Cellophane as a Dialysis Membrane for Studying Nonionic Surfactants

S. J. A. KAZMI and A. G. MITCHELL▲

Abstract □ The permeability of cellophane membranes to the nonionic surfactant cetomacrogol 1000 was investigated using equilibrium dialysis, dynamic dialysis, and an ultrafiltration technique. Cellophane and silicone rubber membranes were compared in an equilibrium dialysis study of the interaction of chlorocresol with cetomacrogol. The permeability and interaction studies showed that the cellophane membranes were permeable to the surfactant. In addition, errors were introduced into the calculation of the binding constants unless changes in volume and in surfactant concentration, which occur as a result of the osmotic differential across the membrane, were taken into account.

Keyphrases □ Cellophane dialysis membranes—permeability to nonionic surfactant, used to study interaction of chlorocresol with cetomacrogol, compared to silicone rubber membranes □ Dialysis membrane permeability to nonionic surfactant, interaction with preservative—cellophane and silicone rubber compared □ Surfactant (nonionic) interaction with preservatives—studied using cellophane dialysis membranes, effect of osmosis on binding constants

Cellophane membranes are widely used in equilibrium (1–7) and dynamic (7–9) dialysis studies involving the interaction of preservatives and drugs with nonionic surfactants. Cellophane has also been used to study the effects of surfactants on the diffusion of drugs across membranes (8–12). Ideally, such investigations require that the membrane be impermeable to the nonionic surfactant while allowing diffusion of the drug and that the osmotic differential across the membrane is negligible.

There is some controversy concerning the permeability of cellophane dialysis membranes to nonionic surfactants. Patel and Kostenbauder (13) reported that Visking cellophane membrane was permeable to polysorbate 80, and they considered this membrane unsatisfactory for equilibrium dialysis work. This observation was supported by Nishida *et al.* (14). Breuninger and Goettsch (2) found that, although Visking cellophane membrane was permeable to polysorbate 80, Fisher cellophane membrane was impermeable to the same surfactant. Matsumoto *et al.* (4) studied the permeability of polysorbate 80 and a polyoxyethylene lauryl ether through Visking cellophane membrane, using dynamic dialysis (without stirring the solutions) under sink and nonsink conditions. They found that Visking cellophane membrane was practically impermeable to the nonionic surfactants and that only impurities, such as low molecular weight polyethylene glycols, passed through it.

Patel (5) compared Fisher cellophane membrane with nylon and rubber membranes in equilibrium dialysis studies, involving the interaction of several preservatives with cetomacrogol 1000 and polysorbate 80. Since close agreement was found between data obtained using Fisher cellophane membrane and those obtained using the rubber or nylon membranes, it was assumed that the cellophane membrane was impermeable to cetomacrogol and polysorbate 80. Ikeda *et al.* (7) used Visking cellophane membrane in equilibrium and dynamic dialysis techniques to study the interaction of barbiturates with a polyoxyethylene ether surfactant. Although the surfactant permeated through the cellophane membrane, the amount passed during 48 hr. was below the CMC of the surfactant.

Short *et al.* (10) reported that Visking cellophane membrane was impermeable to polyoxyethylene surfactants and that only small traces of nonsurface-active impurities passed through it.

Although the permeability of cellophane to polyethylene glycols has been studied less extensively than the nonionic surfactants, it has been shown that cellophane is permeable to polyethylene glycols with a molecular weight less than 20,000 (3, 15). Polyethylene glycols permeate cellophane membranes more readily than nonionic surfactants of similar molecular weight.

Rubber (16) and nylon (17) membranes were used in equilibrium dialysis studies of the interaction of various preservatives with the nonionic surfactant cetomacrogol, since qualitative tests showed that cellophane is permeable to the nonionic surfactant and that volume changes occurred as a result of osmosis. Apart from Matsumoto *et al.* (4), few workers using cellophane appear to have corrected for the volume changes. In view of the continued use of cellophane, it seemed desirable to make quantitative measurements of the permeability of cellophane membranes to cetomacrogol and to assess the effects of surfactant permeation and osmosis on the binding constants for the interaction between the surfactant and a preservative determined using the equilibrium dialysis technique.

