16 Crossover Designs

The principal topic of this chapter is the design and analysis of experiments with different treatments administered in successive periods of time to the experimental units. The analysis of the experiments includes an evaluation of the effect of a treatment that may carry over to affect the response of a treatment in the following period. Designs specifically constructed to efficiently estimate the direct and carryover effects of treatments are presented in this chapter.

16.1 Administer All Treatments to Each Experimental Unit

The **crossover study** describes experiments with treatments administered in sequence to each experimental unit. A treatment is administered to an experimental unit for a specific period of time after which another treatment is administered to the same unit. The treatments are successively administered to the unit until it has received all treatments.

Crossover Designs Can Increase Precision and Reduce Costs

When treatments are compared on the same experimental unit, the between-unit variation is removed from the experimental error. Thus, the individual experimental units are used as blocks in the crossover design to decrease the experimental error and increase efficiency of the experiment. The treatment comparisons in blocked designs are generally more precise than those in unblocked designs because the experimental unit or block variation is removed from comparisons between treatments on the same experimental unit. Thus, the primary advantage of crossover studies is the increased precision of treatment comparisons.

Crossover designs provide an economy of resources when a limited number of units are available for the study. Most commonly, crossover designs are used with human and animal subjects. The expense of maintaining large animals and the difficulties in recruiting adequate numbers of human subjects to achieve sufficient replication make crossover designs more attractive since they require fewer units for an equal amount of treatment replication. The following example of an experiment used a crossover design with beef steers in a feeding trial.

Example 16.1 Digestibility of Feedstuffs in Beef Cattle

Associative effects occur in animal diets when feedstuffs are combined and diet utilization or animal performance is different from that predicted from a sum of the individual ingredients. The addition of roughage to the diets of ruminant animals had been shown to influence various diet utilization factors such as ruminal retention time. However, information about the relative associative effects of different roughage was scarce, especially in mixed feedlot diets.

***Research Hypothesis*:** An animal scientist hypothesized roughage source could influence utilization of mixed diets of beef steers by altering ruminal digestion of other diet ingredients.

***Treatment Design*:** The basic mixed diet for the beef steers was a 65% concentrate based on steam-flaked milo and 35% roughage. Three roughage treatments were used with (A) 35% alfalfa hay as a control treatment, (B) 17.5% wheat straw and 17.5% alfalfa, and (C) 17.5% cottonseed hulls and 17.5% alfalfa.

***Experiment Design*:** Twelve beef steers were available for the study. Each of the three roughage diets was fed to the steers in one of six possible sequences. Each diet in each sequence was fed to two steers for 30 days. The steers were allowed a period of 21 days to adapt to a diet change before any data were collected.

The Neutral Detergent Fiber (NDF) digestion coefficient calculated for each steer on each diet is shown in Table 16.1. The NDF digestion coefficient indicates the percent of dietary fiber digested by the steer.

Table 16.1 NDF digestion coefficients for two steers in each sequence of three roughage diets in a crossover design

	Sequence																	
	1			*2*			*3*			*4*			*5*			*6*		
Steer:		*1*	*2*		*3*	*4*		*5*	*6*		*7*	*8*		*9*	*10*		*11*	*12*
Period I	(A)	50	55	(B)	44	51	(C)	35	41	(A)	54	58	(B)	50	55	(C)	41	46
Period II	(B)	61	63	(C)	42	45	(A)	55	56	(C)	48	51	(A)	57	59	(B)	56	58
Period III	(C)	53	57	(A)	57	59	(B)	47	50	(B)	51	54	(C)	51	55	(A)	58	61

Source: J. Moore, Department of Animal Science, University of Arizona.

Other Examples: A new drug and a standard drug are tested in a crossover design using patients afflicted with acute bronchial asthma to determine whether the new drug improves the patients' breathing over the standard drug. The new drug is administered to a patient during the first week, and the standard drug is administered to the same patient during the second week.

Three front panel designs developed to operate a laboratory instrument are tested in a crossover design with laboratory technicians as operators. The technician operates the instrument with each of the panel designs. Each technician tests the three panel designs on successive days, a different panel each day.

Design to Avoid Confounding Time Period Effects with Treatments

A comparison between two treatments on the same experimental unit is also a comparison between two time periods. Treatments and periods both can contribute to any observed differences. Crossover trials are designed to avoid confounding of period and treatment effects. For example, one group of experimental units will receive the sequence A→B, and a second group of units will receive the sequence B→A. Both treatments are administered in each period, and the treatment comparisons are independent of comparisons between periods.

A Carryover Effect Can Persist After the Treatment Period

A disadvantage of the crossover design is the possibility that a treatment given in one period will influence the response in the following treatment period. Effects of a treatment that continue into the next treatment period are *carryover effects*.

Typically, the treatments are administered in crossover designs for a length of time sufficient to allow the effect of the treatment to be manifested on the subjects. The subject is removed from the treatment for a resting, or washout, period between two treatment periods to bring the subject back to its original physiological or psychological state.

For example, after a drug is administered to a patient the rest period is intended to allow any residual of the drug to "wash out" of the patient's system so that it will not be present in the succeeding treatment period. Although sufficient resting time is allowed for the initial drug to disappear from the system, the physiological state may have been altered sufficiently to have some effect on the responses in the succeeding treatment period. The potential for a carryover effect cannot be ignored in crossover studies.

Since carryover effects are assumed to be present in studies, designs constructed specifically to measure carryover effects will be discussed in this chapter. A variety of designs have been developed for crossover studies to meet specific needs of different research problems. The discussions in this chapter are restricted to a few basic crossover designs to illustrate basic principles for design and analysis of crossover studies to meet specific goals of the research study.

The basic crossover design in Example 16.1 will be used to illustrate the relationship between carryover effects of treatments and other effects present in the

experiment as well as to provide the foundation for the basic statistical model and analysis of crossover studies.

A Balanced Row–Column Design for the Digestibility Study

The crossover design for the roughage diets is a balanced row–column design. The periods and steers are the rows and columns of the design. Each of the roughage diets occurs one time in each steer and four times in each period of the design. The design was referred to as a Latin rectangle design in Chapter 8.

The six sequences of diet treatments are shown in Display 16.1. The six sequences of roughage diets were randomly assigned to the 12 steers. However, the order of diet administration to each of the steers was not randomized. Each of the six sequences of the three treatments must be present with equal frequency to avoid confounding the period effects with the treatment effects and to have a design *balanced* for carryover effects.

Display 16.1 A Crossover Design with Six Treatment Sequences for Three Treatments in Three Periods

	Sequence					
Period	*1*	*2*	*3*	*4*	*5*	*6*
I	A	B	C	A	B	C
	↓	↓	↓	↓	↓	↓
II	B	C	A	C	A	B
	↓	↓	↓	↓	↓	↓
III	C	A	B	B	C	A

Designs to Balance the Carryover Effects

A crossover design is balanced for carryover effects when each treatment follows each of the other treatments an equal number of times. Each treatment occurs equally frequently in each period, and it occurs once with each subject.

The crossover design for the roughage diet study shown in Display 16.1 is a balanced design. Diet A follows diet B twice, once each in sequences 5 and 6; and it follows diet C twice, once each in sequences 2 and 3. Likewise, diets B and C follow each of the other two treatments two times. The balance applies only to first-order carryover effects that alter the response in the first period following administration of the treatment. A second-order carryover effect alters the response in the second period following administration of the treatment.

The 21-day adaptation period in the roughage diet study is equivalent to the washout, or rest period, used in other studies to avoid a carryover effect. In the absence of carryover effects the effects of any previous diet are not manifested in the

digestion physiology of the animal in the current period, and the digestion measurements reflect only the direct effects of the current diet.

The control diet treatment with 35% chopped alfalfa hay was the standard growing diet for feedlot steers. The treatments were designed to address the hypothesis that alternate roughage sources influenced utilization of mixed diets by altering rumen digestion. Diets B and C provided alternate roughage sources of wheat straw or cottonseed hulls mixed with the alfalfa. The remaining parts of the diet including grain and minerals were the same for all diets. If the research hypothesis is true the digestion of dietary fiber will differ among the three treatments.

