BLOOD-BRAIN BARRIER EXPERIMENT

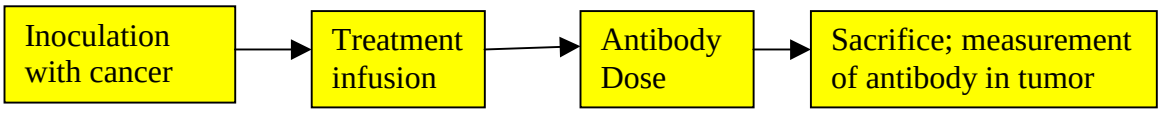
15. Brief Version of the Case Study

15.1 Problem Formulation

The brain is protected from bacteria and toxins, which course through the bloodstream, by a system called the blood-brain barrier. Blood flowing through the brain's capillaries is sealed from outside brain tissue by a single layer of cells. This barrier normally allows only a few substances, including some medications, to reach the brain. Because chemicals used to treat brain cancer have such large molecular size, they cannot pass through the barrier to attack tumor cells. Dr. E. A. Neuwelt from the Oregon Health Sciences University, developed a method of disrupting the barrier by infusing a solution of concentrated sugars.

As a test of the effectiveness of the disruption mechanism, researchers conducted a study on rats. The study was described by P. Barnett et al., "Differential Permeability and Quantitative MR Imaging of a Human Lung Carcinoma Brain Xenograft in the Nude Rat", American Journal of Pathology 146(2), 1995, pages 436-449.

The rats were inoculated with cancer cells to induce brain tumors. After 9 to 11 days they were infused with either the barrier disruption (BD) solution or, as a control, a normal saline (NS) solution. No random mechanism was used to assign the rats to the two treatments. Fifteen minutes later, the rats received a standard dose of the therapeutic antibody L6-$(ab')_2$. After a set time they were sacrificed, and the amounts of antibody in the brain tumor and in normal tissue were measured. The time line for the experiment is shown below:



The data from the experiment are available in the SPSS file *brain.sav* located in the STAT 252 directory on the FTP server.

The following is a description of the variables in the data file:

Column	Name of Variable	Description of Variable
1	BRAIN	Brain Tumor Antibody Count (per gm)

2	LIVER	Liver Antibody Count (per gm)
3	TIME	Sacrifice Time (hours)
4	TREATMENT	BD if infused with the barrier disruption, NS if infused with a normal saline solution (control)
5	DAYS	Days after inoculation
6	SEX	F= Female, M= Male
7	WEIGHT	Initial weight (grams)
8	LOSS	Weight Loss (grams)
9	TUMOR	Tumor Weight (10^{-4} grams)

The brain tumor concentration divided by the liver concentration is a measure of the amount of the antibody that reached the brain relative to the amount of it that reached other part of the body. The ratio Brain/Liver is the response variable in the experiment.

The explanatory variables in the experiment comprise two categories: design variables are those that describe manipulation by the researcher; covariates are those measuring characteristics of the subjects that are not controllable by the researcher. The design variables are sacrifice time (hours) and the type of treatment (BD or NS).

We will use SPSS to answer the following questions using the data:

1. Was the antibody concentration in the tumor increased by the use of the blood-brain barrier disruption infusion? If so, by how much?

2. Do the answers to the two questions in 1 depend on the length of time after the infusion (from 1/2 to 72 hours)?

3. What is the effect of treatment on antibody concentration after weight loss, total tumor weight, sex, and initial weight are accounted for?

15.2 Experiment Design

The blood-brain barrier experiment is not a randomized experiment because no random mechanism was used to assign the rats to the two treatments. Randomization ensures that the rats with different and possibly relevant characteristics are mixed up between the two groups. As the randomization was not used, it is possible that rats with different physical characteristics and different response to the antibody were placed disproportionately in one of the treatment groups.

Thus no cause-and-effect relationships can be drawn from the experiment. It is possible that the estimated relationships may be related to confounding variables over which the experimenter had no control. Causal implications can only be justified on the assumption that the assignment method of the rats to the two treatments had the same effect on the response as if a random assignment would have been made.