EXPERIMENTAL

Materials—Cetomacrogol 1000 BPC[1] of the general formula $CH_3(CH_2)_m(OCH_2CH_2)_nOH$, where m may be 15 or 17 and n may be 19–23, was used. The molecular weight was taken as 1300. The preservative selected was chlorocresol, reagent grade[2]. Three membranes were used: Membrane 1, cellophane, dialysis tubing 3.6-cm. ($1^{47}/_{64}$-in.) flat width[3]; Membrane 2, cellophane, dialysis tubing 2.4-cm. ($^{15}/_{16}$-in.) flat width[4]; and Membrane 3, silicone rubber (silastic sheeting, nonreinforced 0.01 cm. (0.005 in.)[5]. All membranes were washed thoroughly with distilled water before use. Deionized glass-distilled water was used in all studies.

Analysis of Cetomacrogol—The presence of surface-active material in the "surfactant-free" compartment of the dialysis cell was readily detected by the formation of foam on agitation and by the

[1] Glovers Chemicals Ltd., Leeds, England.
[2] British Drug Houses, Poole, England.
[3] Fisher cellophane, Fisher Scientific Co.
[4] Visking cellophane, Union Carbide Ltd.
[5] Dow Corning Corp.

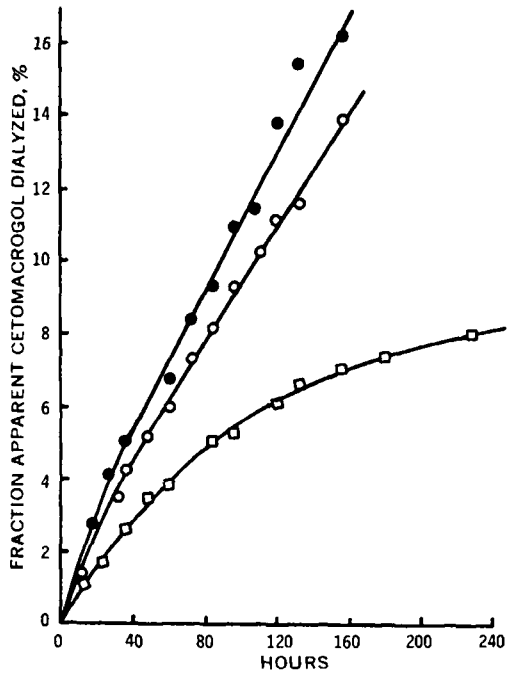

Figure 1—*Permeability of cellophane membranes to cetomacrogol in equilibrium dialysis. Initial cetomacrogol concentrations were:* □, *38.46* × *10*$^{-3}$ M; *and* ○, *76.92* × *10*$^{-3}$ M. *Open and closed symbols represent Membranes 1 and 2, respectively. The fraction dialyzed was not corrected for changes in surfactant concentration due to osmosis.*

precipitation of a phosphomolybdic acid complex in a barium chloride–hydrochloric acid medium (18).

Quantitative analysis of surfactant was made using a polarograph[6]. The analysis was based on the suppression of the oxygen maximum by surfactants. Potassium chloride solution (0.002 *N*) gives a very pronounced oxygen maximum. A comparison of the heights of the maxima in the presence and absence of cetomacrogol and reference to a calibration curve permitted the determination of surfactant down to 1.0 mg./l. (19, 20). Since the method is not specific for cetomacrogol, the results are expressed as apparent cetomacrogol concentrations.

Permeability of Membranes to Cetomacrogol—*Equilibrium Dialysis*—Dialysis cells similar to those described by Patel and Foss (21) were used. The two chambers of the cell were separated by a cellophane or silicone rubber membrane. Twenty milliliters of cetomacrogol solution was pipeted into one chamber, and 20 ml. of distilled water was pipeted into the other. The cells were tumbled in a temperature-controlled water bath; at 12-hr. intervals, equal volumes were pipeted from both chambers and analyzed for cetomacrogol.

Dynamic Dialysis—Twenty-five milliliters of 10% cetomacrogol was transferred to membrane 1 suspended in 200 ml. of distilled water in a jacketed beaker. The solution in the jacketed beaker was stirred with a magnetic stirrer, while the cetomacrogol solution in the cellophane bag was stirred with a glass stirrer. One hundred milliliters of the solution was pipeted from the jacketed beaker at 12-hr. intervals and analyzed for cetomacrogol. The volume of solution in the jacketed beaker was immediately made to 200 ml. with fresh distilled water to maintain sink conditions.

