AN EXAMINATION OF THE MISSING DATA PROBLEM IN EXPERIMENTS ON THE
EFFECT OF DRUGS ON CORONARY LIGATION IN RATS

by

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Abstract

A data set from ongoing experiments on rats to study the effect of certain drugs on clinically induced heart attacks is investigated. After description of the experiment and the data, preliminary analyses examine the relationship between some variables. The problem of missing data inherent to this type of experiment is then approached. Two statistical tests dealing with this problem are proposed and applied. Finally log-linear models are considered to analyse some categorical variables.

Nancy M. Reid

Thesis supervisor

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1. INTRODUCTION

A frequent problem with which the statistician is confronted in practice is that of missing data. In some cases this problem can be circumvented and the standard statistical techniques applied. However the data are often collected or the experiments often designed in such a way that missing values are unavoidable. This was the case for the data submitted by Dr. Walker of U.B.C.'s Department of Pharmacology to the Statistical Consulting Service.

Dr. Walker and his research group are interested in the effect of drugs on the outcome of myocardial infarction. They have developed an experimental method which they think will lead to the detection of drugs capable of ameliorating adverse responses to ischaemia and infarction. The experiment and the data collected are described in Section 2. In Section 3, results of some preliminary analyses undertaken to examine the relationship between the responses are presented.

Briefly, their method consists of inducing a heart attack in rats exposed to various drugs and then measuring different responses during a certain period of time. However, many rats die during the experiment making impossible the measurement of some responses. One natural approach to the missing data problem is to omit all vectors of observation that are incomplete. This is unsatisfactory, especially if few experimental units are available and if many variables are known for an incomplete vector of observation. In Section 4, two

statistical tests which use all the information available on each experimental unit are proposed and results of their applications to Dr. Walker's data are given.

Finally, in the last section, log-linear models are suggested for the analysis of arrhythmias.

2. HISTORY OF THE DATA

Dr. Walker's study concentrated upon the pharmacological and pathological aspects of responses to irreversible myocardial ischaemia and subsequent infarction. From an experimental point of view, irreversible ischaemia can most easily be achieved by ligation of a coronary artery. In order to study the effects of drugs on responses to such ischaemia, a method of producing ligation in conscious rats was developed. The experiment involved measuring cardiovascular, arrhythmic, ECG and mortality responses and cardiac tissue loss resulting from ligation in the prepared rats exposed to various drugs.

This section describes the two parts of the experiment, surgical preparation and ligation technique, the data collected, and enumerates some potential problems related to the data.

2.1 Experiment[1]

Experiments, which are still ongoing, were performed on over 300 male rats since 1979. Approximately half of the rats were of the Wistar(Charles River farms) strain while, more recently, a Sprague-Dawley(Charles River Farm & Taconic) strain has been used. In order to be able to produce ligation in rats, they had to undergo surgical preparation. Rats weighted between 200 and 300 g at the time of operation.

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[1] This section was adapted from Jang et al. (1983), Johnston et al. (1983a,1983b), Macleod et al. (1983).

Surgical Preparation

Operations were performed under clean conditions by one of four technicians. Rats were anaesthetized with halothane and the chest opened by a left lateral incision at the fourth or fifth intercostal space. An occluder which consisted of a (5-0) polypropylene suture was passed around the left anterior descending(LAD) coronary artery and exteriorized in a polythene guide(PE50) in the mid-scapula region of the neck. The site of ligation of the LAD coronary artery was located approximately 3mm from the aortic root. The suture was passed through the myocardium so as to make a loop around the artery such that traction on suture at the exteriorized end of the guide produced complete ligation of the artery.

Permanent ECG leads were implanted with a sub-dermal needle trocar into the pectoralis muscle overlying the chest incision, in the left leg and in both arms. The free ends of leads were exteriorized together with the occluder.

In the early experiments, a permanent catheter was placed in the ventral tail artery and exteriorized at the neck under halothane anaesthesia one day prior the experiment. In later animals, at the time of the thoracotomy, permanent venous and jugular cannulae were implanted in abdominal aorta and external jugular vein by a technique due to Weeks[2]. The cannulae were also exteriorized with the occluder and the ECG wires.

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[2] See Macleod et al. (1983) for further references.

Operative mortality was below one percent with trained technicians. Deaths generally occurred shortly after surgery and were due to blood loss. Animals were allowed to recover for at least 6 days prior to ligation.

Ligation Technique

On the day of the ligation, rats were randomly selected and kept in their home cage. Cannulae and ECG leads were appropriately connected to a Grass Polygraph and infusion pump, and a continuous record made for 30 minutes prior to ligation. Approximatively seven percent of animals were rejected for studies since they showed changes in their ECG's suggestive of cardiac damage, or had blocked cannulae. Ligation was achieved within seconds by exerting sufficient traction on the prolypopylene suture and was made permanent by heat sealing it to the polythene guide.

Various drug treatments were administered to ligated rats. They included infusions begun pre-ligation, intravenous dosing, oral dosing etc. Animals were continuously monitored for 4 hours before being disconnected and returned to the animal house. During recording of blood pressure(BP) and ECG, all arrhythmias were noted. If within the four hours post ligation observation period a severe ventricular arrhythmia occurred and did not spontaneously revert within 10 seconds, an attempt was made to convert the arrhythmia to sinus rhythm by repeatedly tapping the rat's chest.

Twenty-four hours after ligation, cannulae and leads were reconnected and the rats monitored for a further 30 minutes. The animals were then killed by stunning and exsanguination, and the heart removed. Hearts were also removed from animals dying before 24 hours had elapsed.

For all rats, hearts with occluder intact were perfused with Kreb's solution at 37 C and 100mm Hg pressure for 5 min to remove all blood. A bolus of 2.0 ml of cardio-green dye(1.0mg/ml) was used to differentiate perfused(green) from underperfused, or occluded tissue(pink). The underperfused region was immediately cut out and weighed to give a measurement of occluded zone as a percentage of total ventricular weight.

For rats that survived 24 hours, the heart tissue was then sliced longtitudinally into 1.0 mm sections and incubated in tetrazolium dye at 37 C for 30-45 minutes. After incubation, all sections were placed in 10% formaldehyde (in normal saline) for two days before the undyed(white) infarcted tissue was dissected from viable tissue(purple). Infarcted tissue was weighted and expressed as a percentage of total ventricular tissue weight giving a measurement of the infarct zone.

In other words, the occluded zone represents the proportion of heart tissue for which the blood supply was interrupted and the infarct zone the proportion of dead tissue.

2.2 Data

The variables measured during the experiment were: systolic and diastolic pressure, heart rate, ECG, arrhythmias, size of occluded zone, size of infarcted zone and mortality. The small bore of the aortic cannulae often filtered the pressure wave leading to a poor recording of the systolic pressures . According to Dr. Walker the mean of systolic and diastolic represents a more accurate measure of blood pressure(BP). Heart rate was taken from BP or ECG traces using a ratemeter. The ECG was used to detect arrhythmia and for measurement of the size of the major complex(R and RS), time to Q-wave and the height of the S-T segment above the iso-electric line. The size of the occluded and infarcted zone were recorded as described in the previous section.

