Pharm. Sci., **54**, 747 (1965).
(5) G. Levy and L. E. Hollister, *N.Y. J. Med.*, **64**, 3002 (1964).
(6) P. L. Madan and M. Minisci, *Drug Intell. Clin. Pharm.*, **10**, 588 (1976).
(7) G. Levy, *J. Pharm. Sci.*, **52**, 1039 (1963).
(8) P. L. Madan, in "Manual for Pharmacy Technicians," 2nd ed., J. M. Durgin, Z. I. Hanan, and C. O. Ward, Eds., C. V. Mosby, St. Louis, Mo., in press.
(9) E. L. Knoechel, C. C. Sperry, and C. J. Lintner, *J. Pharm. Sci.*, **56**, 116 (1967).

Microsealed Drug Delivery Systems I: *In Vitro–In Vivo* Correlation on Subcutaneous Release of Desoxycorticosterone Acetate and Prolonged Hypertensive Animal Model for Cardiovascular Studies

YIE W. CHIEN *x, LEONARD F. ROZEK ‡, and HOWARD J. LAMBERT *

Received October 18, 1976, from the **Pharmaceutical Research Group, Development Department, and the* ‡*Cardiovascular Group, Biological Research Department, Searle Laboratories, G. D. Searle & Co., Skokie, IL 60076.* Accepted for publication May 2, 1977.

Abstract □ A new generation drug delivery system, named the Microsealed Drug Delivery (MDD) system, was developed. Subcutaneous implantation of MDD's in rats for up to 129 days resulted in a constant release profile of desoxycorticosterone acetate. Three formulations were examined. An excellent *in vitro–in vivo* correlation was established on both the mechanism and the rate of controlled release of desoxycorticosterone acetate from MDD's. A significant degree of hypertension was reproducibly produced within 21 days after implantation and successfully sustained through Day 98. Comparative studies were conducted on MDD's and a previously developed matrix-type silicone device. The elevation of systolic blood pressure initiated by either MDD's or the matrix-type silicone device was essentially the same in pattern, and the difference in the hypertensive responses between these polymeric drug delivery systems was statistically insignificant, although a higher dose, which is time dependent, was administered to rats through the matrix-type silicone device than through MDD's. The bioavailability of desoxycorticosterone acetate and its dose–response relationship apparently were accomplished more effectively *via* the constant drug delivery mechanism of MDD's.

Keyphrases □ Drug delivery systems, microsealed—subcutaneous release of desoxycorticosterone acetate in rats, *in vitro–in vivo* correlation, *compared to matrix-type silicone device* □ Desoxycorticosterone acetate—subcutaneous release from microsealed drug delivery system in rats, *in vitro–in vivo* correlation, compared to matrix-type silicone device □ Dosage forms—microsealed drug delivery system, subcutaneous release of desoxycorticosterone acetate in rats, *in vitro–in vivo* correlation, compared to matrix-type silicone device □ Adrenocortical steroids—desoxycorticosterone acetate, subcutaneous release from microsealed drug delivery system in rats, *in vitro–in vivo* correlation, compared to matrix-type silicone device

Metacorticoid hypertension, induced by the chronic administration of desoxycorticosterone acetate to rats in conjunction with saline loading, simulates, both pathologically and physiologically, the syndrome of essential hypertension in humans (1). A close relationship was observed between salt metabolism and many forms of experimental and clinical hypertension. An increase in total sodium, due in large part to an increase in intracellular sodium, and a fall in intracellular potassium, were reported to occur during the development of hypertension induced by desoxycorticosterone acetate (2).

Induction of experimental hypertension in rats to evaluate the antihypertensive activity of various drugs requires either daily injections of a desoxycorticosterone acetate suspension or implantation of a desoxycorticosterone acetate-containing wax pellet (3) while the rats are maintained on saline. The first technique consistently initiates onset of metacorticoid hypertension within 21–28 days, but the required daily injections are time consuming and may be hazardous for the animals. The second technique, because of inconsistent release rates of desoxycorticosterone acetate from the wax matrix, results in wide variations in hypertensive onset.