The three treatment means calculated from Table 16.1 data are, $\overline{y}_A = 56.6$, $\overline{y}_B = 53.3$, and $\overline{y}_C = 47.2$. The observed mean measures the direct effect of a treatment in the periods it was active on the subjects plus the carryover effects of the other two treatments. The direct effects of diet A are not confounded with periods or steers because they were measured in each of the periods and on each of the steers. In the absence of carryover effects $\overline{y}_A = 56.6$ is an unbiased estimate of μ_A in the balanced design. Since diet A followed diet B in two sequences and diet C in two other sequences the response to diet A could be increased or decreased by any carryover effects of diets B or C administered to the steers in the previous periods.

16.2 Analysis of Crossover Designs

The Linear Model for Crossover Designs

The crossover design has n treatment sequence groups and r_i subjects in the ith group. There are t treatments and each group of subjects receives treatments in a different order for p treatment periods. Let y_{ijk} be the observation of the jth subject of the ith treatment sequence in the kth period.

The linear model for a crossover design is

$$y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \tau_{d(i,k)} + \lambda_{c(i,k-1)} + e_{ijk} \qquad \textbf{(16.1)}$$

$$i = 1, 2, \ldots, n \quad j = 1, 2, \ldots, r_i \quad k = 1, 2, \ldots, p \quad d, c = 1, 2, \ldots, t$$

where μ is the general mean, α_i is the effect of the ith treatment sequence, b_{ij} is the random effect with variance σ_b^2 for the jth subject of the ith treatment sequence, γ_k is the period effect, and e_{ijk} is the random error with variance σ^2 for the subject in period k. The direct effect of the treatment administered in period k of sequence group i is $\tau_{d(i,k)}$, and $\lambda_{c(i,k-1)}$ is the carryover effect of the treatment administered in period $k-1$ of sequence group i. The value of the carryover effect for the observed response in the first period is $\lambda_{c(i,0)} = 0$ since there is no carryover effect in the first period.

For simplicity, the direct and carryover effects of the treatments are identified as $\tau_1, \tau_2, \ldots, \tau_t$ and $\lambda_1, \lambda_2, \ldots, \lambda_t$, respectively. The expected values for observations in the first and second treatment sequences of the roughage diet study are with y_{ik} representing sequence i in period k:

Sequence 1 (A→ B→ C)

$$E(y_{11}) = \mu + \alpha_1 + \gamma_1 + \tau_1$$
$$E(y_{12}) = \mu + \alpha_1 + \gamma_2 + \tau_2 + \lambda_1$$
$$E(y_{13}) = \mu + \alpha_1 + \gamma_3 + \tau_3 + \lambda_2$$

Sequence 2 (B→ C→ A)

$$E(y_{21}) = \mu + \alpha_2 + \gamma_1 + \tau_2$$
$$E(y_{22}) = \mu + \alpha_2 + \gamma_2 + \tau_3 + \lambda_2$$
$$E(y_{23}) = \mu + \alpha_2 + \gamma_3 + \tau_1 + \lambda_3$$

Carryover effects occur only in observations from the second and third period. E.g., $\lambda_{c(1,0)} = 0$ in sequence 1 because no treatment precedes diet A. The observations in periods 2 and 3 contain the carryover effects of diet A and diet B, λ_1 and λ_2, respectively. Likewise, for sequence 2 the carryover effects of diets B and C, λ_2 and λ_3, occur in the observations from the second and third periods, respectively.

Assume the Univariate Model Assumption Is Satisfactory

The observations on each experimental unit are repeated measures in time under different treatment conditions. They represent a multivariate observation on the experimental unit. The assumptions required for the univariate analysis of variance were discussed in Chapter 15, "Repeated Measures Designs." Jones & Kenward (1989) and Diggle et al. (1994) discuss details of repeated measures models for crossover designs beyond the scope of this book.

Whether the univariate analysis of variance can be used for the crossover designs depends on the relationships among variances and covariances of the experimental errors for the repeated measures. The univariate analysis can be used if any of the assumptions for independence, compound symmetry, or the Huynh–Feldt condition is appropriate for the experimental errors.

The Huynh–Feldt condition of equal variances for all possible differences between repeated measures is the least restrictive assumption for the experimental errors. It will be assumed the Huynh–Feldt condition is satisfied for the data.

The Analysis of Variance for Crossover Designs

The Analysis Without Carryover Effects

If the crossover design is a balanced row–column design the analysis of variance described in Chapter 8 can be used *in the absence of carryover effects*. The subjects and time periods are the rows and columns of the design, and the direct treatment effects are orthogonal to the rows and columns. The sums of squares for rows, columns, treatments, and experimental error can be computed with the analysis of variance shown in Table 8.10.

The Analysis with Carryover Effects

The analysis of variance for the model with treatment carryover effects in Equation (16.1) is outlined in Table 16.2. The separation of the sums of squares

Table 16.2 Analysis of variance table for a crossover design with n sequences, p periods, t treatments, and r_i subjects in the ith sequence; $N = \sum_i^n r_i$

Source Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*
Total	$Np-1$	SS Total	
Between subjects:			
Sequence	$n-1$	SSS	MSS
Subjects within sequence	$(N-n)$	SSW	MSW
Within subjects:			
Period	$p-1$	SSP	MSP
Treatments (direct)	$t-1$	SST	MST
Treatments (carryover)	$t-1$	SSC	MSC
Error	$(N-1)(p-1)-2(t-1)$	SSE	MSE

partitions into between- and within-subjects groupings indicates the correspondence to a repeated measures split-plot univariate analysis. The subjects are the whole plots and the repeated measures over the p periods are the subplots.

Treatment and Carryover Effects Are Nonorthogonal

When carryover effects are present the direct treatment effects and carryover effects are not orthogonal, nor are the carryover effects orthogonal to the subject blocks in the balanced row–column design. The relationship between the effects can be observed in Display 16.2. The periods and sequences are each complete block designs for the direct treatment effects. The sequences form a balanced incomplete block design for the carryover effects. Each pair of carryover effects occur together with two of the sequences. The direct and carryover treatment effects are not orthogonal because they do not occur in all possible combinations. The direct and carryover effects of the same treatment never occur together in the same observation.

Display 16.2 Model Effects, τ_i and λ_i, for the Roughage Diet Study

	Sequence					
Period	*1*	*2*	*3*	*4*	*5*	*6*
I	τ_1	τ_2	τ_3	τ_1	τ_2	τ_3
II	$\tau_2+\lambda_1$	$\tau_3+\lambda_2$	$\tau_1+\lambda_3$	$\tau_3+\lambda_1$	$\tau_1+\lambda_2$	$\tau_2+\lambda_3$
III	$\tau_3+\lambda_2$	$\tau_1+\lambda_3$	$\tau_2+\lambda_1$	$\tau_2+\lambda_3$	$\tau_3+\lambda_1$	$\tau_1+\lambda_2$

The significance of carryover effects must be determined before inferences can be made about differences among the direct effects of treatments. Estimates of differences between treatment means, $\mu_i - \mu_j$, need to be adjusted for the carryover effects if they are present in the study.

Adjusted Sums of Squares for Nonorthogonal Effects

The sums of squares for direct and carryover treatment effects in the analysis of variance each must be adjusted for the other.

The adjusted sums of squares are computed from the differences between sums of squares for experimental error from full and reduced models. The sum of squares for carryover effects adjusted for direct effects requires the error sums of squares from the models:

$$\begin{aligned} &\text{Reduced model} && y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \tau_d + e_{ijk} \\ &\text{Full model} && y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \tau_d + \lambda_c + e_{ijk} \end{aligned} \qquad \textbf{(16.2)}$$

The sum of squares for carryover effects adjusted for direct effects is $SSC = SSE_{r(\lambda)} - SSE_f$, where $SSE_{r(\lambda)}$ is the error sum of squares computed from the reduced model without λ_c and SSE_f is computed from the full model.