The variables can be regrouped in three categories according to their time measurement. The first category consists of continuously monitored responses recorded at twelve different time points (-15min, -1min, +1min, +5min, +10min, +15min, +30min, +1hr, +2hr, +3hr, +4hr, +24hr)[3]. Following is a list of these variables, as found in the computer files.

```
SYS: systolic pressure
DIAS: diastolic pressure
HR: heart rate
ST: height of the S-T segment
R: height of the R-wave
RS: height of the RS segment
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[3] Where - and + mean before and after ligation respectively.

DST: ST at time t minus ST at -15min. $(ST(t)-ST(-15))$[a]
DSTR: ST corrected for R. $(ST(t)(R(-15)/R(t))-ST(-15))$[a]
DSTRS: ST corrected for RS. $(ST(t)(RS(-15)/RS(t))-ST(-15))$[a]

The second category of variables includes responses of arrhythmias measured 30 minutes and 4 hours after ligation and mortality measured 4 hours and 24 hours after ligation. Arrhythmias consist of premature ventricular contractions(PVC), ventricular tachycardia flutter(VT), and ventricular fibrillation(VF). Four or more PVC occurring consecutively are considered to be VT. Ventricular tachycardia and ventricular fibrillation are distinguished from each other by looking at the pattern appearing in the ECG trace and the fall in blood pressure.

In order to adjust arrhythmias for duration and mortality by irreversible VF a new variable was created. This variable combines the number and duration of VT, VF,PVC and the incidence of irreversible VF using a 0-8 scoring scale for the 0-30 min or 0-4 hr post-ligation periods. The value 0 was given for 0-50 PVC with no VT or VF over the observation period; 1, for 50-500 PVC only; 2, for 500 PVC or more,or one episode of spontaneously reversible VT or VF; 3, for more than one episode of spontaneously reversible VT and/or VF or, one or more episodes of non-spontaneously reversible VT and/or VF lasting less than 60 sec; 4, for reversible VT and/or VF episodes lasting 60-120 sec; 5, for VT and/or VF episodes lasting more than 120 sec; 6,

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[a] Note that these variables do not have values at time t=-15 min.

for irreversible VF causing death within 15-240 min of ligation; 7,for fatal VF within 4-15 min and finally 8, for fatal VF within 4 min. This score variable called arrhytmia score measures the severity of the arrhythmias.

The variables included in this category are as follows:

    AS30: arrhythmic score at 30 min.
    AS4h: arrhythmic score at 4 hr.
    IVF#30: irreversible vent. fib. 30 min.
    IVF#4: irreversible vent. fib. 4 hr.
    MORT4: mortality after 4 hr.
    MORT24: mortality after 24 hr.

and several measurements 30 min. and 4 hrs. after ligation on length and numbers of spontaneous and non-spontaneous VT and VF episodes.

The last category consists of variables measured only once during the experiment. These variables are:

    OZ: size of occluded zone
    IZ: size of infarcted zone
    QWO: Q-wave occurrence (Y/N)
    QWT: Q-wave time ,time of first appearance (=240 if >240)

As already mentioned, the experiments have been conducted for some time and are still ongoing. The data for seven such experiments are available. Each data set includes several groups of 7 to 13 rats which were administered different drug treatments. Only five out of the seven experiments have a control group i.e. a group of rats that were ligated but did not receive drugs.

Our main concern is the derivation of statistical techniques to deal with problems related to the data, hence throughout this thesis we concentrate our analyses on the data

combining all the control groups (67 rats) and the data of the halothane dose response experiment (55 rats). This experiment was carried out in 1981 to investigate the effect of halothane at different concentrations.

2.3 Problems related to data

Approximately 50% of the rats died during the experiments. This censoring is a major problem in the study of the data. The observation of the variables in the first category is restricted by the value of the mortality variable since no measurements were made after the rat's death. This causes problems especially if the values of the early measurements (i.e. before mortality) contain information about mortality status. Apart from this censoring, we also have other missing data. In some cases, the measurements of some variables, especially BP, were not possible.

Further, the values of variables such as the number of VT or VF, Q-wave occurence and total PVC are also limited by the value of the mortality variable since these variables are measured for a period of time and not at one point in time. The arrhythmia scoring system attempts to compensate for this.

3. PRELIMINARY ANALYSES OF THE DATA

Preliminary analyses of the control groups (67 rats) were undertaken in order to gain understanding of the relationships among the variables. According to Dr. Walker the experiments were performed quite uniformly except for one experiment where the electrode for the ECG was tied directly on the heart leading to a bigger signal. For this group of rats the values of the ECG variables were deleted from the analyses.