Ultrafiltration—Membrane 2 was cut and fitted into an ultrafiltration cell[7]. Thirty-five milliliters of 1.8% cetomacrogol solution was placed in the cell, and a pressure of 18.2 kg. (40 lb.)/in.^{2} was applied until complete filtration of the liquid was attained. The filtrate was analyzed for cetomacrogol.

Interaction of Chlorocresol with Cetomacrogol—The equilibrium dialysis technique was used to study the interaction of chlorocresol with cetomacrogol. The dialysis membrane was either Membrane 1 or 3. An aqueous solution of chlorocresol in cetomacrogol was placed in one side of the cell, and water or water plus chlorocresol was placed in the other. Two glass beads were added to each compartment to ensure continuous mixing, and the cells were rotated in the water bath at 25°. The cholorocresol in both chambers was analyzed spectrophotometrically at 280 nm. after 4 days, and the volume of solution in each chamber was measured.

[6] Radiometer Polariter PO$_4$.
[7] Amicon Corp.

Table I—Change of Surfactant Concentration in Equilibrium Dialysis with Cellophane as a Semipermeable Membrane

Cetomacrogol Concentration, $M \times 10^3$			
M_1, Initial	M_2, Calculated from Volume Change[a]	M_3, Calculated Using Eq. 2[b]	M_4, Permeated at 96 hr.[c]
38.46	33.88	31.60	2.19
76.92	62.06	55.42	7.23

[a] Each value represents the mean of five readings. [b] Values were obtained by substituting n_1K_1 and n_2K_2, calculated from the binding curve obtained using the silicone rubber membrane (Fig. 3), and $[D_b]$ and $[D_f]$, obtained using the cellophane membrane (Fig. 3), into Eq. 2. Each value represents the mean of five readings. [c] From Fig. 1.

RESULTS AND DISCUSSION

Permeability of Membranes to Cetomacrogol—Figure 1 shows the fraction of cetomacrogol dialyzed through Membranes 1 and 2 as a function of time in equilibrium dialysis. Both membranes are permeable to cetomacrogol at approximately the same rate. The silicone rubber membrane, Membrane 3, was completely impermeable to the nonionic surfactant. Figure 2 shows the permeability of Membrane 1 to cetomacrogol using dynamic dialysis under sink conditions. Increases in the volume of solution in the surfactant chamber of the equilibrium dialysis cell and the cellophane bag used in the dynamic dialysis technique showed that osmosis had occurred.

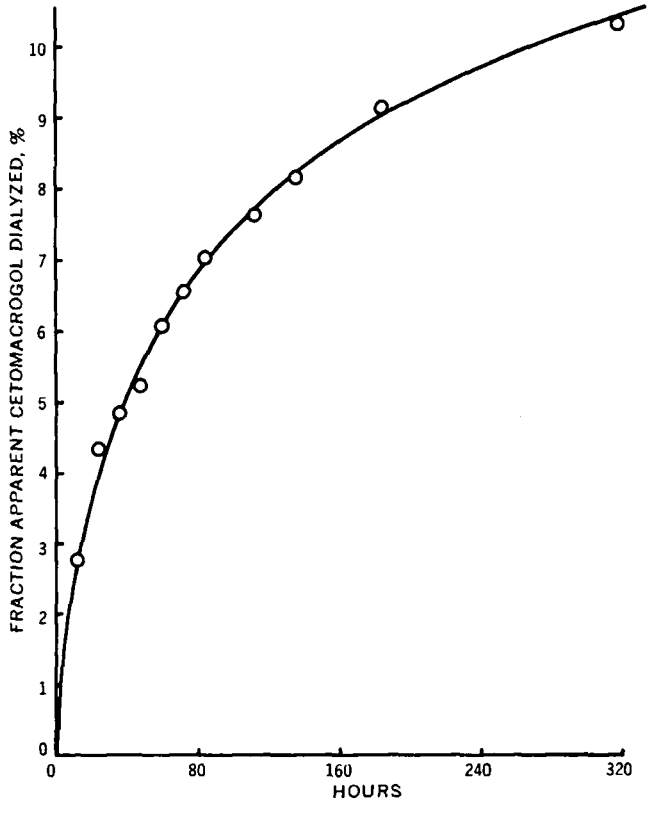

Figure 2—*Permeability of Membrane 1 to cetomacrogol in dynamic dialysis under sink conditions. Initial cetomacrogol concentration was 76.92* × *10*$^{-3}$ M. *The fraction dialyzed was not corrected for changes in surfactant concentration due to osmosis.*