Recent reports (4, 5) demonstrated that experimental hypertension could be reproducibly induced in rats and successfully maintained for a prolonged period of time, *e.g.*, 100 days, by subcutaneous delivery of desoxycorticosterone acetate through a long-acting matrix-type silicone device. The release of desoxycorticosterone acetate was substantially prolonged by homogeneously impregnating the desoxycorticosterone acetate in a silicone polymer matrix. The same drug delivery system was also applied for the intravaginal administration of ethynodiol diacetate to rabbits for sustained contraceptive activity (6). Both the *in vitro* and *in vivo* releases of desoxycorticosterone acetate from silicone devices followed a matrix-controlled process, as defined by Q *versus* $t^{1/2}$ release kinetics (4, 6–11). The release profile of a drug from the matrix-type polymeric devices is high initially and then decreases with time.

In this investigation, a new generation drug delivery system, named the Microsealed Drug Delivery (MDD) system (12), was developed to provide a means for the constant (zero-order) release of desoxycorticosterone acetate. Both the *in vitro–in vivo* release profiles of desoxycorticosterone acetate from MDD's were studied, and their relationship was analyzed. The differences between MDD's and matrix-type silicone devices on the modes of desoxycorticosterone acetate release were examined. The time courses for the production of metacorticoid hypertension in rats also were explored.

Table I—Independence of the *In Vitro* Release Rates of Desoxycorticosterone Acetate on Drug Content in the Medicated Core Matrix of Microsealed Delivery Devices

Drug Content, mg/cm³	Release Rates[a], μg/cm²/day
21.2	50.8 (±2.1)
36.3	56.9 (±3.2)
64.9	53.5 (±1.2)

[a] Mean (± *SD*) of three determinations.

EXPERIMENTAL

Preparation of MDD's—MDD's were fabricated, following the methodology and process outlined earlier (12), to contain 21.1–64.9 mg of desoxycorticosterone acetate[1] in a unit volume of a medicated core matrix. After inspection by photomicrography, UV and IR spectrophotometry, and differential scanning calorimetry, desoxycorticosterone acetate chemical was used as obtained.

***In Vitro* Drug Release Studies**—The *in vitro* drug elution system used in characterizing the mechanism and rates of release of desoxycorticosterone acetate from MDD's was essentially the same as that reported previously (4, 7). Lengths of 16 cm of the MDD were mounted in a circular shape in the arms of a Plexiglas holder. The holder was then rotated at a constant speed (81 rpm) in an elution medium [150 ml of 75% (v/v) polyethylene glycol 400[2] in distilled water] at 37°.

A 50-ml sample of the elution medium was withdrawn each day and was replaced with the same quantity of drug-free elution medium, which was also thermostated at 37°. The sample was then assayed spectrophotometrically at λ_{max} 240 nm. The amount of desoxycorticosterone acetate released daily was calculated and followed for 8 days, and the mechanisms as well as the rates of drug release from MDD's were then analyzed.

***In Vivo* Drug Release Studies**—Sixty male Charles River CD strain rats (with an average weight of 130–190 g and an age of 4–6 weeks) were assigned to three studies. In each study, a control group (implanted with placebo MDD's) was examined in parallel with the treated group (implanted with medicated MDD's). The systolic blood pressure of each rat was indirectly determined using a programmed electrosphygmomanometer[3] at the onset of the study and at weekly intervals thereafter.

Following the determination of initial blood pressure, the rats were anesthetized with ether. A 1-cm cutaneous incision was made in the dorsal thoracic area of each rat. After the formation of a subcutaneous tunnel with forceps, a 3-cm length of the MDD was inserted. The incision was closed with wound clips, and the rats were then returned to their cages for recovery.

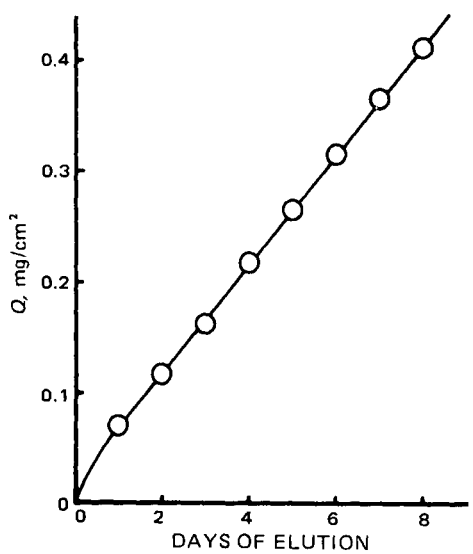

Figure 1—*Time course for the* in vitro *release of oxycorticosterone acetate from MDD 603 in 75% (v/v) aqueous polyethylene glycol 400 solution at 37°. The rate of* in vitro *release, Q/t, was calculated from the slope as 49.34 μg/cm²/day.*