We examined the data using two statistical packages (Midas & BMDP) and keeping in mind important questions which might give some insight into the problems mentioned earlier. First, is it possible to predict the value of a variable at late time periods from early measurements? For instance, is it possible to predict death from heart rate series? Second, is OZ a confounding variable which accounts for difference in treatment groups? Third, is there a relationship existing between the incidence of VT, VF, PVC and the time of mortality?

In this section, results of these preliminary analyses are given.

3.1 Can we predict death ?

For this purpose, the rats were divided into two groups, those that survived (34) the experiment and those that died (33). The mean of BP,HR,ST, R, RS, DST, DSTR, DSTRS, for each point in time were plotted for the two groups. The graphs of

BP, HR,DSTR and DSTRS are given in figures 1 to 4. It is important to note that for the group of rats that died, the sample size decreases with time. For example at the last time point 4 (i.e. 4 hrs after ligation) there are only five rats left in the sample.

For the ECG measurements (ST, R, RS ...etc) the plots for both groups appear to have the same pattern except for DSTRS where at time point 3 (i.e. 1 min after ligation) the difference between the two groups is very large. However, this is due to the extreme value (-7.5 compared to an average of .2) of DSTRS on one rat. In fact when this rat is deleted the curves are quite similar (see figures 4 and 5).

It seems that for rats that died, BP exhibits a greater decrease after ligation than for rats that survived, and then remains lower. We compared (usual t-test) the decrease in BP from the second to the third time point between the two groups and found a p-value of .07.

Heart rate for the two groups have the same pattern for the first time points. At later time points, HR goes down steadily for rats that died while it remains constant for the other group.

An important point to verify is the homogeneity of the two groups before the experiment. This can be done by testing the values of the variables for the first two time points. Using Hotelling's T-square or the t-statistic when appropriate, it

FIGURE 1. Plot of mean BP vs time for rats that died and rats that survived

Legend
△ DIED
✕ SURVIVED

FIGURE 2. Plot of mean HR vs time for rats that died and rats that survived

Legend
△ DIED
✕ SURVIVED

FIGURE 3. Plot of mean DSTR vs time for rats that died and rats that survived
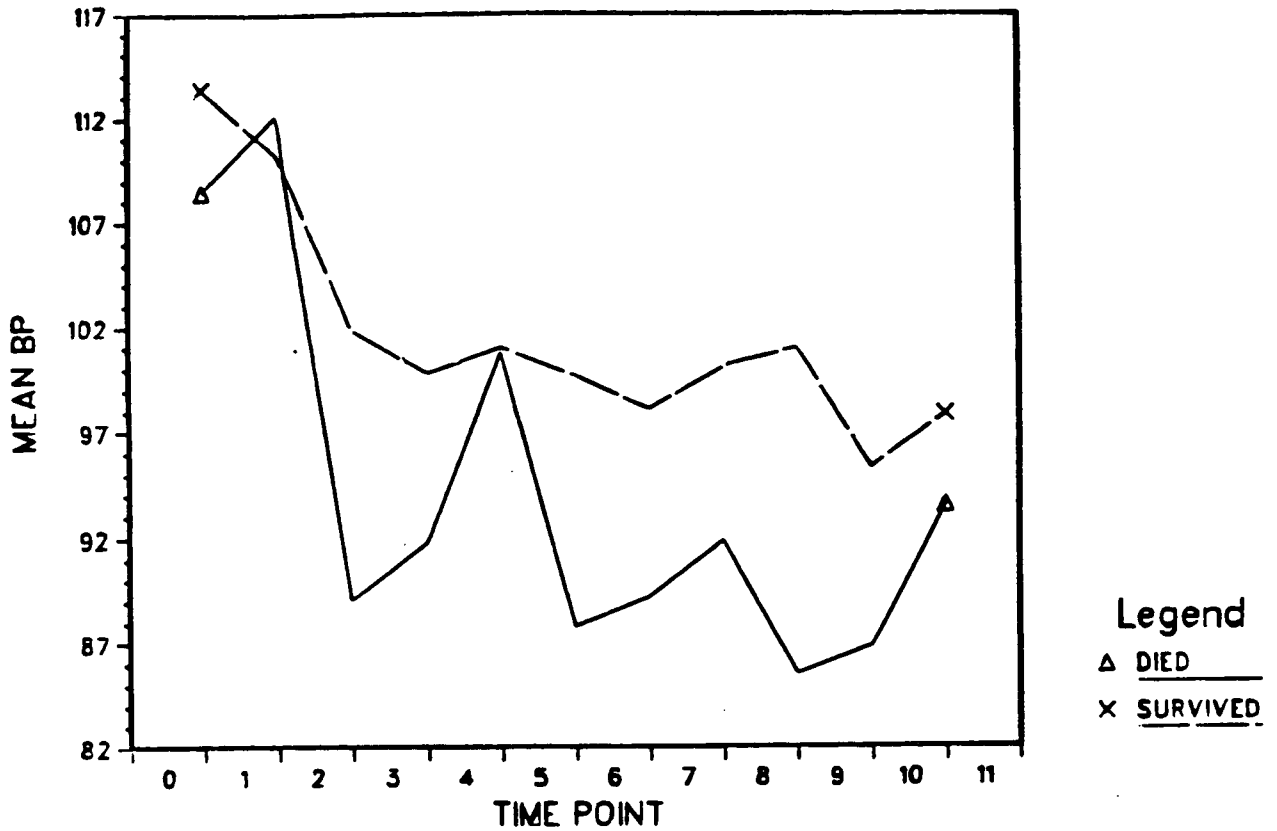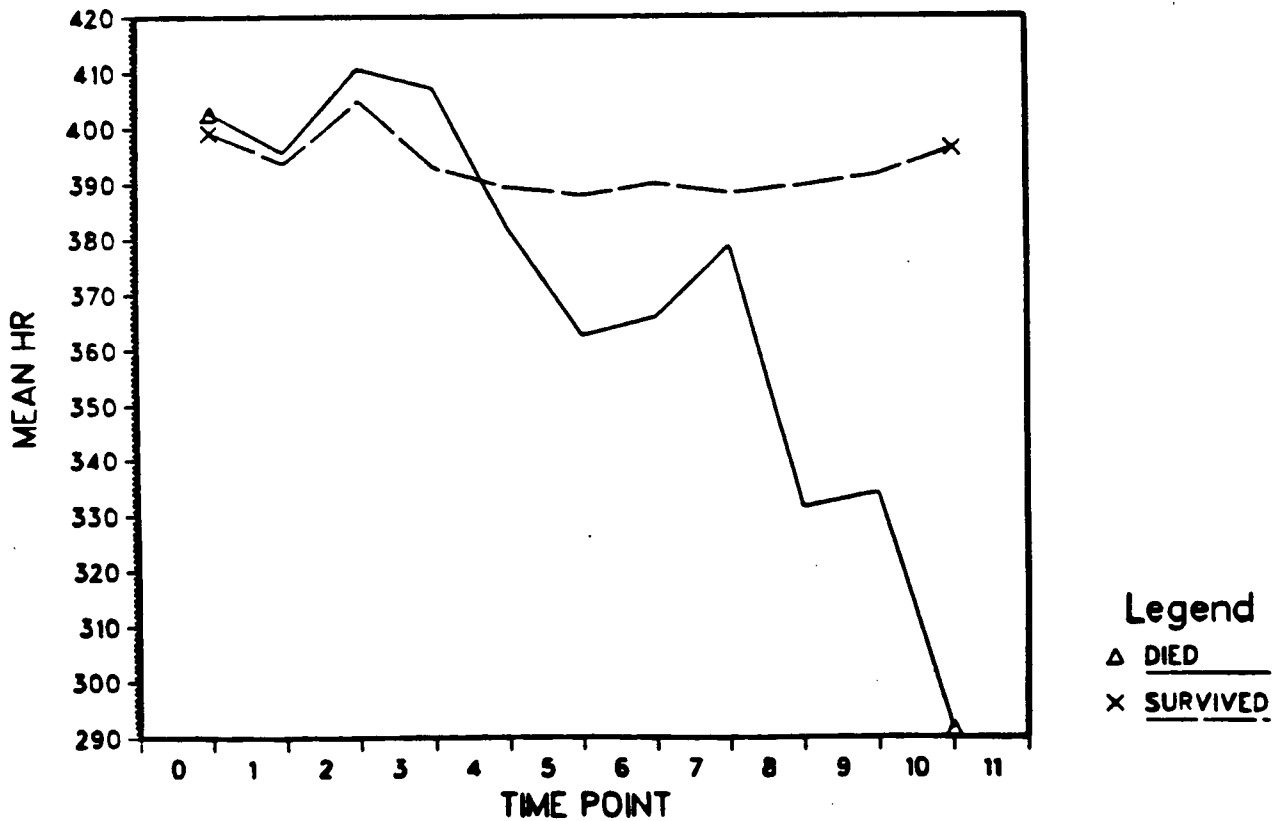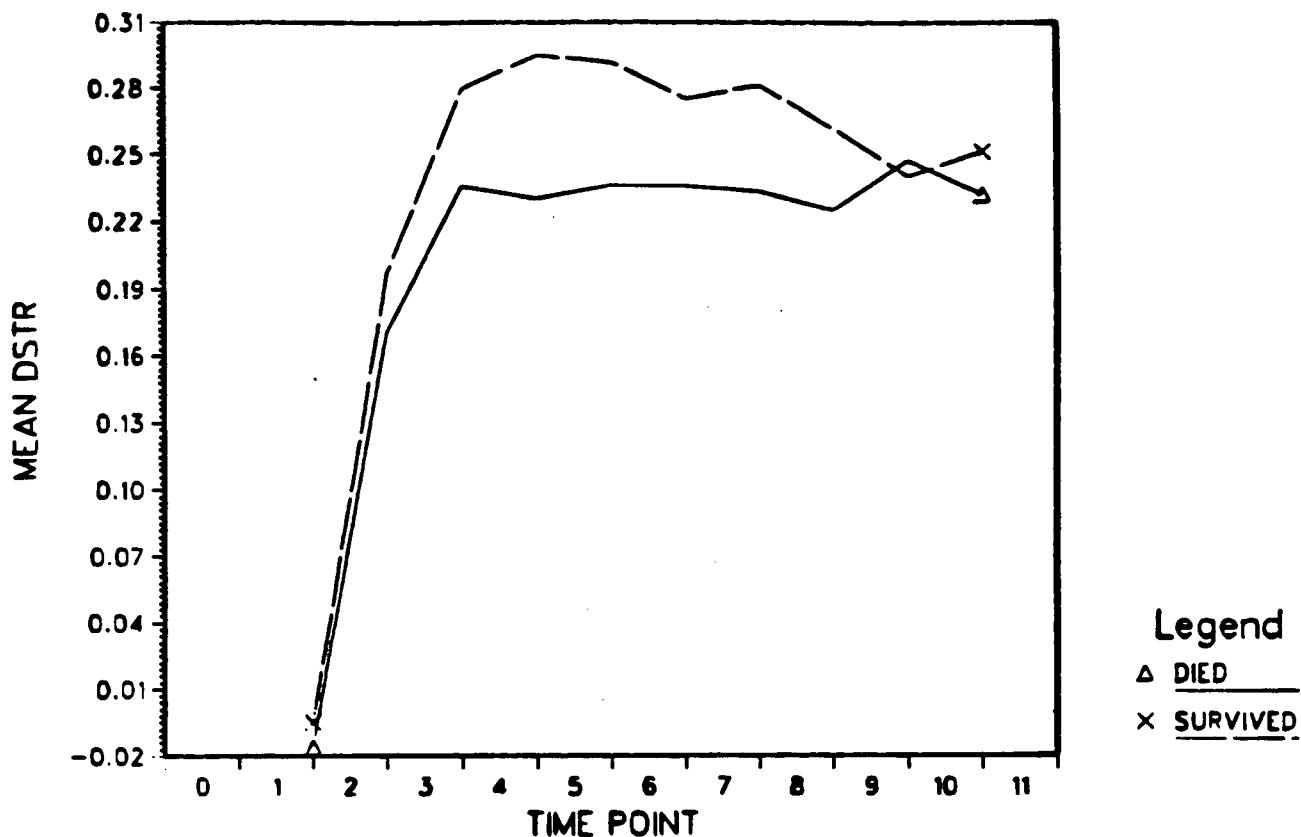

FIGURE 4. Plot of mean DSTRS vs time for rats that died and rats that survived
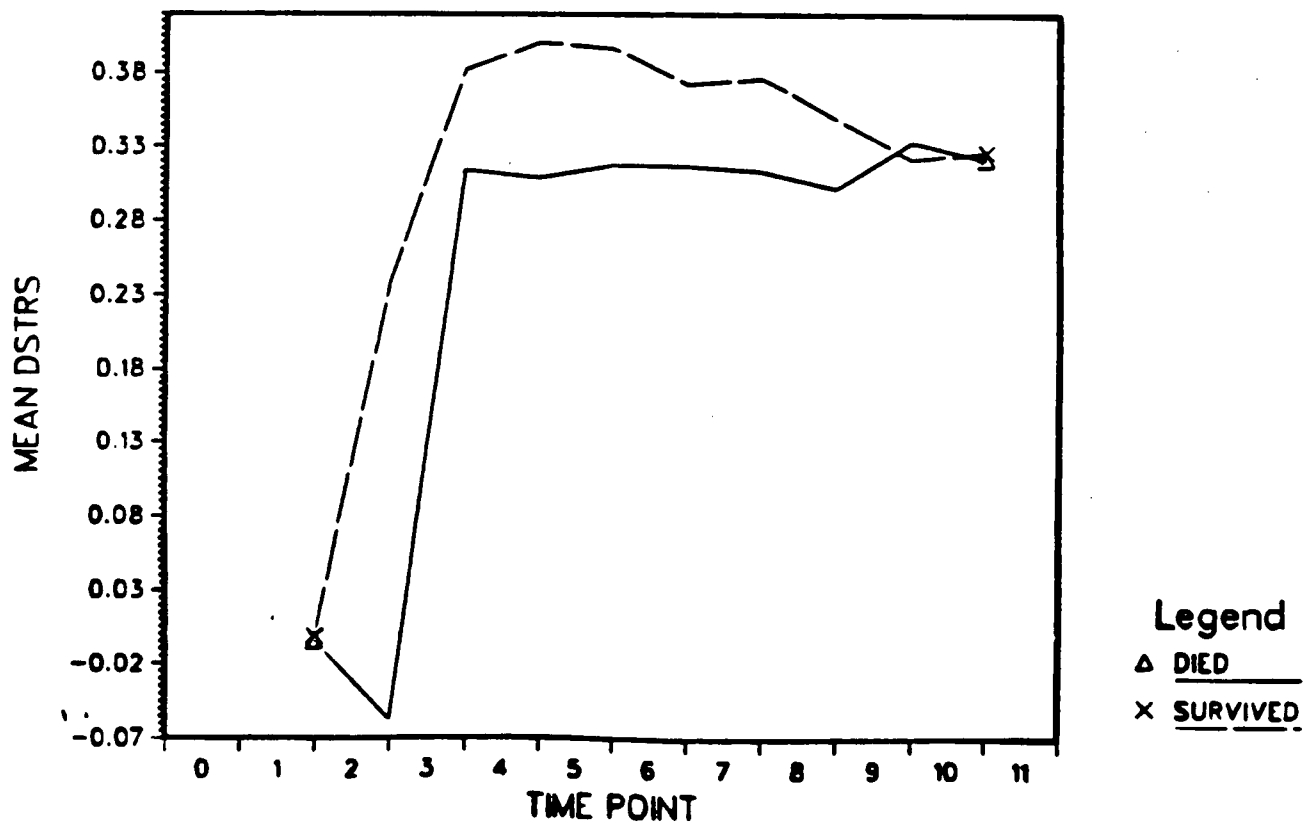
FIGURE 5. Plot of mean DSTRS vs time for rats that died and rats that survived with one rat deleted
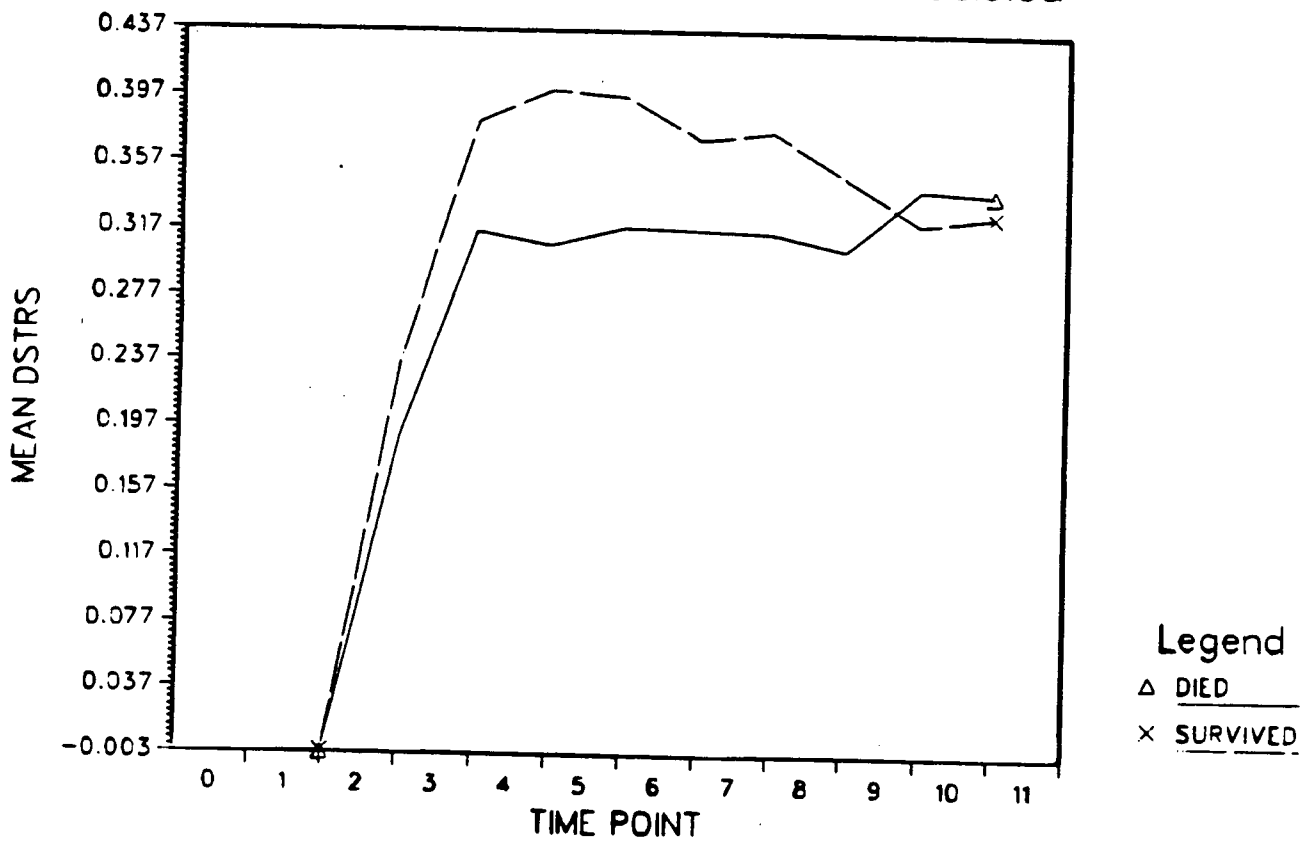
appeared that the two groups were quite homogeneous for all variables except for blood pressure where the p-value was .004. We could not find any reason to explain this unexpected low p-value.

The comparison of means at time point 3 gives the following results:

variables	BP	HR	ST	R	RS	DST	DSTR	DSTRS
p-values	.03	.73	.78	.50	.29	.39	.32	.21

The p-values indicate that the means are not significantly different between the two groups of rats except for BP.

At this stage, it seems that death cannot be predicted with the ECG variables and with the early measurements of heart rate. There is small evidence that the drop in blood pressure just after ligation might be helpful to predict death. However the reliability of this result is questionable, since according to Hotelling's T-square test the two groups were not similar before the experiment.

The results on blood pressure are not very conclusive. To obtain more information on the relationship between blood pressure and mortality, a logistic model was fitted to the proportion of deaths. The logistic function is

$$P = \exp U / (1 + \exp U)$$

where P is the predicted proportion of death and U is a linear function of the variables BP at different time points.

Using BMDP, we performed a stepwise logistic regression on the data. The following p-values were obtained for the significance of the coefficients of the BP variable at time points 1 to 5.

variables	BP1	BP2	BP3	BP4	BP5
p-values	.42	.59	.25	.66	.70

From these results, blood pressure does not seem to be a good predictor for the probability of death.

3.2 Is OZ a confounding variable ?

As a first step, the relationship of OZ with the other variables was examined. A correlation analysis showed that the arrhythmia score and the size of infarcted zone are the only variables which are significantly correlated with the size of occluded zone. The correlation of OZ with AS & IZ are .42 and .44 respectively. The fact that OZ is not correlated with the variables in the first category (i.e. BP,HR, ST,...etc) is also confirmed by the scatter plot of these variables and OZ except in the case of BP. There seems to be a linear relationship between BP and OZ that is not detected by regression because of three outliers (rats) which have unusually low values for OZ.

In fact, when these three rats are deleted the significance level of the regression goes from .1052 to .0002. According to Dr. Walker, these unusually low values for OZ are an indication that something went wrong in the experiment. Hence, we are not introducing any bias when deleting these three values from the data.