The sum of squares for direct effects adjusted for carryover effects requires the error sums of squares from the models:

$$\begin{aligned} &\text{Reduced model} && y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \lambda_c + e_{ijk} \\ &\text{Full model} && y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \lambda_c + \tau_d + e_{ijk} \end{aligned} \qquad \textbf{(16.3)}$$

The sum of squares for direct effects adjusted for carryover effects is $SST = SSE_{r(\tau)} - SSE_f$, where $SSE_{r(\tau)}$ is the error sum of squares computed from the reduced model without τ_d and SSE_f is computed from the full model.

Data Coding for Computing Programs Requires Special Attention

Many statistical computer programs compute the required sums of squares partitions for balanced or unbalanced designs. Special attention must be given to coding the data file to include the carryover effects in the model. Alternative coding strategies for the carryover effects can be found in Ratkowsky, Alldredge, and Cotton (1990) and Milliken and Johnson (1984). The detailed coding for the data file of Example 16.1 is shown in Appendix 16A.1. Formulae for manual calculations are available for selected balanced designs in Cochran and Cox (1957), Petersen (1985), and Gill (1978). A brief outline of critical formulae for adjusted treatment sums of squares for balanced designs appears in Appendix 16A.2.

The Analysis of Variance for Roughage Diets

The analysis of variance for the roughage diet study is shown in Table 16.3. The two analyses illustrate the alternative fitting of full and reduced models to obtain the adjusted sums of squares for carryover and direct treatment effects.

Table 16.3 Analysis of variance for NDF digestion from the roughage diet study in a crossover design

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Sequence	5	331.67	66.33	3.48	.080
Steers in sequence	6	114.33	19.06		
Period	2	288.17	144.08	16.01	.000
Diet, unadj.	2	559.50	279.75*	31.09	.000
Carryover, adj.	2	18.37	9.19†	1.02	.380
Error	18	161.96	9.00		

*Mean Square for direct treatment effects unadjusted for carryover effects, *MST*(unadj.)
†Mean Square for carryover treatment effects adjusted for direct effects, *MSC*(adj.)

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Sequence	5	331.67	66.33	3.48	.080
Steers in sequence	6	114.33	19.06		
Period	2	288.17	144.08	16.01	.000
Carryover, unadj.	2	129.60	64.80*	7.20	.005
Diet, adj.	2	448.28	224.14†	24.91	.000
Error	18	161.96	9.00		

*Mean Square for carryover treatment effects unadjusted for direct effects, *MSC*(unadj.)
†Mean Square for direct treatment effects adjusted for carryover effects, *MST*(adj.)

The first analysis in Table 16.3 provides the mean square for carryover effects adjusted for direct effects, $MS(\text{Carryover, adj.}) = 9.19$, and the second analysis provides the mean square for direct treatment effects adjusted for carryover effects, $MS(\text{Diet, adj.}) = 224.14$. The differences between the adjusted and unadjusted mean squares reflect the nonorthogonal relationship between the carryover effects and direct effects of treatments. The unadjusted mean square for direct treatment effects in the first analysis is $MS(\text{Diet, unadj.}) = 279.75$, and the unadjusted mean square for carryover effects in the second analysis is $MS(\text{Carryover, unadj.}) = 64.80$.

Tests of Hypotheses for Direct and Carryover Effects of Treatments

The null hypothesis for carryover effects is H_0: $\lambda_1 = \lambda_2 = \lambda_3 = 0$. It is the hypothesis of initial interest for the study because inferences about the direct treatment effects are dependent on the presence of carryover effects. The test statistic for the hypothesis of no carryover effects is

$$F_0 = \frac{MS(\text{Carryover, adj.})}{MSE} = \frac{9.19}{9.00} = 1.02$$

with 2 and 18 degrees of freedom. The level of significance for the test is $Pr > F = .380$, and the null hypothesis is not rejected. The carryover effects are not significant in the roughage diet study.

The statistic $F_0 = MS(\text{Diet, adj.})/MSE = 224.14/9.00 = 24.90$ with 2 and 18 degrees of freedom tests the null hypothesis for direct treatment effects. The null hypothesis is rejected with significance level $Pr > F = .000$. There are significant differences in NDF digestion among the three roughage diets.

The significance of carryover effects unadjusted for direct treatment effects in the second analysis of variance in Table 16.3 again reflects the nonorthogonal relationship between direct and carryover effects. The correlation between their estimates leads to difficulties in distinguishing between their separate contributions. The sum of squares for carryover effects unadjusted for direct effects,

$$SS(\text{Carryover, unadj.}) = 129.60$$

is considerably larger than the adjusted sum of squares,

$$SS(\text{Carryover, adj.}) = 18.37$$

because the direct effects are intermingled with the carryover effects in the unadjusted sum of squares. Consequently, the significance of the unadjusted mean square is due to a combination of variation in direct and carryover effects, and it is not possible to determine which set of effects is contributing to the significance.

If the carryover effects are not significant it is common practice to base all inferences on the treatment means unadjusted for carryover effects. The analysis for Latin square designs in Chapter 8 would be used in this case. However, a detailed study by Abeyasekera and Curnow (1984) on the consequences of ignoring any adjustments for carryover effects led them to recommend always adjusting the treatment means for carryover effects regardless of the significance of the test for carryover effects.

Interpretation of Treatments with Multiple Contrasts

According to the research hypothesis the rumen digestion efficiency is dependent on the source of roughage. The standard or control diet A included 35% chopped alfalfa hay. The altered diets had half that amount of alfalfa and 17.5% wheat straw (diet B) or 17.5% cottonseed hulls (diet C). The null hypothesis of no change in NDF digestion by the altered diets can be tested with comparisons of the control

diet with the altered diets using the Dunnett method. The estimates of the contrasts between the adjusted means of altered diets and diet A, $\widehat{\mu}_A - \widehat{\mu}_B$ and $\widehat{\mu}_A - \widehat{\mu}_C$, are shown in Table 16.4, along with the 95% simultaneous confidence intervals for comparisons of control with other treatments using the Dunnett method.

Table 16.4 Estimates of contrasts between the control and altered diets for the roughage diet study in a crossover design

Contrast	*Estimate*	*Standard Error*	*95% SCI* (L, U)
Diet A–Diet B	4.06	1.37	(0.77, 7.35)
Diet A–Diet C	9.63	1.37	(6.34, 12.92)

The estimated standard error of the difference between two adjusted means is 1.37 in the third column of Table 16.4. The two-sided Dunnett statistic for two comparisons is $d_{.05,2,18} = 2.40$. The 95% SCI indicate that the replacement of half the alfalfa hay by cottonseed hulls (diet C) in the diet reduced the NDF digestion to a greater extent than replacement by wheat straw (diet B) since the lower limit of the interval for diet C is further removed from 0 than is that for diet B.

The critical value for a confident inequalities test with the Dunnett method is $|\widehat{\mu}_A - \widehat{\mu}_i| > D(2, .05) = 1.37(2.40) = 3.28$. The difference for diet A versus diet B, 4.06, and the difference for diet A versus diet C, 9.63, are both significant because they exceed the critical value. The replacement of half the alfalfa hay by cottonseed hulls (diet C) in the diet reduces the NDF digestion by an estimated 9.63%, and the replacement by wheat straw (diet B) reduces the NDF digestion by an estimated 4.06%.

16.3 Balanced Designs for Crossover Studies

Practical considerations dictate whether a crossover study is appropriate for the research problem. The designs are most effective when treatments manifest an effect on the subject in a reasonably short period of time to provide a study time of manageable length. Extensive discussions of crossover studies can be found in Jones and Kenward (1989) and Ratkowsky, Evans, and Alldredge (1993).