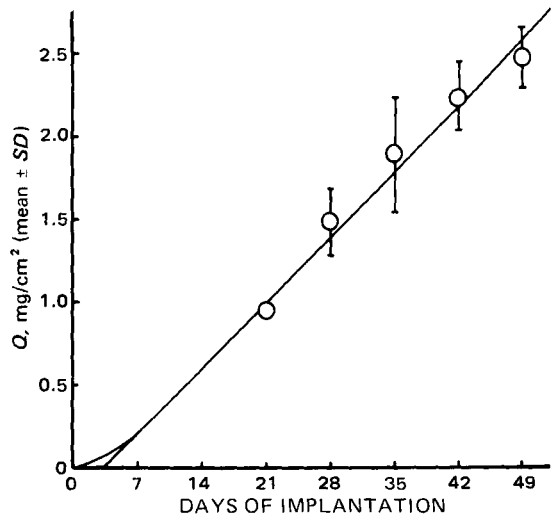

Figure 2—*Time course for the subcutaneous release of desoxycorticosterone acetate from MDD 603 in rats. The rate of* in vivo *release, Q/t, was calculated from the slope as 53.7 μg/cm²/day. Each data point represents the mean* (±SD) *of three to five determinations.*

Both the control and treated groups were maintained on a normal laboratory rat diet with a fluid ration of 1% saline solution throughout the study.

At scheduled intervals, five rats were randomly selected from the treated group for removal of the MDD's. After measurement of the systolic blood pressure, the MDD's were removed. Their residual desoxycorticosterone acetate content was thoroughly extracted with methanol and assayed spectrophotometrically.

The placebo MDD's were removed from the control group at the end of each study following the final reading of systolic blood pressure.

RESULTS AND DISCUSSION

***In Vitro* Release of Desoxycorticosterone Acetate from MDD's**—A typical set of data on the *in vitro* release of desoxycorticosterone acetate from an MDD is illustrated in Fig. 1. The cumulated amount, *Q*, of desoxycorticosterone acetate released from a unit surface area of the MDD was linearly proportional to the duration (in days) of elution in the elution solution. Apparently, a constant (zero-order) release profile is achieved using the MDD.

The release profile shown in Fig. 1 was generated from an MDD containing 64.9 mg of desoxycorticosterone acetate/cm³. From the slope of the linear *Q versus t* plot, the rate of release, *Q/t*, of desoxycorticosterone acetate was calculated to be 49.34 μg/cm²/day. The magnitude of the desoxycorticosterone acetate release rates, *Q/t*, was independent of the desoxycorticosterone acetate content in the medicated core matrix of the MDD (Table I). This observation provides additional evidence that release of desoxycorticosterone acetate from MDD's follows a zero-order rate process.

***In Vivo* Release of Desoxycorticosterone Acetate from MDD's in Rats**—Subcutaneous implantation of MDD's in the dorsal thoracic area of rats for various lengths of time also resulted in a constant (zero-order) release of desoxycorticosterone acetate (Fig. 2). This constant release rate was in agreement with the *in vitro* observations. From the slope of the linear *Q versus t* relationship, the *in vivo* release rate, *Q/t*, was computed to be 53.7 μg/cm²/day. This magnitude of the *in vivo* release rate was very close to the value of the *in vitro* release rate (49.3 μg/cm²/day) obtained from the same MDD formulation (MDD 603).

Several MDD formulations were developed to give a range of *in vitro* release rates for desoxycorticosterone acetate. Three were used for the subcutaneous drug release study in rats to establish their *in vitro–in vivo* correlation (Fig. 3). The *in vivo* release rates of desoxycorticosterone acetate were well correlated to their *in vitro* release rates for the corresponding MDD formulations. The establishment of this correlation allows one to predict the mechanisms and rates of long-term drug release in *in vivo* conditions (up to 129 days) on the basis of the data generated from a short-term (8-day) *in vitro* drug release study.

Induction of Prolonged Experimental Hypertension by

[1] Searle Chemicals, Chicago, Ill.
[2] MC/B Manufacturing Chemists, Norwood, Ohio.
[3] Model PE-300, Narco Biosystems, Houston, Tex.