It appears that the size of occluded zone varies with the strain of the rats. A p-value of .0002 is obtained when comparing the means using the usual t-test. However, the difference in OZ between the two strains of rats is not too important since in any one experiment the same strain was used throughout. However when combining data from many experiments a covariate representing the strains of the rats should be included in the model considered.

A small difference in the size of occluded zone is also detected between rats that died and rats that survived. A comparison of the mean of OZ between these two groups gives a p-value of .06. In the preceeding section, we found that at the third time point the mean blood pressure in the two groups of rats were also different. It would then be interesting to see if this difference could be explained by the difference in OZ. In the following table, we compare the results of the analysis of variance of BP with the results of the covariance analysis of BP using OZ as a concomitant variable.

| | all rats | | 3 rats deleted | |
	died	survived	died	survived
means	88.98	101.82	90.77	102.82
ANOVA p-value	.03		.04	
adjusted means	89.81	100.95	94.09	99.57
ANOCOV p-value	.07		.35	

From this table, we can see that the difference in blood pressure between the two groups of rats can be attributed to the confounding effect of OZ. It should be pointed out that in both cases the assumption about the equality of slope (i.e. the coefficient of OZ) was not violated.

It is now important to determine if differences in treatments could also be explained by the difference in the size of occluded zone. For this purpose, we used the data of the halothane dose response experiment. In this experiment, halothane was administered at concentrations of 0(control), 0.25, 0.50, 1.0 and 2.0 percent.

In this experiment, the regression of BP on OZ is less significant than with the data on control groups. The effect of halothane seems to tangle the effect of OZ on BP. The slope of the regression increases steadily from -2.13 at dose 0 to .48 at dose 2%. In fact, at dose 1% and 2% the regression of BP on OZ

is not significant at the 5 percent level.

According to an analysis of variance, the mean of BP appears to be significantly different among treatments and there is also a slight difference in the mean of OZ between treatments. However as opposed to the results found for the control groups, a covariance analysis on BP using OZ as a concomitant variable did not reduce the difference in BP between treatments. This might be due to the fact that difference in OZ between treatments is not very significant and also that the relation between BP and OZ is not the same in each treatment. The results are shown in the following table.

		treatments				
		control	0.25%	0.50%	1.0%	2.0%
ANOVA	means	101.82	103.75	89.09	82.05	50.46
	p-value	< .00005				
ANOCOV	adjusted means	104.85	102.87	84.18	83.42	51.76
	p-value	< .00005				
ANOVA	mean of OZ	35.95	29.42	25.63	33.79	33.71
	p-value	.0925				

A covariance analysis was also performed on HR, ST...etc at

time point 3 with OZ as a concomitant variable. The conclusion is that in this experiment the size of occluded zone is not a significant concomitant variable and does not account for difference in treatments.

3.3 Are arrhythmias related to time of mortality ?

We already mentioned that the number of VT or VF, Q-wave occurrence and total PVC were limited by the value of the mortality since they were measured over a period of time. To verify this assertion, tables 1-3 illustrating the relation between those variables and the time of mortality are shown below.

In the first table, the mean of log PVC increases with the time of death. However the mean for the rats that survived and the mean for those that died between 3 and 24 hours are about the same. This is probably an artifact since the number of total PVC are recorded up to 4 hours only. The log transformation is used to make the distribution of PVC more normal. In the second table, the total number of arrhythmias increases with the time of death and in the third table the longer a rat survives the larger is the chance that a Q-wave occurs.

Table 1. Log of total PVC at 4 hours.

time of death	mean	SD
(1) before 10 min	2.75	1.31
(2) between 10min&3hr	5.21	1.73
(3) between 3hr&24hr	6.96	0.90
(4) survive	6.67	1.06

Table 2. Total number of arrhythmias at 4 hours.

time of death	total # of arrhytmias					total
	0	1	2 to 4	5 to 9	10 and +	
(1)	7	3	4	1	0	15
(2)	0	0	1	2	4	7
(3)	0	1	0	4	4	9
(4)	2	4	8	3	11	28
total	9	8	13	10	19	59

Table 3. Q-wave occurrence

Q-wave occurrence	time of death			
	before 10min	bet 10min&4hr	bet 4hr&24hr	survive
yes	0	9	5	31
no	16	4	0	2
total	16	13	5	33

In summary, it does not seem possible to predict the death of a rat from early measurements of variables such as BP, HR, ST, etc. This statement is stonger in the cases of HR and ECG measurements than in the case of BP. Also, the differences in treatments in the halothane dose response experiment could not be explained by the size of occluded zone. However, this is not the case when comparing BP between groups of rats that died and survived, indicating that OZ is a very important variable to consider when analysing this type of data. Finally, as expected, the arrhythmias are related to the time of mortality.

In the next sections we analyse the set of repeated BP and DSTR measurements using manova types models and analyse the derived variable arrhythmia score using log-linear models.