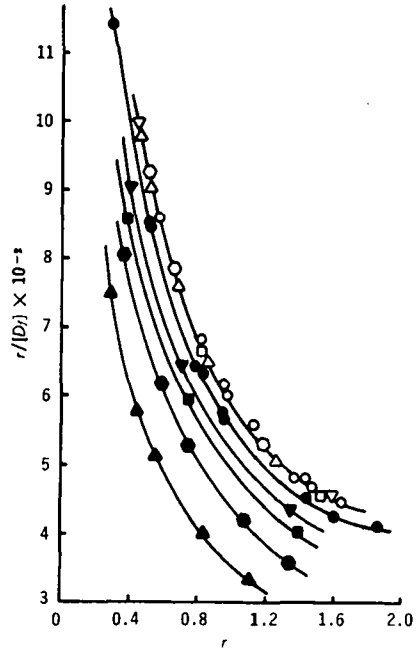

Figure 3—*Scatchard plot for the interaction of chlorocresol with cetomacrogol. Initial cetomacrogol concentrations were:* ○, *7.69* × *10⁻³* M; ▽, *15.3* × *10⁻³* M; □, *23.07* × *10⁻³* M; ○, *38.46* × *10⁻³* M; *and* △, *76.92* × *10⁻³* M. *Closed and open symbols represent data obtained using Membranes 1 and 3, respectively.*

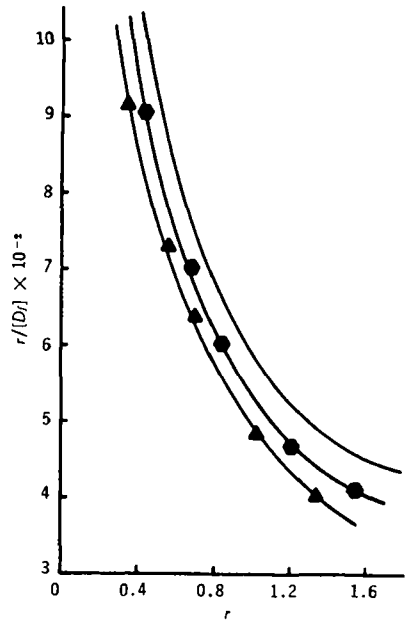

Figure 4—*Scatchard plot for the interaction of chlorocresol with cetomacrogol. The upper binding curve was obtained using the silicone rubber membrane as in Fig. 3. Cellophane membrane data were corrected for changes in surfactant concentrations due to osmosis. Initial concentrations were as listed in Fig. 3; corrected concentrations are shown in Table I.*

Ultrafiltration of cetomacrogol solution (1.8%) through Membrane 2 showed that 2.7% of the total surfactant passed through the membrane.

Interaction of Chlorocresol with Cetomacrogol—The binding results in the form of a Scatchard plot (22) are shown in Fig. 3. The binding of chlorocresol with cetomacrogol is independent of surfactant concentration when silicone rubber is used as the dialysis membrane. Similar independence of surfactant concentration was reported previously for the binding of several other preservatives with cetomacrogol when nylon was used as the dialysis membrane (17). The binding constants were estimated from the slope of the curve in the region of interest. In this work, however, the curve was characterized according to Eq. 1 on the assumption that two classes of binding sites are involved in the interaction:

$$\frac{[D_b]}{[M]} = r = \frac{n_1K_1[D_f]}{1 + K_1[D_f]} + \frac{n_2K_2[D_f]}{1 + K_2[D_f]} \qquad \text{(Eq. 1)}$$

where:

$[D_f]$ = concentration of free preservatives in the aqueous phase
$[D_b]$ = concentration of preservative bound to surfactant
$[M]$ = concentration of surfactant
n_1 = number of independent binding sites of Class I on the surfactant molecule
n_2 = number of independent binding sites of Class II on the surfactant molecule
K_1 = intrinsic association constant for the binding of a molecule of preservative to one of the binding sites of Class I
K_2 = intrinsic association constant for the binding of a molecule of preservative to one of the binding sites of Class II

The experimental data are indicated by points, while the solid lines were fitted according to the n and K values computed from Eq. 1 using a nonlinear regression program.