15.3 Multiple Linear Regression Model

We found in Section 7 that the covariates are not significant when the design variables are also included in the model. Moreover, we also found that the design variables are significant when the covariates are included in the model.

In this section we will apply multiple regression to blood-brain barrier data. Sacrifice time is treated as a factor with four levels corresponding to the four sacrifice time values: 0.5, 3, 24, and 72. As there are 4 levels, then 4-1=3 indicator variables are needed as explanatory variables. Selecting the first level, 0.5 hours, as the reference level, the multiple linear regression model is

$$LNRATIO = \beta_0 + \beta_1 * D3 + \beta_2 * D24 + \beta_3 * D72 + \beta_4 * TREAT + ERROR.$$

The dummy variables D3, D24, and D72 are defined as follows:

TIME	**D3**	**D24**	**D72**
0.5	0	0	0
3	1	0	0
24	0	1	0
72	0	0	1

The dummy variables can be incorporated into the SPSS data file, by using the *Recode into Different Variables* feature in the *Transform* menu. The SPSS output for the regression model is displayed below:

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.975[a]	.951	.944	.5328

a. Predictors: (Constant), TREAT, D72, D24, D3

b. Dependent Variable: LNRATIO

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	158.573	4	39.643	139.646	.000[a]
	Residual	8.233	29	.284		
	Total	166.806	33			

a. Predictors: (Constant), TREAT, D72, D24, D3

b. Dependent Variable: LNRATIO

The value of the F statistic is equal to 139.646 with the corresponding p-value of 0 provides very strong evidence of the utility of the model.

The estimates and standard errors are displayed in the next table:

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	-4.302	.205		-21.010	.000	-4.720	-3.883		
	D3	1.134	.252	.226	4.501	.000	.619	1.650	.676	1.480
	D24	4.257	.259	.815	16.431	.000	3.727	4.787	.691	1.447
	D72	5.154	.259	.987	19.892	.000	4.624	5.684	.691	1.447
	TREAT	.797	.183	.180	4.346	.000	.422	1.172	.993	1.007

a. Dependent Variable: LNRATIO

In particular, the coefficient of the indicator variable for the blood-brain barrier disruption treatment is 0.797. So, expressed in accordance with the interpretation for log-transformed responses, the median ratio of antibody concentration in the brain tumor to antibody concentration in the liver is estimated to be exp(0.797) = 2.22 times greater for the blood-brain diffusion treatment than for the saline control. The estimate is a little bit smaller than the estimate of 2.33 obtained for the model discussed in Section 7.

Moreover, according to the above table, the 95% confidence interval for the ratio is from exp(.422) = 1.56 to exp(1.172) = 3.15.

As the values of tolerance are not very small and VIF not very large, there is no evidence any problem with collinearity in this case. The regression diagnostics for the model is discussed in detail in Section 9.

15.4 Analysis of Variance Model

The effects of design variables and covariates on the response can also be investigated by general factorial analysis of variance available in SPSS. This very useful statistical tool encompasses both analysis of variance and regression. It will be discussed in class after multiple regression techniques are covered. Thus you can skip this section now to return to the material later after the analysis has been covered in your lectures.

The blood-brain barrier experiment is an example of a factorial experiment. A factorial experiment consists of several factors (sacrifice time, treatment) which are set at different levels, and a response variable (concentration ratio). The purpose of the experiment is to assess the impact of different combinations of the levels of sacrifice time and treatment type on the concentration ratio.

Analysis of variance allows us to test the null hypothesis that the design variables and covariates have no impact on the response. There are four sources of variation in the experiment: the main effects of design variables and covariates, the interaction effects, and the error variation. Corresponding to these four sources, there are several null hypotheses that may be tested. In particular, we test the following hypotheses:

1. H_0: No main effect of *Sacrifice Time,*
2. H_0: No main effect of *Treatment,*
3. H_0: No interaction effect between *Sacrifice Time* and *Treatment.*

The design variables in this experiment, sacrifice time and treatment type can be both treated as categorical variables, which means they should be entered as factors in the GLM General Factorial procedure.