4. EXAMINATION OF TIME-RESPONSE VARIABLES

The time-response variables recorded during the experiment can be analysed using growth curves model without restriction on growth i.e. on the slope of the curves. A variety of methods for the comparison of growth curves has been developed (e.g. Box (1950), Potthoff and Roy (1964), C.R. Rao (1965,1966), Grizzle and Allen (1969)). Unfortunately most of these methods are not appropriate for our data since they require complete data.

Koziol et al. (1981) proposed a distribution-free statistical methodology for the comparison of growth curves that may be used with incomplete data. Furthermore Koziol and Yuh (1982) developed a score test, as defined by Cox and Hinkley (1974), for testing group differences when observations are missing.

Both of these methods assumed that missing data are missing at random. In other words, the assumption is that the variables are missing without regard to values that would have been observed. The results of the preliminary analyses seem to indicate that this assumption is not violated in our data.

In this section, the two methods suggested above are described and the results of their applications using the appropriate data are presented.

4.1 Manova approach

The score statistic derived by Koziol and Yuh is based on a multivariate analysis of variance model which allows for missing observations. This test is described below.

4.1.1 Description of the score test

In general, several measurements (p) are collected for each experimental unit in a set of k treatments. Those measurements are assumed to be independent observations on p-dimensional multivariate normal variates with mean vectors μ_1, ... , μ_k and a common unknown covariance matrix Ω. Moreover, it is assumed that the pattern of missing values is such that the data matrix has a monotonic or nested pattern: for each line representing an experimental unit when one observation is missing all the other observations to its right are also missing.

It should be pointed out that this special case of missing data allows us, as shown in the Appendix, to find explicit expressions for the maximum likelihood estimates of the mean, variances and covariances of the underlying normal population.

In the framework of this model, we would like to determine whether the mean vectors differ among the treatments groups. For this purpose the following form of the score statistic was derived by Koziol and Yuh

$$W_u = \sum_{i=1}^{k} (\bar{Y}^i - \hat{\mu})' \hat{\Omega}^{-1} \overset{*}{S}_i^{-1} \hat{\Omega}^{-1} (\bar{Y}^i - \hat{\mu}) \tag{4.1}$$

where

$$\overset{*}{S}_i = (n_i)^{-2} \sum_{j=1}^{ni} \begin{pmatrix} \hat{\Omega}_{joo}^{i^{-1}} & 0 \\ 0 & 0 \end{pmatrix} .$$

Here, $\hat{\mu}$ and $\hat{\Omega}^{-1}$ are the maximum likelihood estimates under the null hypothesis of the mean vector and the variance-covariance matrix respectively, $\hat{\Omega}_{joo}$ is the matrix of the elements of $\hat{\Omega}$ corresponding to the variables observed for the j^{th} unit, $\bar{Y}^i$ is the estimate of the mean vector for treatment i, and n_i is the number of rats in treatment i.

More details concerning the notation used are presented in Appendix. In addition, the maximum likelihood (ML) statistic and the score statistic for complete data and the ML statistic for incomplete data are derived in the appendix. It is found that with complete data the ML and the score statistics are exactly the same. With incomplete data the ML statistic cannot be reduced to a simple form so that the comparison of the two statistics is not straightfoward. However these statistics are asymptotically equivalent and either could be used for our analysis.

The test we used in this section is based on the score statistic. Under the null hypothesis that the treatment means μ_i are equal, W_u has approximately a chi-square distribution with $p(k-1)$ degrees of freedom. Hence large values of W_u compared to a $\chi^2(pk-p)$ will indicate that the treatment means vectors are significantly different. We can now apply this test to our data.

4.1.2 Application of the score test

First we should verify if multivariate normality of the variables is a reasonable assumption for our time-response data. We only checked for normal marginals assuming that we could extend it to the joint distribution. For this purpose ,normal probability plots of BP, HR, R, RS, DSTR [1] for the first four time points were produced. The plots suggested that the assumption of normality seems acceptable only for BP and HR. Some transformations to normality were attempted on the other variables but without success. The normal plots of BP and DSTR for the control groups and the halothane data at time point 3 are presented in figures 6 to 9 respectively.

The analysis reported here is for the data on blood pressure. There are five treatment groups; one control group and four groups with halothane administered at a concentration

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[1] The variables DST,DSTR,DSTRS represent different corrections to ST and DSTR is the one Dr. Walker usually considers.

Figure 6. Normal plot of BP at time point 3 for the control groups data.
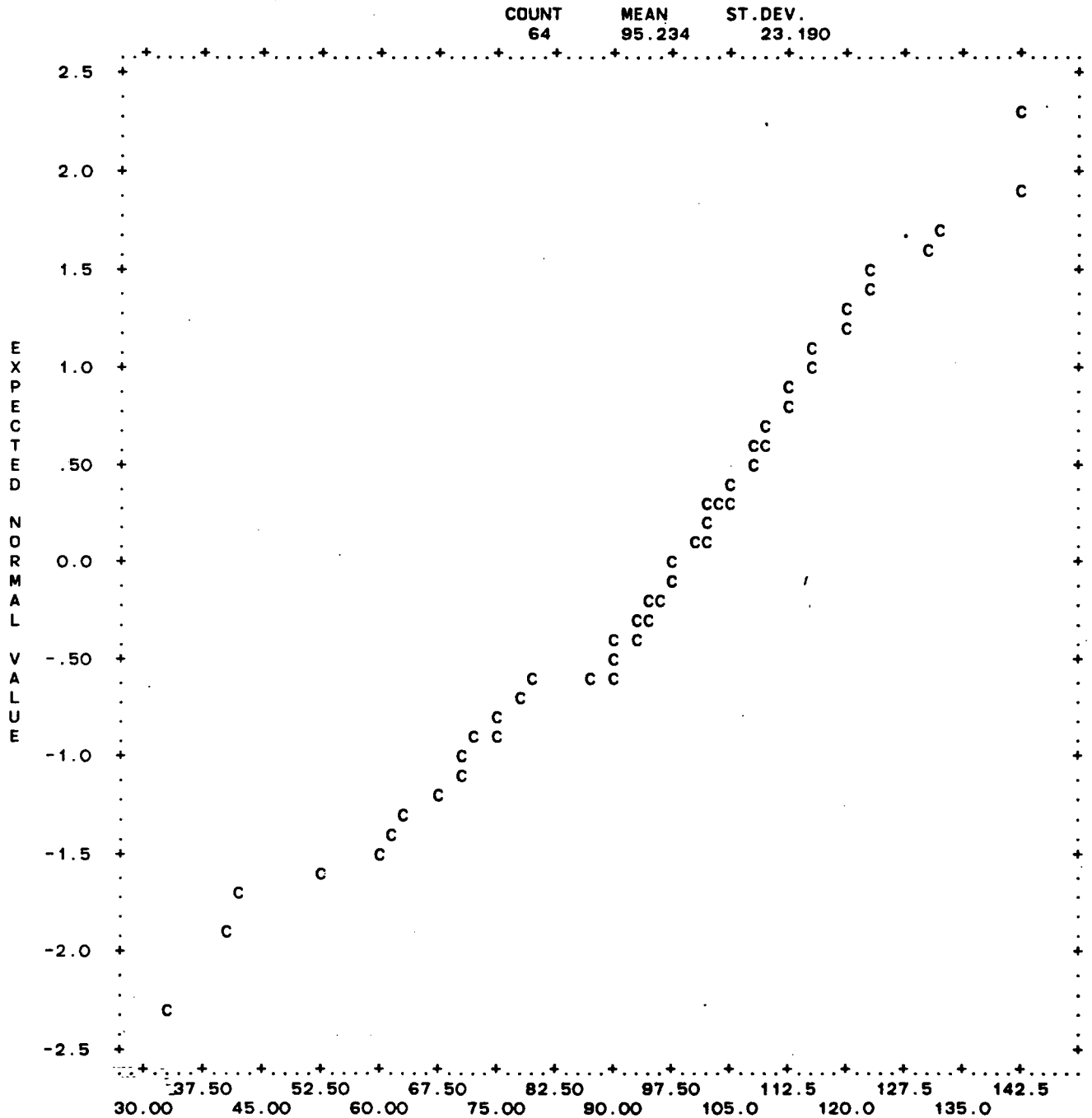

NORMAL PLOT OF VARIABLE

Figure 7. Normal plot of DSTR at time point 3 for the control groups data.

NORMAL PLOT OF VARIABLE

	COUNT	MEAN	ST.DEV.
	58	0.183	0.143