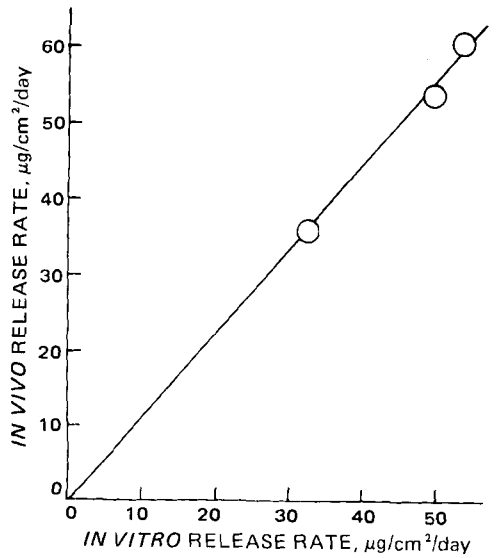

Figure 3—*Linear correlation of* in vivo *with* in vitro *release rates of desoxycorticosterone acetate (64.9 mg/cm³) from three MDD formulations. An* in vivo–in vitro *correlation factor of 1.13 was estimated.*

MDD's—The final objective of this investigation was to examine the possibility of producing a reproducible, prolonged metacorticoid hypertension in experimental animals by continuous administration of a constant, low dose of desoxycorticosterone acetate *via* long-term subcutaneous implantation of an MDD formulation. Figure 4 shows a typical result.

The mean systolic blood pressure of both the treated and control groups increased following implantation of the devices. This increase is normally seen following control subcutaneous injections or implantation in rats maintained on a 1% NaCl fluid ration (4). These effects are thought to occur as a result of the increased sodium intake and the growth of the animals. Blood pressure normally will plateau as the animals reach maturity (as seen in the control group in Fig. 4).

At Day 21, the elevation of systolic blood pressure in the treated rats was significantly greater than that in the control group. This hypertensive state was maintained and sustained up to Day 98. The development of such a prolonged experimental hypertensive animal model should facilitate the routine evaluation program of antihypertensive drugs.

The long-acting hypertensive state (Fig. 4) resulted from the subcutaneous implantation of an MDD, which continuously released to each rat a dose of desoxycorticosterone acetate as low as only 168.1 μg/day for 129 days. This dose level is significantly lower than the minimum effective

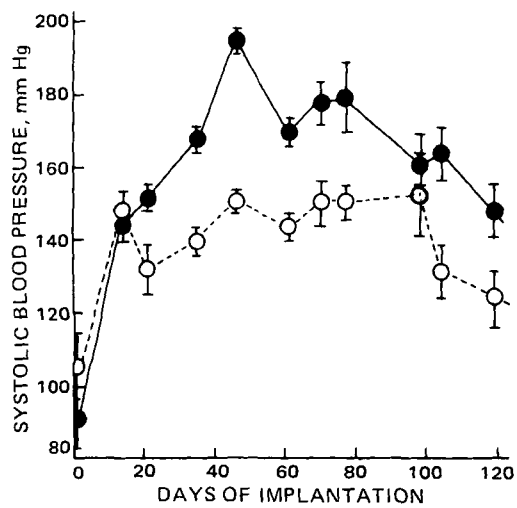

Figure 4—*Time course for the prolonged elevation of systolic blood pressure (mean ± SD) in rats after the long-term subcutaneous implantation of MDD 603-1 (3 cm long). Key: ●, medicated MDD (with an* in vivo *release rate of 168.1 μg/day or 35.9 μg/cm²/day); and ○, placebo MDD. The implants were inserted on Day 0 and removed on Days 59, 70, 104, or 129 after implantation.*