To produce the output for this model, from the menus choose:

Statistics

General Linear Model

GLM- General Factorial…

- Dependent: *LNRATIO*
- Fixed Factor(s): *TIME, TREAT*
- Covariate(s): *DAYS, SEX, WEIGHT, LOSS, TUMOR*

Observe that the dependent variable is log-transformed concentration ratio, not ratio itself to make the assumption of equal variances satisfied. As we observed in Section 4.2, the variance of RATIO increases as TIME increases. The log transformation helps to compress the RATIO values uniformly over the range of TIME.

Model

♦ Full

The following output will be displayed:

Tests of Between-Subjects Effects

Dependent Variable: LNRATIO

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	160.121[a]	12	13.343	41.918	.000
Intercept	1.816E-02	1	1.816E-02	.057	.814
DAYS	4.982E-03	1	4.982E-03	.016	.902
SEX	3.830E-06	1	3.830E-06	.000	.997
WEIGHT	3.492E-03	1	3.492E-03	.011	.918
LOSS	1.219	1	1.219	3.831	.064
TUMOR	.509	1	.509	1.600	.220
TIME	70.570	3	23.523	73.898	.000
TREAT	5.356	1	5.356	16.826	.001
TIME * TREAT	.498	3	.166	.522	.672
Error	6.685	21	.318		
Total	232.373	34			
Corrected Total	166.806	33			

a. R Squared = .960 (Adjusted R Squared = .937)

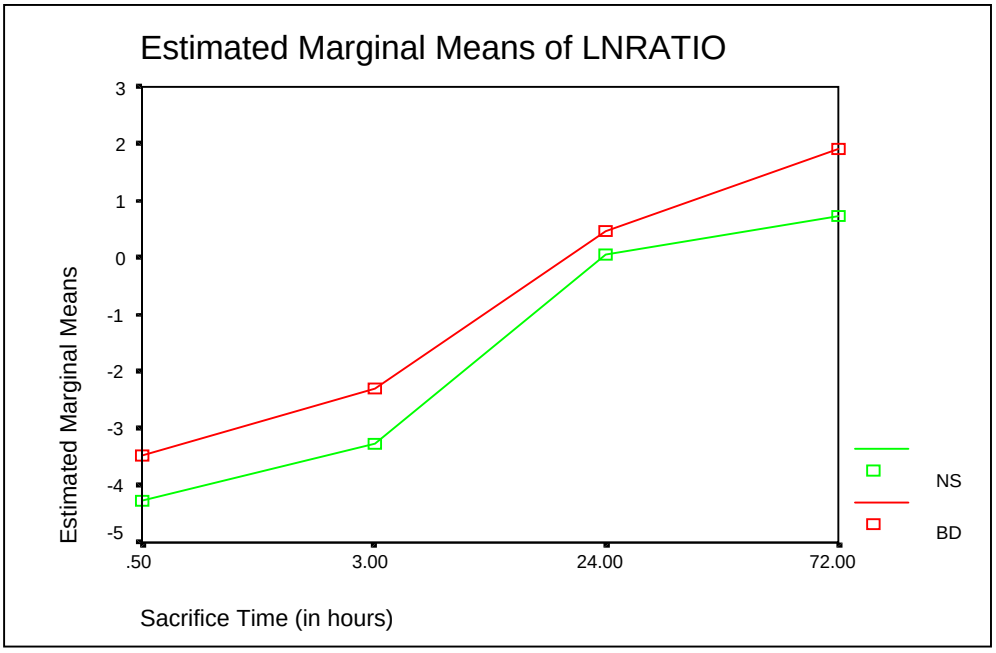
The table contains rows for the components of the model that contribute to the variation in the dependent variable. The row labeled *Corrected Model* contains values that can be attributed to the regression model, aside from the intercept. The sources of variation are identified as *Days*, *Sex*, *Weight*, *Loss*, *Tumor*, *Time*, *Treat*, *Time*Treat* (interaction), and *Error*. *Error* displays the component attributable to the residuals, or the unexplained variation. *Total* shows the sum of squares of all values of the dependent variable. *Corrected Total* (sum of squared deviations from the mean) is the sum of the component due to the model and the component due to the error.