```
         ...+....+....+....+....+....+....+....+....+....+....+....+....+....+....+....
   2.5  +                                                                          +
        .                                                                    C    .
   2.0  +                                                                          +
        .                                            C                             .
        .                                    C                                     .
   1.5  +                                  C                                       +
        .                              C                                           .
        .                            C                                             .
E       .                          C                                               .
X  1.0  +                        C                                                  +
P       .                      CC                                                  .
E       .                      CC                                                  .
C       .                    CC                                                    .
T  .50  +                    C                                                     +
E       .                    C                                                     .
D       .                   C                                                      .
        .                  C                                                       .
N       .                 C                                                        .
O       .               CC                                                         .
R  0.0  +               CC                                                         +
M       .               C                                                          .
A       .               CC                                                         .
L       .              C                                                           .
V -.50  +              C                                                           +
A       .              C                                                           .
L       .             CC                                                           .
U       .             C                                                            .
E       .             C                                                            .
  -1.0  +             C                                                            +
        .            C                                                             .
        .          C                                                               .
        .          C                                                               .
        .          C                                                               .
  -1.5  +         C                                                                +
        .        C                                                                 .
        .      C                                                                   .
  -2.0  +                                                                          +
        .                                                                          .
        .     C                                                                    .
  -2.5  +                                                                          +
        ...+....+....+....+....+....+....+....+....+....+....+....+....+....+....+....
        -.06     .06      .18      .30      .42      .54      .66      .78
            0.0      .12      .24      .36      .48      .60      .72      .84
```
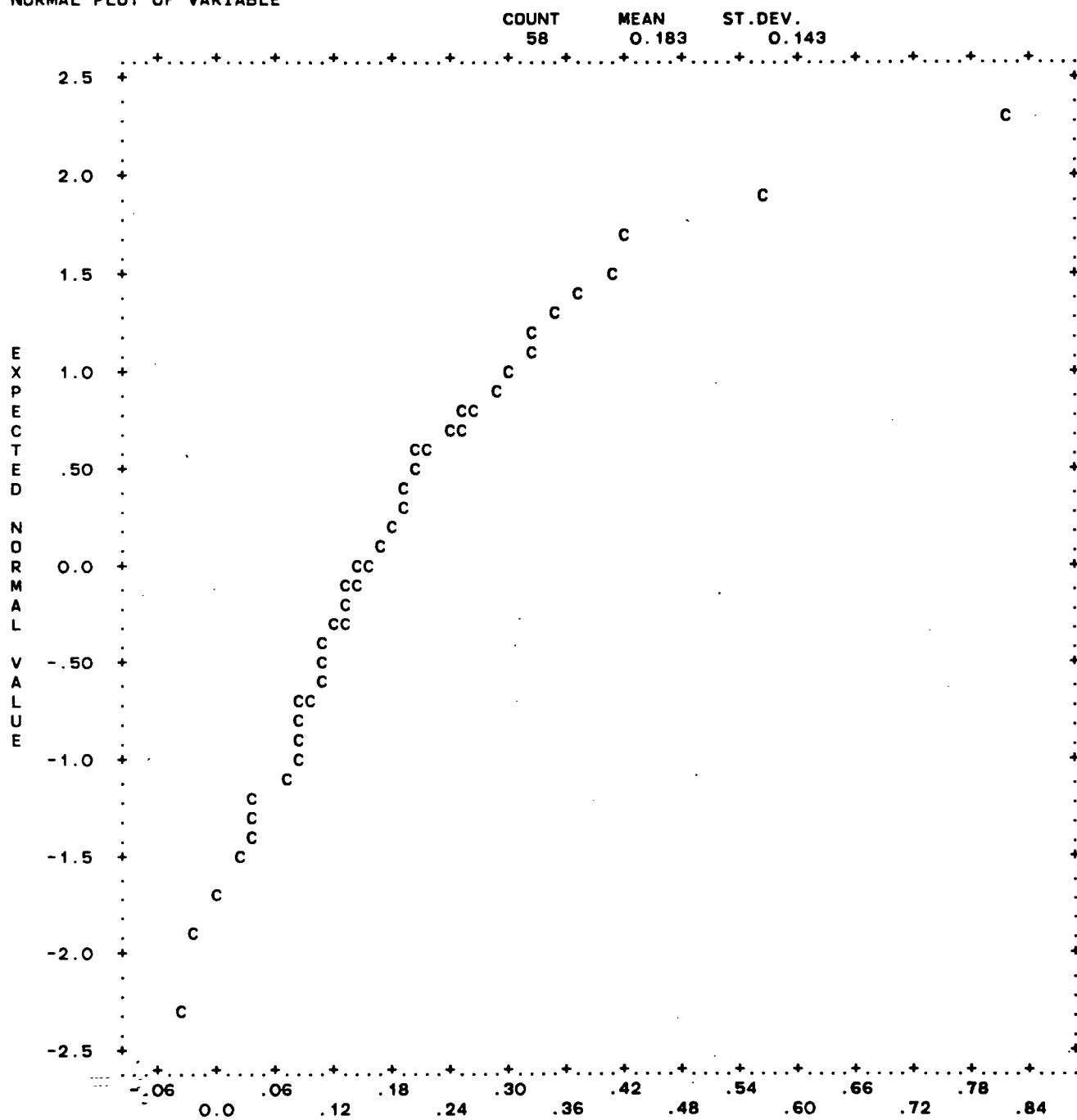
Figure 8. Normal plot of BP at time point 3 for the halothane data.

NORMAL PLOT OF VARIABLE

Figure 9. Normal plot of DSTR at time point 3 for the halothane data.
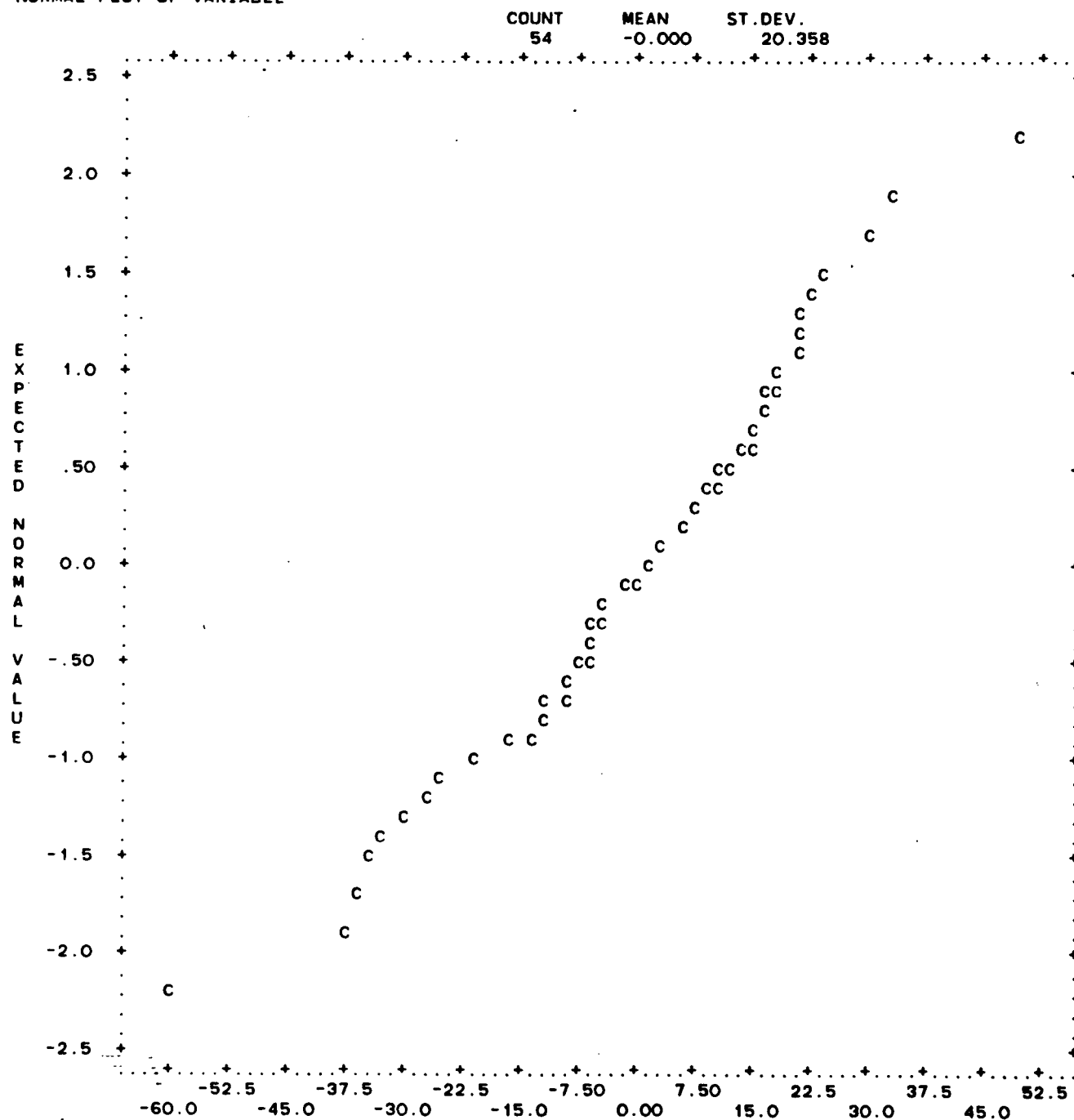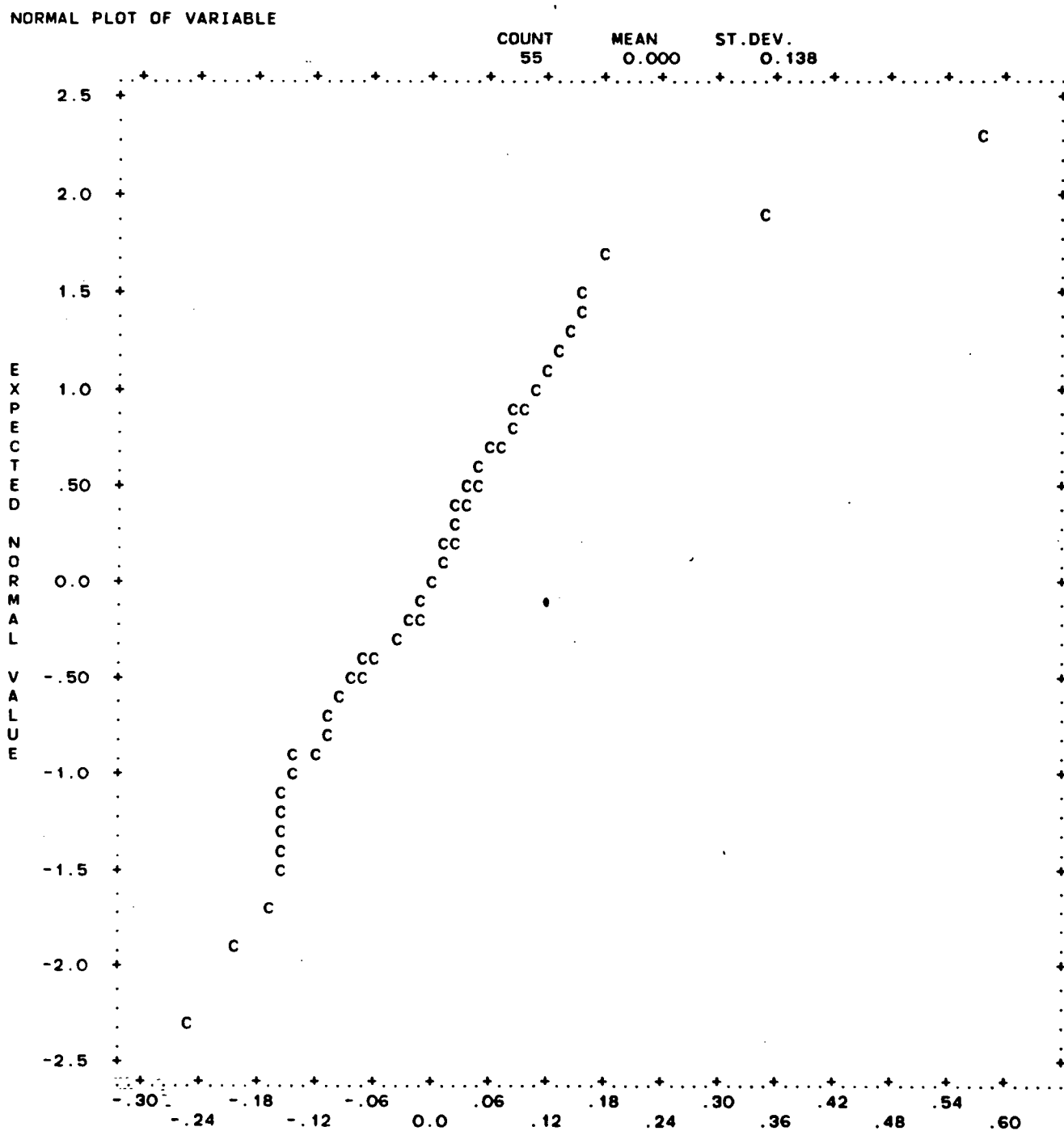
NORMAL PLOT OF VARIABLE

of 0.25, 0.50, 1.0 and 2 percent. Some characteristics of these data are worth mentioning. In the experiment, all the rats that were administered halothane at 2% concentration died before the end of the experiment and none of them survived more than one hour after ligation. For this reason the score test was performed on two data sets separately. The first one included BP at time points 3 to 7 (i.e. 1 to 30 min after ligation) for all five treatments and the second included BP at time points 3 to 11 only for the first four treatments. The measurements 24 hours after ligation were not reliable and therefore were not included. In those two data sets, one rat had missing values other than by death. In order that the data matrix had a nested pattern, this rat was deleted.

The two data sets are given in tables 4 and 5; the results obtained from Koziol's program are summarized below.

Table 4. Blood pressure data for all five treatments at time points 3 to 7.

treat #	3	4	time points 5	6	7
1	112.50	100.50	102.50	102.50	107.50
1	92.50	102.50	105.00	100.00	110.00
1	132.50	125.00	115.00	112.50	110.00
1	110.00	110.00			
1	122.50	127.50			
1	102.50	107.50	107.50	102.50	90.00
1	42.50	42.50			
1	107.50	80.00			
1	110.00	130.00	115.00	105.00	112.50
1	97.50	97.50	80.00	82.50	82.50
1	90.00	70.00	85.00	85.00	92.50
2	115.00	115.00	107.50	107.50	112.50
2	120.00				
2	125.00	125.00	120.00	120.00	117.50
2	95.00	90.00	95.00	90.00	100.00
2	97.50	70.00			
2	87.50	65.50	85.00	90.00	105.00
2	90.00	87.50	97.50	95.00	100.00
2	97.50	92.50	57.50	55.00	90.00
2	107.50	107.50	145.00	110.00	105.00
2	102.50	130.00	85.00	80.00	127.50
3	107.50	107.50	102.50	102.50	102.50
3	67.50	20.00			
3	97.50	108.50	94.50	102.50	102.50
3	105.00	105.00			
3	85.00	60.00			
3	100.00	105.00	105.00	105.00	110.00
3	95.00	95.00	90.00	100.00	100.00
3	85.00	92.50	92.50	92.50	90.00
3	82.50	77.50	75.00	65.50	65.00
3	92.50	75.00	40.00	35.00	
3	62.50	75.00	115.00	110.00	100.00
4	70.00	67.50	67.50	77.50	77.50
4	45.00	37.50	45.00	45.00	47.50
4	52.50	22.50	90.00	65.00	60.00
4	100.00	100.00	100.00	100.00	97.50
4	47.50	30.00			
4	102.50	90.00			
4	115.00	110.00	100.00	110.00	105.00
4	97.50	97.50	97.50	105.00	95.00
4	95.00	125.00	130.00	125.00	115.00
4	72.50	87.50	65.00	57.50	92.50
4	105.00	105.00	105.00	105.00	102.50
5	52.50	52.50	57.50	52.50	44.00
5	15.00	10.50			