When Crossover Designs Can Be Successful

Studies that measure responses to changes in animal diets have been successfully completed with crossover designs. Crossover designs reduce the costs of maintaining the larger numbers of animals required to provide equivalent treatment replication with other designs. The cost savings are especially pertinent in large animal studies. Human factors studies can use crossover designs to remove subject- to-subject variability in stress or anxiety responses to treatments. Differential

physiological or psychological stresses are measured readily on each subject in experiments designed to compare different systems of component assembly or instrument operation with each subject.

When Crossover Designs May Not Be Successful

The designs should not be used in medical clinical trials for acute conditions such as postoperative pain. If a treatment in the sequence cures the acute condition nothing remains to treat in the succeeding periods. The designs can be used effectively to study the treatment of persistent conditions such as arthritis when treatments alleviate the symptoms rather than cure when administered to the individual.

Practical Considerations Influence Design Choice

Other practical considerations influence the choice of a design if the crossover study is feasible. The likelihood of subject loss during the course of the study increases with the number of periods. The more desirable designs, those balanced for carryover effects, increase in complexity as more treatments are added to the study. Consequently, the correct order of treatment administration to subjects becomes more difficult to manage in large studies. The ability to interpret results can diminish with the complexity of the design.

Many different designs have been developed for crossover studies to meet specific requirements for the practical research problem or to have good statistical properties. Three general categories of designs introduced in this section are useful for a variety of research studies that can appropriately use crossover studies. They are restricted to balanced designs for three or more treatments and three or more periods. Two-period designs are not efficient for estimation of treatment means. Designs for two treatments require special orchestration of treatment sequences to estimate all of the effects of interest in the study. Designs for two treatments are discussed in a separate section.

The categories of designs discussed in the remainder of this section are based on the relationship between the number of treatments t and the number of periods p. They are designs with $p = t, p = t + 1$, and $p < t$.

Compare Efficiency of Crossover Designs with Latin Squares

The efficiency of a design for statistical estimation of direct and carryover effects is an important consideration in the choice of design. The variance of the difference between two treatment effects, σ_d^2, forms the basis for efficiency measures. The Latin square design, in the absence of carryover effects, provides the minimum variance, $2\sigma^2/r$, and is the standard for comparison. The ratio of the variance for the Latin square to the crossover design measures the relative efficiency of the crossover design as

$$RE = \frac{2\sigma^2/r}{\sigma_d^2} \times 100 \qquad \textbf{(16.4)}$$

The relative efficiency measure is applied to either direct or crossover effects. The variance of the difference, σ_d^2, pertains to either treatment effect adjusted for the other.

The most desirable designs have variance balance where the variance of the difference between two treatment effects, direct or carryover, is the same for all treatment pairs. The balance defined by the relationship between direct and carryover effects is the other desirable feature of a design. The design is balanced if the direct effect of each treatment is associated equally frequently with the first-order carryover effect of each other treatment. Patterson and Lucas (1962) presented some general formulae to compute efficiencies for balanced designs.

Designs for Equal Numbers of Periods and Treatments, $p = t$

The Williams Designs Constructed from Latin Squares

Williams (1949) presented methods to construct balanced crossover designs from Latin square arrangements. If the number of treatments is even, $t = 4, 6, \ldots$, a balanced design can be constructed from one particular Latin square. Two particular squares are required if the number of treatments is an odd number, $t = 3, 5, 7, \ldots$.

Two 3×3 Latin squares that produced six treatment sequences were used for the design of the roughage diet study of Example 16.1. The design is reproduced in Display 16.3 showing the two 3×3 Latin squares. One Latin square was used for the first three sequences of treatments in the experiment, while the second square was used for the last three sequences.

Display 16.3 A Balanced Crossover Design from Two 3×3 Latin Squares

	Square 1			*Square 2*		
Period	*1*	*2*	*3*	*4*	*5*	*6*
I	A	B	C	A	B	C
	↓	↓	↓	↓	↓	↓
II	B	C	A	C	A	B
	↓	↓	↓	↓	↓	↓
III	C	A	B	B	C	A
	$E_d = 80.0$			$E_c = 44.44$		

Williams designs for $t = 4$, 5, and 6 treatments and balanced for carryover effects are shown in Display 16.4. Each treatment follows each of the other treatments one time in the designs with one Latin square for an even number of treatments, and the direct effects of each treatment associate once with the first-order carryover effects of all other treatments. For the designs with an odd number of treatments each treatment follows the others twice, and the direct effects of each treatment associate twice with the first-order carryover effect of all other treatments. The relative efficiency of the Williams designs for direct effects E_d and the relative efficiency E_c for carryover effects are shown below each of the designs in Displays 16.3 and 16.4.

Display 16.4 Balanced Latin Square Crossover Designs for Four, Five, or Six Treatments

Four Treatments

	Sequence Group			
Period	*1*	*2*	*3*	*4*
I	A	B	C	D
II	D	A	B	C
III	B	C	D	A
IV	C	D	A	B
	$E_d = 90.91$		$E_c = 62.50$	

Six Treatments

	Sequence Group					
Period	*1*	*2*	*3*	*4*	*5*	*6*
I	A	B	C	D	E	F
II	C	D	E	F	A	B
III	B	C	D	E	F	A
IV	E	F	A	B	C	D
V	F	A	B	C	D	E
VI	D	E	F	A	B	C
	$E_d = 96.55$		$E_c = 77.78$			

Five Treatments

	Sequence Group 1					*Sequence Group 2*				
Period	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
I	A	B	C	D	E	A	B	C	D	E
II	B	C	D	E	A	C	D	E	A	B
III	D	E	A	B	C	B	C	D	E	A
IV	E	A	B	C	D	E	A	B	C	D
V	C	D	E	A	B	D	E	A	B	C
				$E_d = 94.74$		$E_c = 72.00$				

Designs from Orthogonal Sets of Latin Squares

A complete set of orthogonal Latin squares provides a crossover design balanced for all orders of carryover effects. An orthogonal set of Latin squares for t treatments requires $t - 1$ squares. Two Latin squares are orthogonal when the two squares are superimposed and each treatment of one square occurs once with each treatment of the other square.

Two orthogonal 3×3 Latin squares were used for the roughage diet study. They make up the Williams design shown in Display 16.3.

Note when square 1 is imposed over square 2 that the treatment label A in square 1 will occur one time each with treatment labels A, B, and C in square 2.

The same occurrence pattern is true of the labels B and C from square 1 when superimposed on square 2.

The complete set of orthogonal squares requires $t(t-1)$ subjects for any number of treatments. For example, the orthogonal set of three Latin squares for four treatments requires 12 subject groups.

The efficiency of the design for estimating direct and carryover effects from the complete set of orthogonal squares is the same as that for the Williams designs. The Williams designs require only $t-1$ subjects for t even and $2(t-1)$ subjects for t odd, thus the only real advantage of a complete orthogonal set of Latin square designs over the Williams designs is that the complete set is balanced for all orders of carryover effects up to order $(p-1)$. For example, the design with four treatments is balanced for first-, second-, and third-order carryover effects.

When only first-order carryover effects are present in the study the Williams designs have a clear savings in number of treatment sequences to maintain with more than three treatments.

Extra-Period Designs for Orthogonal Direct and Carryover Effects, $p = t+1$

The direct and carryover effects are not orthogonal in designs where the number of periods equals the number of treatments. Lucas (1957) pointed out that the nonorthogonality of the balanced designs resulted in much lower efficiency for estimating carryover effects than for estimating direct effects of treatments. The differences in efficiencies for estimating the two effects can be seen in Display 16.4. The addition of an extra period to the balanced design can remove the nonorthogonality and provide designs with independent estimates of direct and carryover effects that have more equal precision for estimates of direct and carryover effects. Lucas (1957) provided the first formal description of extra-period crossover designs.

The simplest extra-period design is derived from a balanced crossover design by repeating in period $(p+1)$ the treatment administered to the subject in period p. An extra-period design for $t = 4$ treatments in $p = 5$ periods derived from the Williams design for four treatments is shown in Display 16.5.

