 N acetic acid). Amino acid analysis: Cys-O$_3$H, 1.95; Asp, 1.01; Glu, 1.02; Pro, 1.01; Gly, 1.00; Ile, 0.96; Leu, 1.02; Tyr, 0.9; NH$_3$, 1.97. High pressure LC demonstrated it to be pure (elution volume 137 mL) and free of oxytocin. Comparative elution volumes were determined when a sample of oxytocin was simultaneously chromatographed.

Biological Assays. Rat uterotonic assays were performed on isolated horns from virgin rats in natural estrus according to the method of Holton, as modified by Munsick with the use of Mg^{2+}-free van Dyke–Hastings solution as bathing fluid.[20] For dose–response determinations on the rat uterus in vitro the conditions were those of above or had an added Mg^{2+} concentration of 1.0 mM in the bathing fluid, or the Ca^{2+} ion concentration of the bath was varied from the standard 0.5 mM to lower concentrations (0.3, 0.15, and 0.05 mM). The individual injection method[22] was used with doses being increased geometrically according to a $^1/_2$ log 10 procedure until a maximal response was reached. Details of the experimental procedure have been previously published.[40] Avian vasdopressor assays were performed on conscious White Leghorn roosters by the method of Coon, as described with modifications.[23] Antidiuretic[24] and pressor[25] assays were performed on anesthetized Sprague–Dawley male rats.

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(1) All optically active amino acids are of the L configuration. Abbreviations used follow the recommendations of the

IUPAC–IUB as found in *Biochemistry*, **14**, 449 (1975), and *Biochem. J.*, **126**, 773 (1972). Other abbreviations used are: Asp[$N(CH_3)_2$], N^4,N^4-dimethylasparagine; DMF, dimethylformamide; Me_2SO, dimethyl sulfoxide; LC, liquid chromatography.
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Book Reviews

Serotonin in Health and Disease. Volume II. Physiological Regulation and Pharmacological Action. Edited by W. B. Essman. Spectrum Publications, New York, N.Y. 1978. 443 pp. 16 × 23.5 cm. $37.50.

The first volume in this series described the availability, localization, and distribution of serotonin. In this, the second volume, a physiological role of serotonin in a number of systems is assessed together with relevant drug action.

The first four chapters are concerned with "Thermoregulation", "Early (Prenatal and Perinatal) Development", "Rhthmicity and Periodic Functions of the Central Nervous System", and "Regulation of Sleep". These chapters provide most readable reviews and are concerned with relevant drug action on serotonin as a secondary consideration. Chapter 5 surveys the drugs affecting serotonin. Most of the agents covered in this chapter are already mentioned in the previous chapters and it is difficult for any author to achieve a novel approach in such familiar territory. Nevertheless the review is concise, interesting, up-to-date, and includes details on the latest serotonin reuptake inhibitors. Chapter 6 is entitled, rather inappropriately, "Clinical Pharmacology of Serotonin". In reality, the chapter relates to a potential relevance of serotonin to antidepressant, minor tranquillizer, central stimulant, anorectic, and narcotic action. The chapter comprises only 25 pages which necessitates a concise but superficial approach. The conclusion to this chapter remains an excercise in bland semantics which is perhaps inevitable. Both Chapters 7 and 8 are essentially concerned with the role of sertonin in drug addiction with respect to chronic opiate action and alcoholism. Both chapters are well written and informative. While the selection of topics for inclusion in this book may be questioned, the text should prove interesting and useful to both the casual and serious reader.

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