5	17.50	17.50	14.50	10.50	
5	25.00	27.50			
5	52.50	47.50			
5	65.00	65.50	65.50	47.50	27.50
5	75.00	62.50			
5	42.50	27.50	40.00		
5	65.00	67.50	67.50	20.00	
5	45.00	15.00			
5	100.00	97.50	62.50		

Table 5.   Blood Pressure data for the four treatments excluding halothane at 2.0% at time points 3 to 11.

treat #	\ time points 3	4	5	6	7	8	9	10	11
1	112.50	100.50	102.50	102.50	107.50	107.50	95.00	102.50	100.50
1	92.50	102.50	105.00	100.00	110.00	117.50	97.50	102.50	112.50
1	132.50	125.00	115.00	112.50	110.00	110.00	127.50		
1	110.00	110.00							
1	122.50	127.50							
1	102.50	107.50	107.50	102.50	90.00	112.50	107.50	110.00	112.50
1	42.50	42.50							
1	107.50	80.00							
1	110.00	130.00	115.00	105.00	112.50	110.00	115.00	102.50	92.50
1	97.50	97.50	80.00	82.50	82.50	102.50	100.00	95.00	95.00
1	90.00	70.00	85.00	85.00	92.50	97.50	107.50	97.50	90.00
2	115.00	115.00	107.50	107.50	112.50	107.50	112.50	107.50	107.50
2	120.00								
2	125.00	125.00	120.00	120.00	117.50	125.00	122.50	120.00	120.00
2	95.00	90.00	95.00	90.00	100.00	107.50	100.00	100.00	92.50
2	97.50	70.00							
2	87.50	65.50	85.00	90.00	105.00	90.00	85.00	87.50	100.00
2	90.00	87.50	97.50	95.00	100.00	95.00	102.50		
2	97.50	92.50	57.50	55.00	90.00	97.50	110.00	115.00	105.00
2	107.50	107.50	145.00	110.00	105.00	112.50			
2	102.50	130.00	85.00	80.00	127.50	97.50	117.50	102.50	127.50
3	107.50	107.50	102.50	102.50	102.50	97.50	98.50	102.50	92.50
3	67.50	20.00							
3	97.50	108.50	94.50	102.50	102.50	107.50	117.50	112.50	
3	105.00	105.00							
3	85.00	60.00							
3	100.00	105.00	105.00	105.00	110.00	110.00	115.00	107.50	105.00
3	95.00	95.00	90.00	100.00	100.00	100.00	95.00	90.00	100.00
3	85.00	92.50	92.50	92.50	90.00	110.00	100.00	102.50	87.50
3	82.50	77.50	75.00	65.50	65.00	72.50	72.50	67.50	67.50
3	92.50	75.00	40.00	35.00					
3	62.50	75.00	115.00	110.00	100.00	100.00			
4	70.00	67.50	67.50	77.50	77.50	77.50	72.50	65.00	55.00
4	45.00	37.50	45.00	45.00	47.50	45.00	50.00	45.00	50.00
4	52.50	22.50	90.00	65.00	60.00	65.50	52.50	47.50	57.50
4	100.00	100.00	100.00	100.00	97.50	92.50			
4	47.50	30.00							
4	102.50	90.00							
4	115.00	110.00	100.00	110.00	105.00	105.00	105.00	105.00	105.00
4	97.50	97.50	97.50	105.00	95.00	92.50	92.50	92.50	92.50
4	95.00	125.00	130.00	125.00	115.00	117.50	110.00	105.00	102.50
4	72.50	87.50	65.00	57.50	92.50	82.50	57.50	50.00	50.00
4	105.00	105.00	105.00	105.00	102.50	100.00	95.00	92.50	87.50

Maximum likelihood estimate of the mean vector
for each treatment

time	data set # 1					data set # 2			
	control	0.25%	0.50%	1.0%	2.0%	control	0.25%	0.50%	1.0%
3	101.82	103.75	89.09	82.05	50.45	101.77	103.75	89.09	82.05
4	99.36	100.23	83.73	79.32	44.64	99.36	100.38	83.72	79.32
5	97.31	98.50	85.24	85.52	49.84	98.04	98.47	86.18	86.52
6	94.42	93.78	84.10	83.64	38.25	95.36	93.65	85.18	84.92
7	97.28	105.40	86.52	84.52	43.42	98.35	105.34	88.84	85.98
8						103.80	103.41	92.22	85.13
9						103.00	106.34	91.57	80.15
10						100.70	103.57	89.24	76.33
11						99.68	105.83	87.63	76.35

Results of the score test

__data set #1__

$\chi^2(20) = 51.445$

p-value = .00014

__data set #2__

$\chi^2(27) = 30.854$

p-value = .277

These results suggest that no drugs and small dose (i.e. <
2%) of halothane have no different effect on blood pressure for
rats that have a heart attack. However, when administered at 2%
concentration halothane clearly lowered down the blood pressure.
This was expected from inspection of the raw data.

Other analyses such as pairwise comparisons of treatments
would probably provide more information on the effect of
halothane on BP. It would also be interesting to see if the
effect of halothane on BP is the same whether the rat has a
heart attack or not. Unfortunately those analyses could not be
performed because of the inaccessibility of the program.

Another approach that is not rigorous but might be an alternative to the score test has been considered and the results compared to those obtained with the score test. The method consists of replacing the missing values by their regression estimates. This was done using the BMDP:PAM program where each missing value for a variable is estimated by regressing it on all variables that have non-missing values in the case. Once all the missing values are replaced we can perform a one-way multivariate analysis of variance on the new dataset using any program or statistical package available. The results obtained for the BP data using this method were as follows.

data set #1

$F(20,150) = 3.28$

p-value < .00005

MAXROOT = .6239

p-value < .00005

data set #2

$F(27,91) = 1.36$ [2]

p-value = .1426

MAXROOT = .5301 [3]

p-value = .0250

The p-values obtained with this test appear to be too small. This was expected since with this method we did not take into account the loss of degrees of freedom due to estimating the missing data from the observed data. Unfortunately the formulas for the estimated degrees of freedom of the F statistic

---

[2] F corresponds to a transformation of Wilk's lambda that can be compared with the F distribution (Rao 1973).
[3] MAXROOT is Roy largest root statistic (Morrison,1976).

are such that no straightforward calculation could be made to adjust them for missing values. The significance level of the approximate F-test is closer to the significance level of the score test. This may not be surprising since both statistics are developed through the likelihood ratio principle.

Rigorous hypothesis testing cannot be performed with this approach. Nevertheless, it still provided some good indications about the significance of treatments.

4.2 Non-parametric approach

The test described in the preceeding section assumes multivariate normality of the variables which as we have seen is not a reasonable assumption for some variables (R, RS, DSTR). For any analysis which includes variables we then need a test which involves no explicit assumptions about the distribution of the observations. Koziol et al. (1981) have developed a test, based on a multivariate rank statistic for the comparison of growth curves, which is distribution-free. This test may be used with incomplete observations and in situations where parametric models for growth may not be appropriate. This procedure is appropriate for the variables mentioned above.

4.2.1 Description of the multivariate rank test