According to the output, the model sum of squares is 160.121 and the error sum of squares is 6.685. The total sum of squares (corrected total) is 167.806. Notice a very small contribution of error in the total sum of squares. The p-value of the F-test for the model is reported as 0.000 indicating convincing evidence of an effect of at least one of the factors on the response.

The sum of squares for the treatment factor is estimated to be 5.356. The value of the F-statistic equal to 5.356 and p-value of the F-test reported as 0.000 indicate very strong evidence of effect of treatment on the response. Although *Treatment* main effects are also statistically significant, they are not that strong as the main effects due to brand factor.

The p-value of the interaction term *Time*Treatment* is equal to 0.672, indicating no evidence of an interaction between the two factors. The table also shows that the covariates are not significant when the design variables are also included in the model.

The same conclusion about no interaction between the two factors can be reached by examining the interaction effects with a profile plot. A profile plot is a line plot in which each point indicates the estimated marginal mean of a dependent variable at one level of a factor. The plot for our data is displayed below.



The plot indicates that the rats subjected to the BD treatment had higher concentration ratios than those subjected to the control treatment for all four sacrifice time levels. The lines corresponding to the two levels of treatment are almost parallel. The parallelism in this chart indicates that there is little or no interaction between the two factors indicating no interaction between treatment type and sacrifice time. In other words, the effect of the BD treatment is approximately the same for the four sacrifice time levels.

Now we estimate the effect of blood-brain diffusion treatment (BD) on the effectiveness of the disruption method measured by *LNRATIO*. As we want to compare the BD to NS effects on the response variable *LNRATIO*, simple contrast will be used.

The SPSS output for the simple contrast applied to the *TREAT* factor is

Contrast Results (K Matrix)

TREAT Simple Contrast[a]			Dependent Variable: LNRATIO
Level 1 vs. Level 2	Contrast Estimate		-.870
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		-.870
	Std. Error		.394
	Sig.		.036
	95% Confidence Interval for Difference	Lower Bound	-1.681
		Upper Bound	-6.03E-02

a. Reference category = 2

Test Results

Dependent Variable: LNRATIO

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1.515	1	1.515	4.878	.036
Error	8.077	26	.311		

The p-value of two-sided test about the contrast is 0.036. Thus the p-value for the one-sided test is 0.036/2 = 0.018. This provides strong evidence of the effectiveness of the blood-brain diffusion treatment.

The point estimate of the contrast is -0.870 (NS versus BD). So, expressed in accordance with the interpretation for log-transformed responses, the median ratio of antibody concentration in the brain tumor to antibody concentration in the liver is estimated to be exp(.870) = 2.3869 times greater for the blood-brain diffusion treatment than for the saline control.

15.5 Summary

The goal of the experiment is to test the effectiveness of a new method to disrupt the natural blood-brain barrier. The disruption is crucial in order to allow some medications to reach the brain.

The experiment was conducted on rats. The data collected includes two design variables: sacrifice time and treatment, and several covariates. The response variable defined in the experiment measures the effectiveness of the new method.

We used both multiple regression and general factorial procedure to examine the effects of the design variables and covariates on the response. It was found that both sacrifice time and treatment are highly significant, although there is a weak interaction between the two factors. More precisely, the median ratio of antibody concentration in the brain tumor to antibody concentration in the liver is estimated to be 2.3 times greater for the blood-brain diffusion treatment than for the saline control.

Can we then conclude that the disruption method is effective?

Unfortunately, randomization was not used to assign rats to treatment groups. This raises the possibility that the estimated relationships might be related to confounding variables over which the experimenters had no control. In other words, no cause and effect conclusions can be drawn from the data. Causal implications can only be justified if we assume that the assignment method used had no effect on the response.