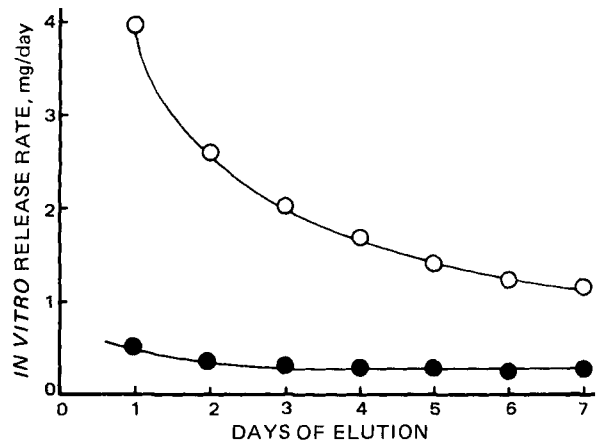

Figure 5—*Comparison of the 7-day* in vitro *release rates of desoxycorticosterone acetate (146.9 mg/cm³) from the matrix-type silicone device (4.845 cm²) (○) and the MDD (4.686 cm²) (●).*

dose of 400 μg/day/rat established earlier by a study of subcutaneous bolus injection (4). This observation indicates that the administration of the MDD with a constant drug release profile optimizes the bioavailability and dose–response relationship of desoxycorticosterone acetate. It also appears that the concept of constant drug availability, or input, substantially minimizes the dose requirement of a treatment.

Comparative Studies between MDD's and Matrix-Type Silicone Device—Recently, the authors examined the possibility of producing a sustained metacorticoid hypertension in rats by the continuous administration of desoxycorticosterone acetate through a 3-cm implant of a matrix-type silicone device subcutaneously implanted in the dorsal thoracic area (4). The dose–response relationship was also investigated. The dose dependency of the differential systolic blood pressure was essentially the same as that exhibited by daily bolus injection, although the modes of administration of desoxycorticosterone acetate between silicone device implantation and daily bolus injection were not identical.

For comparison, the release profiles of desoxycorticosterone acetate from both an MDD and a matrix-type silicone device were investigated under identical conditions. The results of the short-term *in vitro* and long-term *in vivo* release profiles of desoxycorticosterone acetate are compared in Figs. 5 and 6, respectively. The data clearly demonstrated that the release of desoxycorticosterone acetate from the matrix-type silicone device was not constant but was time dependent. Both the *in vitro* and *in vivo* release profiles of desoxycorticosterone acetate were high initially and then decreased with time. This release pattern was best described by a Q *versus* $t^{1/2}$ relationship (4, 6–11). On the other hand,

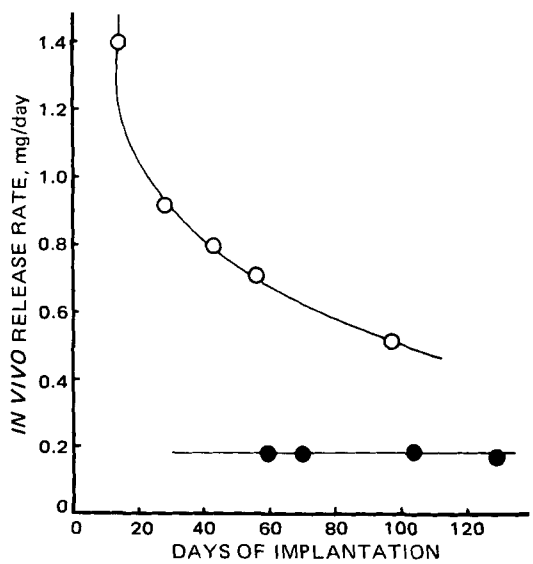

Figure 6—*Comparison of the long-term* in vivo *release rates of desoxycorticosterone acetate (146.9 mg/cm³) from the matrix-type silicone device (4.845 cm²) (○) and the MDD (4.686 cm²) (●).*

Table II—Time Course for the Elevation of Systolic Blood Pressure in Rats following Prolonged Administration of Desoxycorticosterone Acetate *via* Two Drug Delivery Systems

Days	Systolic Blood Pressure, Mean ± *SEM*		
		Treatment Groups	
	Control Group[a]	MSD[b]	MDD[c]
0	105 ± 13	110 ± 8	91 ± 9
14	148 ± 4	149 ± 6	144 ± 4
21	132 ± 7	163 ± 7	152 ± 3
35	140 ± 4	160 ± 5	168 ± 4
46	151 ± 3	210 ± 10	195 ± 3
61	144 ± 4	178 ± 3	170 ± 4
70	151 ± 7	182 ± 4	178 ± 6
77	151 ± 5	197 ± 5	179 ± 10
98	153 ± 12	158 ± 5	161 ± 9
104	132 ± 8	155 ± 5	164 ± 7
119	125 ± 9	160 ± 6	148 ± 7

[a] Each value is a mean (± *SE*) of four measurements in rats implanted with control (placebo) device. [b] Each value is a mean (± *SE*) of five measurements in rats implanted with the matrix-type silicone device (MSD). [c] Each value is a mean (± *SE*) of four measurements in rats implanted with the MDD.

the release rate profiles of desoxycorticosterone acetate from MDD's with identical dimensions were constant and independent of time.