Figure 3 shows that when a cellophane dialysis membrane was used, the binding is apparently not independent of cetomacrogol concentration and a series of curves are obtained. Theoretically, such results indicate that an increase in the concentration of cetomacrogol results in a decrease in the number of binding sites on the cetomacrogol molecule. However, since the results obtained with the silicone rubber membrane are independent of surfactant concentration, it is suggested that changes in cetomacrogol concentration due to dilution as a result of osmosis and/or to permeation of surfactant through the cellophane membrane provide a more likely explanation. Permeation of sufficient cetomacrogol into the surfactant-free chamber of the dialysis cell would lead to an increase in the apparent value of $[D_f]$ due to micellar interaction with the chlorocresol and, therefore, decrease the ratio $r/[D_f]$ for a given r value. Moreover, loss of surfactant by permeation through the membrane would also decrease r due to the uncorrected decrease in the value of $[M]$ and the decrease in $[D_b]$. Thus, a plot of $r/[D_f]$ *versus* r for different concentrations of cetomacrogol would result in a series of curves, each representing a given surfactant concentration. Dilution of cetomacrogol as a result of osmosis would produce a similar displacement of the binding curves.

The surfactant concentration required to produce the observed displacement of the binding curves was calculated from a rearrangement of Eq. 1:

$$[M] = \frac{[D_b](1 + K_1[D_f] + K_2[D_f] + K_1K_2[D_f]^2)}{[D_f](n_1K_1 + n_2K_2 + n_1K_1K_2[D_f] + n_2K_1K_2[D_f])} \qquad \text{(Eq. 2)}$$

where $[D_b]$ and $[D_f]$ are the experimental values for the cellophane membrane (Fig. 3) and n_1, K_1, n_2, and K_2 are the binding constants obtained using the silicone rubber membrane. The data in Table I show that the changes in surfactant concentration (*i.e.*, differences between the initial and calculated cetomacrogol concentrations, $M_1 - M_3$) were considerably greater than could be accounted for solely by loss of surfactant as a result of permeation through the cellophane membrane, M_4. However, when the binding curves are replotted using surfactant concentrations corrected for volume changes in the chambers of the dialysis cell, M_2, the discrepancies between the curves determined using the silicone rubber membrane and the cellophane membrane are markedly reduced (Fig. 4).

Since the difference between M_2 and M_3 is of the same order of magnitude as M_4, the residual displacement of the binding curves can be attributed to the decrease in $[M]$ due to permeability of the cellophane membrane to cetomacrogol.

Hence, it can be concluded that the observed displacement of the binding curves obtained in the equilibrium dialysis study using cellophane membranes is due both to osmosis and to permeability of the membrane to the surfactant, with dilution of surfactant as a result of the osmotic differential across the membrane being the major factor. The use of cellophane as a membrane will introduce appreciable errors into interaction and transport studies involving nonionic surfactants unless corrections are made for osmosis.