Each treatment administered to a subject in period IV is repeated on the same subject in period V. Each treatment is preceded by each of the other treatments in the design including itself. For example, treatment A is preceded by itself in sequence 3, by B in sequence 2, by C in sequence 4, and by D in sequence 3.

The extra-period Williams design is completely balanced for carryover effects since each carryover effect occurs an equal number of times in each sequence and with each direct effect. Thus, the estimates of the direct effects are the same whether or not the carryover effects are in the model, and vice versa. The addition of an extra period makes the design unbalanced with respect to sequences and direct effects because treatments appear an unequal number of times within each sequence. Note in Display 16.5 that one of the treatments appears two times in a sequence while each of the other treatments appears only once in the same sequence.

The efficiencies for orthogonal sets of Latin squares with one extra period are the same as those for the Williams designs with one extra period. The advantage of

Display 16.5 Extra-Period Crossover Design for Four Treatments

	Sequence Group			
Period	*1*	*2*	*3*	*4*
I	A	B	C	D
	↓	↓	↓	↓
II	D	A	B	C
	↓	↓	↓	↓
III	B	C	D	A
	↓	↓	↓	↓
IV	C	D	A	B
	↓	↓	↓	↓
V	C	D	A	B

$E_d = 96.00$ $E_c = 80.00$

increasing the precision for estimating carryover effects must be weighed against the disadvantage of extending the length and cost of the experiment with the extra period. The designs should only be used if there is a strong indication that the carryover effects are likely to occur in the study. Some bias in the responses can occur if either the subjects or the investigators are aware that the last two measurement periods involve the same treatment.

Designs for Less than a Full Treatment Cycle, $p < t$

Each subject received all of the treatments in the balanced designs discussed in the previous sections. Practical or ethical considerations can prevent adminstration of the full cycle of treatments to each of the subjects. It may be more practical to administer only part of the treatments to each subject to reduce the chances of subjects dropping out of the study. Ethical considerations may prevent the investigator from administering too many treatments to patients in a clinical trial.

Patterson (1951, 1952) described methods to construct balanced designs for studies that required the number of periods to be less than the number of treatments, $p < t$. The subjects are incomplete blocks in the design because they receive less than the full complement of treatments. The incomplete designs may be derived by deleting one or more periods from a complete set of orthogonal Latin squares. The incomplete designs also may be obtained from the incomplete Latin squares or Youden squares discussed in Chapter 9 for row–column designs. Finally, they can be constructed from regular balanced incomplete block designs. For illustration, a design for seven treatments and four periods is shown in Display 16.6.

Display 16.6 Incomplete Design for Seven Treatments and Four Periods

	Sequence						
Period	*1*	*2*	*3*	*4*	*5*	*6*	*7*
1	A	B	C	D	E	F	G
2	B	C	D	E	F	G	A
3	D	E	F	G	A	B	C
4	G	A	B	C	D	E	F

	Sequence						
Period	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1	A	B	C	D	E	F	G
2	G	A	B	C	D	E	F
3	E	F	G	A	B	C	D
4	B	C	D	E	F	G	A

Tables of incomplete Latin square designs for crossover studies can be found in Petersen (1985) and Patterson and Lucas (1962). Jones and Kenward (1989) listed the most efficient incomplete designs for $t = 3, 4, 5, 6$, and 7 treatments.

16.4 Crossover Designs for Two Treatments

Perhaps the most widely used design for crossover studies has been the two-treatment, two-sequence, two-period design. One group receives the treatment sequence A→B and another group receives the treatment sequence B→A, as shown in Display 16.7. Each of the treatments appears in each sequence and in each period of the 2×2 Latin square arrangement. Thus, the treatment effects are not confounded with the effects of sequences or periods, the row and column equivalents of the Latin square design.

Display 16.7 The 2×2 Crossover Design

	Period		
Sequence	I		II
1	A	→	B
2	B	→	A

The Linear Model for Two Treatments

The linear model for the design with two treatments is

$$y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \tau_d + \lambda_c + e_{ijk} \qquad (16.5)$$
$$i, k, d, c = 1, 2 \quad j = 1, 2, \ldots, r_i$$

where μ is the general mean, α_i is the sequence effect, b_{ij} is the random subject effect with mean 0 and variance σ_b^2, γ_k is the period effect, τ_d is the direct treatment effect, λ_c is the carryover effect, and e_{ijk} is the independent, random error with mean 0 and variance σ^2.

Carryover Effects Confounded in the 2×2 Design

The 2×2 crossover design has one major deficiency. The carryover effects are confounded with other effects in the study, and the significance of the carryover effects cannot be tested from an analysis of the model in Equation (16.5). The observed means for the subjects in each period of each sequence of the design, $\overline{y}_{11}$, $\overline{y}_{12}, \overline{y}_{21}, \overline{y}_{22}$, contain all of the information available in the study to estimate the design factor effects for sequences and periods and the direct and carryover treatment effects. The expected values of the cell means, $E(\overline{y}_{ik}) = \mu_{ik} = \mu + \alpha_i + \gamma_k + \tau_d + \lambda_c$, are shown in Display 16.8.

Display 16.8 Expected Cell Means for the 2×2 Crossover Design

	Expected Cell Mean, μ_{ij}	
Sequence	*Period I*	*Period II*
A→B	$\mu + \alpha_1 + \gamma_1 + \tau_1$	$\mu + \alpha_1 + \gamma_2 + \tau_2 + \lambda_1$
B→A	$\mu + \alpha_2 + \gamma_1 + \tau_2$	$\mu + \alpha_2 + \gamma_2 + \tau_1 + \lambda_2$

Simplified expectations follow upon application of the relationship $\tau_1 + \tau_2 = 0$ based on our definition of treatment effects. The sum of the two treatment effects is 0 so that $\tau_2 = -\tau_1$ and $-\tau_1$ can be substituted for τ_2 in the expectations. Similar substitutions can be made for the other factor effects. Given the substitutions $\alpha_2 = -\alpha_1$, $\gamma_2 = -\gamma_1$, and $\lambda_2 = -\lambda_1$, the expectations are

$$E(\overline{y}_{11}) = \mu_{11} = \mu + \alpha_1 + \gamma_1 + \tau_1$$
$$E(\overline{y}_{12}) = \mu_{12} = \mu + \alpha_1 - \gamma_1 - \tau_1 + \lambda_1$$
$$E(\overline{y}_{21}) = \mu_{21} = \mu - \alpha_1 + \gamma_1 - \tau_1$$
$$E(\overline{y}_{22}) = \mu_{22} = \mu - \alpha_1 - \gamma_1 + \tau_1 - \lambda_1$$

The five parameters to be estimated are μ, α_1, γ_1, τ_1, and λ_1, and only four observed means are available for estimation of the parameters. It is not possible to obtain estimates of parameters for some of the effects without involving the parameters of other effects. In particular, it is not possible to disentangle the sequence effect α_1 or the direct treatment effect τ_1 from the carryover effect λ_1 in the analysis. If one of the parameters can be eliminated from the model, the remaining parameters can be estimated from the four cell means.

Eliminate Some Model Parameters to Estimate Effects

A number of alternative models and methods of analysis have been proposed for the 2×2 crossover design (Grizzle, 1965, 1974; Hills & Armitage, 1979; Milliken & Johnson, 1984). Regardless of the model one parameter has to be eliminated to estimate the remaining parameters and test hypotheses about them. Under certain circumstances it may be possible to provide arguments for the elimination of sequence or period effects from the model. Eliminating either of those parameters puts the analysis at risk because inferences are made under the generous assumption of either no period or no sequence effect. Effects are left in the model because it is necessary to test their significance in the study and not because it is believed they are actually present in the study.