The time courses for the evaluation of systolic blood pressure in rats following the long-term (120-day) administration of desoxycorticosterone acetate *via* MDD's and the matrix-type silicone device are compared in Table II along with the control group. The statistical significance of the difference in systolic blood pressures between treatment groups and the control group as well as between these two treatment groups was analyzed using the Student *t* test (Table III). The systolic blood pressure in both treatment groups was statistically greater than that in the control group after Day 21, with the exception of Day 98.

Table III—Results of Student *t* Statistic

Days	Unpaired *t* Values[a]		
	T-1/C[b]	T-2/C[c]	T-1/T-2
0	0.37	−0.90	1.62
14	0.15	−0.61	0.61
21	3.33*	2.64*	1.45
35	2.76*	4.96**	−1.17
46	5.12**	9.85**	1.26
61	6.55**	4.09**	1.57
70	3.80**	2.84*	0.52
77	6.36**	2.48*	1.78
98	0.41	0.55	−0.35
104	2.38*	2.93*	−1.06
119	3.33*	2.07	1.29

[a] The asterisks shown on the *t* value denote that the difference in the systolic blood pressure between groups is statistically significant: *, $p < 0.05$; and **, $p < 0.01$. [b] T-1 represents the treatment group tested with the matrix-type silicone device. [c] T-2 represents the treatment group treated with the MDD.

The difference in systolic blood pressure between the treatment groups was not significant statistically (Table III), although the daily dose of desoxycorticosterone acetate delivered to each rat by the matrix-type silicone device was substantially higher than that administered by MDD's (Figs. 5 and 6). The results suggested that the dose–response relationship of desoxycorticosterone acetate in the initiation of metacorticoid hypertension was optimized by the constant drug delivery mechanism of MDD's.

REFERENCES

(1) H. C. Stanton and J. B. White, *Arch. Int. Pharmacodyn. Ther.*, **154,** 351 (1965).

(2) S. M. Friedman, M. Nakashima, and C. L. Friedman, *Proc. Soc. Exp. Biol. Med.*, **150,** 171 (1975).

(3) F. M. Sturdevant, *Ann. Intern. Med.*, **49,** 1281 (1958).

(4) Y. W. Chien, L. F. Rozek, and H. J. Lambert, in "Controlled Release Polymeric Formulations," D. R. Paul and F. W. Harris, Eds., ACS Symposium Series, vol. 33, American Chemical Society, Washington, D.C., 1976, pp. 72–86.

(5) H. F. Ormsbee, III, and C. F. Ryan, *J. Pharm Sci.*, **62,** 255 (1973).

(6) Y. W. Chien, S. E. Mares, J. Berg, S. Huber, H. J. Lambert, and K. F. King, *ibid.*, **64,** 1776 (1975).

(7) Y. W. Chien, H. J. Lambert, and D. E. Grant, *ibid.*, **63,** 365 (1974).

(8) Y. W. Chien and H. J. Lambert, *ibid.*, **63,** 515 (1974).

(9) Y. W. Chien, H. J. Lambert, and T. K. Lin, *ibid.*, **64,** 1643 (1975).

(10) Y. W. Chien and E. P. K. Lau, *ibid.*, **65,** 488 (1976).

(11) Y. W. Chien, in "Controlled Release Polymeric Formulations," D. R. Paul and F. W. Harris, Eds., ACS Symposium Series, vol. 33, American Chemical Society, Washington, D.C., 1976, pp. 53–71.

(12) Y. W. Chien and H. J. Lambert, U.S. pat. 3,946,106 (Mar. 23, 1976).

ACKNOWLEDGMENTS

The authors express their appreciation to Ms. D. M. Jefferson and Ms. L. Zeitlin for technical assistance, to Ms. B. Sullivan, Ms. D. Warren, and Ms. M. L. Sachen for manuscript preparation, and to Dr. David T. K. Lin for helpful discussions.