REFERENCES

(1) D. C. Chakravarty and J. L. Lach, *Drug Stand.*, **27**, 6(1959).
(2) W. B. Breuninger and R. W. Goettsch, *J. Pharm. Sci.*, **54**, 1487(1965).
(3) B. N. Kabadi and E. R. Hammarlund, *ibid.*, **55**, 1072(1966).
(4) H. Matsumoto, H. Matsumura, and S. Iguchi, *Chem. Pharm. Bull.*, **14**, 385(1966).
(5) N. K. Patel, *Can. J. Pharm. Sci.*, **2**, 97(1967).
(6) N. K. Patel and J. M. Romanowski, *J. Pharm. Sci.*, **59**, 372 (1970).
(7) K. Ikeda, K. Kato, and T. Tukamoto, *Chem. Pharm. Bull.*, **19**, 2510(1971).
(8) H. Matsumoto, H. Matsumura, and S. Iguchi, *ibid.*, **19**, 391 (1966).
(9) H. Matsumoto, *ibid.*, **19**, 398(1966).
(10) P. M. Short, E. T. Abbs, and C. T. Rhodes, *J. Pharm. Sci.*, **59**, 995(1970).
(11) P. M. Short and C. T. Rhodes, *Nature, New Biol.*, **236**, 44 (1972).
(12) R. Withington and J. H. Collett, *J. Pharm. Pharmacol., Suppl.*, **24**, 131P(1972).
(13) N. K. Patel and H. B. Kostenbauder, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 289(1958).
(14) M. Nishida and S. Iguchi, *Yakuzaigaku*, **24**, 53(1964); through *Reference 4*.
(15) C. B. Shaffer, F. H. Critchfield, and J. H. Nair, *J. Amer. Pharm. Ass., Sci. Ed.*, **39**, 344(1950).
(16) A. G. Mitchell and K. F. Brown, *J. Pharm. Pharmacol.*, **18**, 115(1966).
(17) S. J. A. Kazmi and A. G. Mitchell, *ibid.*, **23**, 482(1970).
(18) J. Oliver and C. Preston, *Nature*, **164**, 242(1949).
(19) I. Vavruch, *Anal. Chem.*, **22**, 930(1950).
(20) H. Jehring, *Akad. Wiss. Berlin Kl. Chem., Geol. Biol.*, **6**, 197 (1966).
(21) N. K. Patel and N. E. Foss, *J. Pharm. Sci.*, **53**, 94(1964).
(22) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660(1949).

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Tremor Induction by Intracaudate Injections of Bretylium, Tetrabenazine, or Mescaline: Functional Deficits in Caudate Dopamine

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Abstract □ Tremor responses were evoked by intracaudate injections of tetrabenazine, bretylium, or mescaline in cats with chronically implanted recording electrodes and microinjection cannulas. The characteristics of the maximal tremors with each agent closely resembled those induced by increased levels of cholinergic activity in the caudate (previously reported). These maximal tremors, like the cholinergic tremors, were suppressed by local injections of catecholamines (dopamine and epinephrine), scopolamine, or hemicholinium but were intensified by intracaudate serotonin. Although local acetylcholine had no effect on established tremor activity, tremors abolished by hemicholinium were reestablished by small doses of acetylcholine. These results suggested that interference with local dopamine inhibitory mechanisms ("functional dopamine deficiency") was the basis for the tremorgenic actions of bretylium, tetrabenazine, and mescaline; the findings also indicated that sustained endogenous acetylcholine activity in the caudate was a necessary condition for the development and maintenance of tremor activity. These data lend support to the hypothesis that an imbalance in the caudate between dopamine inhibition and acetylcholine excitation in favor of the latter results in tremors.

Keyphrases □ *Dopamine*—functional deficiency in caudate tremor mechanisms, role in tetrabenazine-, bretylium-, and mescaline-induced tremor □ Tetrabenazine—tremor evoked by intracaudate injection □ Bretylium—tremor evoked by intracaudate injection □ Mescaline—tremor evoked by intracaudate injection □ Catecholamine—inhibition of tetrabenazine-, bretylium-, and mescaline-induced tremor □ Scopolamine—inhibition of tetrabenazine-, bretylium-, and mescaline-induced tremor □ Hemicholinium—inhibition of tetrabenazine-, bretylium-, and mescaline-induced tremor □ Serotonin—intensification of tetrabenazine-, bretylium-, and mescaline-induced tremor

Resting tremors have been induced consistently by carbachol or anticholinesterases injected directly into the head of the caudate nucleus in unanesthetized cats prepared with permanently implanted recording electrodes and microinjection cannulas. These tremors were inhibited by intracaudate injection of agents that interfere with either postsynaptic receptor actions (atropine and scopolamine) or presynaptic synthesis (hemicholinium-3) of acetylcholine, or they were suppressed by intracaudate injection of catecholamines (epinephrine and dopamine) (1, 2). From these findings and from a consideration of the high concentrations of both acetylcholine and dopamine in the caudate, it has been postulated that the functions of acetylcholine (excitatory) and dopamine (inhibitory) are critically balanced in the caudate so as to comprise a local tremor regulatory mechanism (3–5).

Clinically, the involuntary movement disorders associated with Parkinson's disease have been linked to deficiencies in caudate dopamine (6). Also, the extra-