The test of significance for carryover effects from the 2×2 crossover design is possible only under the questionable assumption that some other effect is nonexistent. Under these circumstances the design should not be used. Jones and Kenward (1989) consider the design to be appropriate if the sequence, period, and carryover effects are negligible in size relative to the direct treatment effect. On the other hand, a panel for the U.S. Food and Drug Administration has recommended the design not be used in drug evaluation.

A Little Help from Baseline and Washout Observations

Adjuncts to the 2×2 crossover design reviewed in detail by Jones and Kenward (1989) include the addition of baseline observations and washout period observations. The baseline observations taken prior to any treatment administration enable the estimation of carryover effects and the test for their significance. However, the direct and carryover effects are still nonorthogonal in the design and the estimates of the direct and carryover effects are highly correlated, $\rho = 0.87(\sigma^2/n)$. Although the two effects are no longer confounded, the high correlation between their estimates leads to difficulties in distinguishing between their separate contributions. When estimates of effects are highly correlated, contradictory tests of significance can occur in the analysis. One effect may be nonsignificant when a second effect is in the model but significant when the second effect is removed from the model.

The addition of observations on the washout period between the two-treatment period reduces the correlation between estimates of the two effects to $\rho = 0.50(\sigma^2/n)$. Although the correlation is reduced, substantial nonorthogonality remains between the direct and carryover effects. The estimates are uncorrelated if observations are included from a second washout period after the second treatment is terminated. Thus, a completely orthogonal design for two treatments is only possible with added baseline and washout data that require a total of five measurement periods.

Add an Extra Period to Estimate Carryover Effects

The other adjuncts for the 2×2 crossover design reviewed by Jones and Kenward (1989) included designs for the two treatments administered in various combinations of sequences and periods. The addition of extra sequences or extra periods of treatment increases the ability to estimate the important effects in the crossover study. Two of the designs for two sequences in three periods will be discussed in this section.

The *switchback* design for two treatments, shown in Display 16.9, has been used frequently as an adjunct to the 2×2 design to obtain estimates of the direct and carryover effects. However, the *extra-period* design for two treatments, also shown in Display 16.9, is a better design for the estimation of the treatment effects.

Display 16.9 Switchback and Extra Period Designs for Two Treatments

	Switchback Period				*Extra Period*			
Sequence	*I*	*II*	*III*	*Sequence*	*I*	*II*	*III*
1	A	B	A	1	A	B	B
2	B	A	B	2	B	A	A

The first two periods of both designs provide the basic 2×2 crossover design, but the designs differ in the treatment administered during the third period. The third period treatment reverts to the first period treatment in the switchback design, whereas the second period treatment is repeated in the third period of the extra-period design.

Both designs have six cell means for the estimation of design and treatment factor effects, and the analysis of variance in Table 16.2 based on the model in Equation (16.5) can be used for the sum of squares partitions. An analysis of either design provides valid tests for all of the effects in the model.

Extra-Period Design Superior to the Switchback Design

Recall from the previous section on balanced designs that direct and carryover treatment effects are orthogonal in the extra-period design so that the estimates of direct and carryover treatment effects are uncorrelated. The effects are not orthogonal in the switchback design, and the correlation between the direct and carryover effects is $\rho = 0.87(\sigma^2/n)$.

The additional superiority of the extra-period design resides in its greater efficiency for the estimation of direct and carryover treatment effects. The variances of the estimates for direct and carryover effects in the extra-period design are

$$\sigma^2_{\hat{\tau}} = 0.19\left(\frac{\sigma^2}{n}\right) \quad \text{and} \quad \sigma^2_{\hat{\lambda}} = 0.25\left(\frac{\sigma^2}{n}\right)$$

whereas the variances for the effects in the switchback design are

$$\sigma^2_{\hat{\tau}} = 0.75\left(\frac{\sigma^2}{n}\right) \quad \text{and} \quad \sigma^2_{\hat{\lambda}} = 1.0\left(\frac{\sigma^2}{n}\right)$$

The statistical properties of the extra-period design are superior to those of the switchback design since the estimates of the direct and carryover treatment effects are uncorrelated and the variances of the estimates are smaller.

The choice of design to this point has focused on estimation and significance tests for the primary design and treatment factors in the crossover design: the sequence, period, direct treatment, and carryover treatment effects. Other designs can be constructed to facilitate the estimation of additional effects that are potentially important in some studies. These effects include interactions between the direct and carryover treatment effects and interactions between the treatment effects and the design factors such as periods and sequences. Discussions on designs for the estimation and tests for these additional model effects can be found in Jones and Kenward (1989).

EXERCISES FOR CHAPTER 16

1. A pharmaceutical manufacturer conducted a crossover study with an anticonvulsant drug (DPH) used in the management of grand mal and psychomotor seizures. A single dose of DPH was given to a subject, and the plasma level of the drug was measured 12 hours after the drug was administered. The four treatments were (A) 100 mg generic DPH product in solution, (B) 100 mg manufacturer DPH in capsule, (C) 100 mg generic DPH product in capsule, and (D) 300 mg manufacturer DPH in capsule.

   A 4×4 Latin square crossover design was used for the study. Two subjects were assigned to each of the four treatment sequences. A single dose of each treatment was administered to each subject with an interval of two weeks between treatments. The subject's plasma levels of DPH are shown in the table at the end of the exercise.

   a. Is the design balanced for carryover effects? Explain.
   b. Write a linear model for the study, explain the terms, and state the assumptions necessary for the analysis.
   c. Compute the analysis of variance for the data, and test the significance of the direct and carryover effects.
   d. The manufacturer wanted to know if the bioavailability of DPH in the capsule form was as high as that for the liquid form, whether the bioavailability of the generic product was equivalent to their product, and whether there was a dosage effect (300 mg versus 100 mg) on the bioavailability. Devise a meaningful set of contrasts among the four treatments, test their significance, and interpret the results.
   e. Obtain the residual plots for the analysis, and interpret them.

Sequence	Subject	Period I	Period II	Period III	Period IV
ABDC	1	2.3	1.7	1.8	4.5
	2	1.0	1.2	1.1	3.3
BCAD	3	1.9	4.4	1.4	1.5
	4	0.9	2.1	1.0	0.8
CDBA	5	3.2	0.8	0.8	0.9
	6	2.2	0.8	0.9	0.8
DACB	7	1.4	1.2	3.6	1.1
	8	0.9	0.8	2.9	0.8

Source: K. S. Albert et al. (1974), Bioavailability of diphenylhydantoin. *Clinical Pharmacology and Therapeutics*, 16, 727–735.

2. A crossover design was used to compare drugs for the control of hypertension. Two drugs, A and B, were used alone and in combination. The combination of the two drugs was labeled as drug C in the experiment. Subjects were randomly assigned to one of six sequences of the drug treatments. Each treatment period lasted four weeks with no washout period between treatments. The systolic blood pressure of the subjects measured at the end of each period is shown in the table at the top of the next page.
   a. Is the design balanced for carryover effects? Explain.
   b. Write a linear model for the study, explain the terms, and state the assumptions necessary for the analysis.
   c. Compute the analysis of variance for the data, and test the significance of the direct and carryover effects.
   d. Compute the contrasts between drug C (the combination of A and B) and drugs A and B separately along with the standard error. Is the systolic blood pressure for the combination of the two drugs significantly different from that of the two drugs alone? Do drugs A and B differ significantly with respect to systolic blood pressure?
   e. Obtain the residual plots for the analysis, and interpret them.

3. A digestion trial with beef steers was conducted in an extra period Latin square crossover design to evaluate the effects of low-quality roughage on feed digestion. The low-quality roughages used in the trial were (A) cottonseed hull, (B) bermuda straw, and (C) wheat straw, and the high-quality roughage used as a control was (D) alfalfa hay. One steer was randomly assigned to each sequence of four diets. The steer remained on each diet for 30 days and measurements on dry matter digestion were made during the last week of the trial allowing a 21-day adjustment to each diet. The

		Period		
Sequence	*Subject*	*I*	*II*	*III*
ABC	1	174	146	164
	2	145	125	130
	3	230	174	200
	4	240	130	195
ACB	5	192	150	160
	6	194	208	160
	7	175	152	175
	8	202	160	180
BAC	9	184	192	176
	10	140	150	150
	11	155	230	226
	12	180	185	190
BCA	13	136	132	138
	14	145	154	166
	15	194	210	190
	16	180	180	190
CAB	17	206	220	210
	18	160	180	145
	19	188	200	190
	20	185	197	182
CBA	21	180	180	208
	22	210	160	226
	23	185	180	200
	24	190	145	160

Source: B. J. Jones and M. G. Kenward (1989), *Design and Analysis of Cross-Over Trials*. London: Chapman Hall.

roughage diet fed in the fourth period was repeated during the fifth period. The data on dry matter digestion for each steer in each sequence are shown in the table.

	Period				
Steer	*I*	*II*	*III*	*IV*	*V*
1	75(A)	76(B)	79(C)	81(D)	79(D)
2	79(C)	73(A)	79(D)	75(B)	77(B)
3	81(D)	79(C)	75(B)	72(A)	73(A)
4	76(B)	79(D)	72(A)	76(C)	73(C)

a. Is the design balanced for carryover effects? Explain.
b. Is there an advantage to this design over a four-period design? Explain.
c. The linear model for this extra-period crossover design is

$$y_{ij} = \mu + \alpha_i + \gamma_j + \tau_d + \lambda_c + e_{ij}$$

$$i = 1, 2, \ldots, 4 \quad j = 1, 2, \ldots, 5 \quad d, c = 1, 2, \ldots, 4$$

where μ is the general mean, α_i is the steer sequence effect, γ_j is the period effect, τ_d is the direct treatment effect, λ_c is the carryover effect, and e_{ij} is the normally distributed independent random error with variance σ^2.
d. Compute the analysis of variance for the data. Make the computations with your program using a sequential fit of carryover and direct effects. Notice the sums of squares for direct and carryover effects are the same regardless of their order with respect to one another in the sequential fit of terms. Why is this so?
e. Test the significance of the direct and carryover effects.
f. Use the Dunnett method to compare the control diet, alfalfa hay, with each of the low-roughage diets, and interpret the results.
g. Obtain the residual plots for the analysis and interpret them.

4. An extra-period crossover design was used to compare two drugs for the control of hypertension. Subjects were randomly assigned to one of two sequences of the drug treatments. Each treatment period lasted six weeks with a one-week washout period between treatments. The diastolic blood pressures of the subjects measured at the end of each period are shown in the table.

		Period		
Sequence	*Subject*	*I*	*II*	*III*
ABB	1	73	92	75
	2	90	90	80
	3	95	75	75
	4	80	80	90
	5	90	90	70
	6	45	60	45
	7	70	60	80
	8	122	101	90
BAA	9	60	70	100
	10	100	85	80
	11	50	45	70
	12	65	70	70
	13	88	88	88
	14	70	70	80
	15	60	90	70

a. Is the design balanced for carryover effects? Explain.

b. Is there an advantage to this design over a simple two-period design without using the third period as shown in the data table? Explain.
c. Write a linear model for the study, explain the terms, and state the assumptions necessary for the analysis.
d. Compute the analysis of variance for the data, and test the significance of the direct and carryover effects.
e. Compute the contrast between drugs A and B along with the standard error. Do drugs A and B differ significantly with respect to diastolic blood pressure?
f. Obtain the residual plots for the analysis, and interpret them.

5. A crossover study was conducted to evaluate four keyboard layouts. Twelve volunteers experienced in a common keyboard configuration were used in the study. Each subject used the four test layouts in sequence. Each subject was randomly assigned to a sequence of layouts. Each layout was used for four days in their ordinary data and text entry activities. On the fifth day they were all given a common task to perform with their assigned layout, and the number of errors on the task were recorded. None of the subjects knew they were being tested on the final day. The number of errors recorded for each subject on each layout are shown in the table.

	Period			
Subject	*I*	*II*	*III*	*IV*
1	7(D)	2(B)	1(A)	5(C)
2	1(A)	4(C)	6(D)	3(B)
3	6(C)	1(A)	3(B)	7(D)
4	3(B)	6(D)	3(C)	1(A)
5	4(C)	5(D)	1(A)	2(B)
6	6(D)	4(C)	2(B)	0(A)
7	1(A)	3(B)	4(C)	5(D)
8	2(B)	2(A)	7(D)	4(C)
9	5(D)	0(A)	3(C)	3(B)
10	0(A)	4(D)	2(B)	3(C)
11	3(C)	2(B)	7(D)	0(A)
12	2(B)	4(C)	0(A)	6(D)

a. Is the design balanced for carryover effects? Explain.
b. Write a linear model for the study, explain the terms, and state the assumptions necessary for the analysis. Do you think an assumption of normal distribution for the observations is valid for this study? Explain.
c. Compute the analysis of variance for the data, and test the significance of the direct and carryover effects.
d. Obtain the residual plots for the analysis, and interpret them. Do you think a transformation of the data will improve the analysis? If so, which transformation might be appropriate?

16A.1 Appendix: Coding Data Files for Crossover Studies

Carryover Effects Coded as Factor Levels

This coding scheme was suggested for the GLM program in SAS by Ratkowsky, Alldredge, and Cotton (1990). The correct sum of squares partitions are produced also by the MANOVA program in SPSS with this coding scheme. The estimates of the adjusted treatment means may differ by some constant amount between programs because of their differing computing algorithms. However, estimates of contrasts among the treatment means and standard errors for the contrasts will be consistent among the programs. Some programs will not complete the analysis with this coding scheme.

The basic codes for levels of a factor in a data file ordinarily are a sequence of integers, $1, 2, 3, \ldots$. A code for the level of each factor is required for each observation in the data file. However, the carryover effects do not occur with observations in the first period of the crossover study, and there is no "level" to code for carryover effects. Some programs will allow a code for the factor even though the factor does not occur for the observation. For the examples in this chapter the levels of the carryover factor were identified with the levels $0, 1, 2, \ldots, t$, where the "0" code was used for the first-period observations because there were no carryover effects present in the first period. The observation was given a code of "1" if treatment 1 occurred in the previous period, a code of "2" if treatment 2 occurred in the previous period, and so forth.

The complete coding for the data file used in Example 16.1 included columns of codes for the period (P), sequence (S), animal within sequence (A), diet (D), and carryover (C) factors as well as the column of data for the response variable NDF.

P	*S*	*A*	*D*	*C*	*NDF*	*P*	*S*	*A*	*D*	*C*	*NDF*	*P*	*S*	*A*	*D*	*C*	*NDF*
1	1	1	1	0	50	2	1	1	2	1	61	3	1	1	3	2	53
1	1	2	1	0	55	2	1	2	2	1	63	3	1	2	3	2	57
1	2	1	2	0	44	2	2	1	3	2	42	3	2	1	1	3	57
1	2	2	2	0	51	2	2	2	3	2	45	3	2	2	1	3	59
1	3	1	3	0	35	2	3	1	1	3	55	3	3	1	2	1	47
1	3	2	3	0	41	2	3	2	1	3	56	3	3	2	2	1	50
1	4	1	1	0	54	2	4	1	3	1	48	3	4	1	2	3	51
1	4	2	1	0	58	2	4	2	3	1	51	3	4	2	2	3	54
1	5	1	2	0	50	2	5	1	1	2	57	3	5	1	3	1	51
1	5	2	2	0	55	2	5	2	1	2	59	3	5	2	3	1	55
1	6	1	3	0	41	2	6	1	2	3	56	3	6	1	1	2	58
1	6	2	3	0	46	2	6	2	2	3	58	3	6	2	1	2	61

Note the coding for the carryover factor C is "0" in the first period ($P = 1$). The carryover factor code in the second period ($P = 2$) is identical to the diet (D)

code for the same steer in the previous period ($P = 1$). Likewise, the diet codes for the second period are those used for the carryover code in the third period.

Carryover Effects Coded as Covariates

Milliken and Johnson (1984) introduced the carryover effects into the model as covariates. The method of coding is sometimes referred to as *indicator* or *dummy* variable codes. The coding method is based on the knowledge that a restriction must be placed on the solutions for factor effects in the normal equations. One restriction on the relationship among the solutions that can be used for the carryover effects is $\hat{\lambda}_1 + \hat{\lambda}_2 + \cdots + \hat{\lambda}_t = 0$. The restriction indicates that knowledge of $t - 1$ effects automatically provides the value for the remaining effect. The relationship is $\hat{\lambda}_1 + \hat{\lambda}_2 + \hat{\lambda}_3 = 0$ with three-carryover treatment effects. Knowledge of the values for two-carryover effects automatically provides the value for the third. Therefore, $\hat{\lambda}_3 = -\hat{\lambda}_1 - \hat{\lambda}_2$ and carryover effects have 2 degrees of freedom.

Only $t - 1$ covariates are required for the linear model to include parameters for carryover effects because of the restrictions on the carryover effects. The model for the roughage diet study is used as an example. The model expressed with two covariates for carryover effects is

$$y_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \tau_d + \lambda_1 X_1 + \lambda_2 X_2 + e_{ijk} \qquad \textbf{(16A.1)}$$

$$i = 1,2,\ldots,6 \quad j = 1,2 \quad k = 1,2,3 \quad d = 1,2,3$$

One method of coding the covariates for the carryover effects in period k is

$$X_1 = \begin{cases} 1 & \text{if } d = 1 \text{ in period } (k-1) \\ -1 & \text{if d} = 3 \text{ in period } (k-1) \\ 0 & \text{otherwise} \end{cases}$$

Thus, $X_1 = 1$ when diet A occurs in the previous period and $X_1 = -1$ when diet C occurs in the previous period.

Also,

$$X_2 = \begin{cases} 1 & \text{if } d = 2 \text{ in period } (k-1) \\ -1 & \text{if d} = 3 \text{ in period } (k-1) \\ 0 & \text{otherwise} \end{cases}$$

Thus, $X_2 = 1$ when diet B occurs in the previous period and $X_2 = -1$ when diet C occurs in the previous period.

The complete coding for the data file for Example 16.1 includes columns of codes for the period (P), sequence (S), animal within sequence (A), diet (D), and covariates for the carryover effects X_1 and X_2, as well as the column of data for the response variable NDF.

There is no carryover effect in the first period ($P = 1$), and the covariates are coded $X_1 = X_2 = 0$. The observation on diet B in the second period ($P = 2$) of the first sequence ($S = 1$) follows diet A from the first period; thus, a carryover effect for diet A is required and the covariate codes are $X_1 = 1$ and $X_2 = 0$. The observation on diet A in the third period ($P = 3$) of the second sequence ($S = 2$) follows diet C from the second period; thus, a carryover effect for diet C is required with covariate codes $X_1 = -1$ and $X_2 = -1$.

The sum of squares for the two covariates, X_1 and X_2, will be the sum of squares for carryover effects adjusted for direct effects. This method for the analysis can be used with any statistical program that allows the inclusion of covariates in the model. Each program will have unique syntax for the representation of added covariates in the model.

P	S	A	D	X_1	X_2	NDF	P	S	A	D	X_1	X_2	NDF	P	S	A	D	X_1	X_2	NDF
1	1	1	1	0	0	50	2	1	1	2	1	0	61	3	1	1	3	0	1	53
1	1	2	1	0	0	55	2	1	2	2	1	0	63	3	1	2	3	0	1	57
1	2	1	2	0	0	44	2	2	1	3	0	1	42	3	2	1	1	−1	−1	57
1	2	2	2	0	0	51	2	2	2	3	0	1	45	3	2	2	1	−1	−1	59
1	3	1	3	0	0	35	2	3	1	1	−1	−1	55	3	3	1	2	1	0	47
1	3	2	3	0	0	41	2	3	2	1	−1	−1	56	3	3	2	2	1	0	50
1	4	1	1	0	0	54	2	4	1	3	1	0	48	3	4	1	2	−1	−1	51
1	4	2	1	0	0	58	2	4	2	3	1	0	51	3	4	2	2	−1	−1	54
1	5	1	2	0	0	50	2	5	1	1	0	1	57	3	5	1	3	1	0	51
1	5	2	2	0	0	55	2	5	2	1	0	1	59	3	5	2	3	1	0	55
1	6	1	3	0	0	41	2	6	1	2	−1	−1	56	3	6	1	1	0	1	58
1	6	2	3	0	0	46	2	6	2	2	−1	−1	58	3	6	2	1	0	1	61

16A.2 Appendix: Treatment Sum of Squares for Balanced Designs

The quantities shown next for manual calculation of the adjusted sums of squares for direct and carryover effects in the balanced crossover designs and balanced extra-period designs are valid for equal numbers of subjects per treatment sequence. Following the notation of Cochran and Cox (1957) the required formulae are

T_i = treatment totals, $i = 1,2,\ldots,t$
R_i = total of observations in the periods immediately following treatment i
F_i = total of sequences in which treatment i is the final treatment
P_1 = total of period 1
G = grand total of all observations

Also, let t = the number of treatments, r = the number of subjects per sequence, and m = the number of Latin squares used for the design.

Williams Designs: For the Williams designs compute

$$\widehat{T}_i = (t^2 - t - 1)T_i + tR_i + F_i + P_1 - tG$$

for the adjusted sum of squares for direct treatment effects

$$SST(\text{adjusted}) = \frac{\Sigma \widehat{T}_i^2}{rmt(t^2 - t - 1)(t + 1)(t - 2)}$$

Compute

$$\widehat{R}_i = tT_i + t^2R_i + tF_i + tP_1 - (t + 2)G$$

for the adjusted sum of squares for carryover treatment effects

$$SSC(\text{adjusted}) = \frac{\Sigma \widehat{R}_i^2}{rmt^3(t + 1)(t - 2)}$$

Compute the usual unadjusted sums of squares for periods, sequences, subjects within sequences, and treatments according to the Latin rectangle design in Chapter 8. The sum of squares for experimental error to complete the sum of squares partition for the analysis of variance as shown in Table 16.2 is

$$SSE = \text{Total } SS - SSS - SSW - SSP - SST(\text{unadjusted}) - SSC$$

The estimates of differences between the adjusted treatment means can be found as

$$\widehat{\mu}_i - \widehat{\mu}_j = \frac{\widehat{T}_i - \widehat{T}_j}{rmt(t + 1)(t - 2)}$$

The standard error estimate for the contrast with adjusted means from a balanced crossover design is

$$s_{(\widehat{\mu}_i - \widehat{\mu}_j)} = \sqrt{\frac{2MSE}{N} \cdot \frac{(t^2 - t - 1)}{(t + 1)(t - 2)}}$$

where N is the number of replications per treatment.

Extra-Period Williams Design: Adjustments to the calculations required for the extra-period Williams designs are

$$\widehat{T}_i = (t + 1)T_i - F_i - G$$

with the adjusted sum of squares for treatments as

$$SST(\text{adjusted}) = \frac{\Sigma \widehat{T}_i^2}{mt(t + 1)(t + 2)}$$

Compute

$$\widehat{R}_i = tR_i + P_1 - G$$

for the adjusted sum of squares for carryover effects as

$$SSC(\text{adjusted}) = \frac{\Sigma \widehat{R}_i^2}{mt^3}$$

The estimated difference between two adjusted treatment means is

$$\widehat{\mu}_i - \widehat{\mu}_j = \frac{\widehat{T}_i - \widehat{T}_j}{mt(t + 2)}$$

with standard error estimate

$$s_{(\widehat{\mu}_i - \widehat{\mu}_j)} = \sqrt{\frac{2MSE}{mt} \cdot \frac{(t + 1)}{(t + 2)}}$$