Let F_i denote the p-variate continuous cumulative distribution function for the i^{th} group. The hypothesis of

interest is

$$H_o : F_i = F \quad \text{for all } i=1,2,\ldots,k$$

against the alternative that $F_i < F_j$ for some $i,j=1,2,\ldots,k$; that is, $F_{it}(\cdot) \leq F_{jt}(\cdot)$ for each $t=1,2,\ldots,p$, with at least one strict inequality. The test proposed by Koziol et al. (1981) for the detection of this kind of alternative i.e. stochastic ordering of distributions, is analogue to the rank tests suggested for randomized block and makes use of the basic rank permutation principle. According to this principle all permutations of the N vectors of observations are equally likely under the null hypothesis (see Puri and Sen 1971).

Now let n_i be the initial number of experimental units (rats in our case) in group i with $N = \sum_{i=1}^{k} n_i$. Let $y_{tj}^{(i)}$ denote the value of the time-response variable of the j^{th} rat in group i at time t. Note that $y_{tj}^{(i)}$ may not be observed so that in general only n_{it} of the n_i rats in group i yield observations at time t. If $y_{tj}^{(i)}$ is observed, let $R_{tj}^{(i)}$ denote its rank among the $n_{.t} = \sum_{i=1}^{k} n_{it}$ available values at time t. Define for $i=1,\ldots,k$

$$S_{it} = (n_{it})^{-1} \sum_{j=1}^{ni} a_t (R_{tj}^{(i)}) ,$$

here the a_t , $t=1,2,\ldots,T$ are the univariate score functions. Without loss of generality, the score functions are chosen such that $\bar{a}_t = \sum_{k=1}^{n.t} a_t(k) = 0.$ When $y_{tj}^{(i)}$ is not observed then

$$a_t (R_{tj}^{(i)}) = 0.$$

From the rank permutation principle, it can be shown that

$$E(S_{it} \mid P_N) = 0$$

and

$$\text{cov}(S_{it}, S_{ms} \mid P_N) = (n_{it} n_{ms})^{-1} n_i \{\delta_{im} - (n_m/N)\}(N-1)^{-1}$$

$$\times \sum_{i=1}^{k} \sum_{j=1}^{ni} a_t (R_{tj}^{(i)}) a_s (R_{sj}^{(i)}) \qquad (4.2)$$

where δ_{im} denotes the Kronecker delta: $\delta_{im} = 1$ if $i=m$ and 0 otherwise, and P_N represents the permutation probability measure generated by $N!/ \prod_{i=1}^{k} n_i !$ possible distinct permutations of the observed data vectors.

The test proposed for testing the hypothesis formulated previously is based on the following multivariate statistic

$$M_N = S_N^\intercal \, V_N^- \, S_N \qquad (4.3)$$

where $S_N^\intercal = (S_{1.}, S_{2.}, \ldots, S_{I.})$ with $S_{i.} = \sum_{i=1}^{p} S_{it}$ and V_N^- denotes a generalized inverse of V_N, the covariance matrix of S_N as calculated from (4.2).

Under P_N and H_o, the permutation distribution of M_N is distribution-free. To carry out the test, we would need to study all the $N! / \prod_{i=1}^{k} n_i!$ distinct values of M_N. Except for small values of N and p an exact application of the test is difficult because of the large amount of computation. Nevertheless, this permutation test procedure can be simplified in large samples. It can be proved that under rather general conditions the joint conditional asymptotic distribution of the S_{it} is multivariate normal. Using this fact it can be shown that the quadratic form M_N has approximately a chi-square distribution with degrees of freedom equal to the rank of V_N. Hence large values of M_N relative to $\chi^2(k)$ would lead to the rejection of H_o. In the special case where $n_{it} = n_i$ for all t the rank of V_N is $k-1$.

4.2.2 Application of the multivariate rank test

The data on DSTR in the halothane experiment was chosen for the application of this test (see tables 6 and 7). As before, two data sets were analysed separately; the first data set contained observations at time points 3 to 7 for the 5 treatments and the second contained observations at time points 3 to 11 for the first four groups.

The Wilcoxon score was chosen for a_t ;

$$a_t(j) = j - (n_{.t} + 1)/2 \; , \; j=1,2,\ldots,n_{.t} \quad t=1,2,\ldots,p.$$

With the first data set, it was found that

$$S_N^T = (14.69, 27.36, -26.87, -9.33, -4.49)$$ with corresponding

permutation covariance matrix

$$V_N = \begin{bmatrix} 333.93 & -74.25 & -73.88 & -72.49 & -134.81 \\ -74.25 & 264.83 & -65.83 & -64.61 & -119.14 \\ -73.89 & -65.83 & 261.85 & -64.25 & -118.78 \\ -72.49 & -64.61 & -64.25 & 252.25 & -116.39 \\ -134.81 & -119.14 & -118.78 & -116.39 & 889.00 \end{bmatrix} .$$

Hence from (3.3) M_N = 5.52, giving an approximate p-value of .36.

Table 6. DSTR for all five treatments and at time points 3 to 7.

treat #	\[time points\] 3	4	5	6	7
1	.82	1.08	1.02	.98	.98
1	.17	.24	.22	.23	.22
1	.08	.28	.22	.27	.24
1	.32	.40			
1	.13	.33			
1	.35	.20	.25	.24	.24
1	.09	.12			
1	.29	.31			
1	.41	.49	.49	.45	.42
1	.00	.08	.15	.13	.15
1	.11	.34	.34	.38	.37
2	.12	.23	.11	.03	.18
2	.16	.08	.10	.10	.01
2	.20				
2	.23	.33	.33	.33	.39
2	.36	.33	.32	.34	.31
2	.23	.38			
2	.26	.51	.52	.52	.53
2	.17	.27	.32	.35	.31
2	.19	.27	.24	.29	.28
2	.31	.39	.37	.41	.37
2	.28	.34	.61	.51	.57
3	.24	.39	.34	.33	.29
3	-.02	.20			
3	-.01	.07	.07	.09	.10
3	.18	.21			
3	.14	.20	.27	.26	.28
3	.21	.27	.29	.28	.28
3	.25	.32	.31	.32	.30
3	.19	.23	.23	.22	.23
3	.18	.18	.18	.18	.18
3	.06	.14	.15	.17	
3	.04	.10	.11	.12	.13
4	.16	.25	.31	.30	.29
4	.33	.56	.81	.47	.48
4	.06	.09	.25	.37	.31
4	.20	.24	.25	.26	.26
4	.21	.25			
4	.16	.21			
4	.06	.06	.06	.06	.06
4	.15	.21	.24	.26	.27
4	.32	.32	.32	.31	.32
4	.02	.10	.19	.21	.24
4	.18	.22	.23	.23	.23
5	.52	.84	.88	.87	.96
5	-.03	.17			
5	.02	.08	.13	.09	
5	.08	.10			
5	.18	.29			
5	.01	.02	.03	.03	.03
5	.16	.28			
5	.20	.23	.28		
5	.35	.35	.35	.35	
5	.20	.27			
5	.17	.28	.46		

Table 7. DSTR for the four treatments excluding halothane at 2.0% for the time points 3 to 11.

treat #	\-	\-	\-	\-	time points	\-	\-	\-	\-
	3	4	5	6	7	8	9	10	11
1	.82	1.08	1.02	.98	.98	.97	.99	.98	1.01
1	.17	.24	.22	.23	.22	.22	.21	.22	.22
1	.08	.28	.22	.27	.24	.22	.25		
1	.32	.40							
1	.13	.33							
1	.35	.20	.25	.24	.24	.22	.20	.21	.20
1	.09	.12							
1	.29	.31							
1	.41	.49	.49	.45	.42	.42	.36	.39	.43
1	.00	.08	.15	.13	.15	.08	.11	.11	.10
1	.11	.34	.34	.38	.37	.37	.35	.33	.40
2	.12	.23	.11	.03	.18	.11	-.01	.03	-.05
2	.16	.08	.10	.10	.01	.04	-.01	.04	.16
2	.20								
2	.23	.33	.33	.33	.39	.33	.33	.38	.35
2	.36	.33	.32	.34	.31	.32	.33	.35	.30
2	.23	.38							
2	.26	.51	.52	.52	.53	.54	.52	.54	.49
2	.17	.27	.32	.35	.31	.34	.34		
2	.19	.27	.24	.29	.28	.26	.23	.23	.23
2	.31	.39	.37	.41	.37	.35			
2	.28	.34	.61	.51	.57	.53	.57	.53	.53
3	.24	.39	.34	.33	.29	.30	.31	.29	.22
3	-.02	.20							
3	-.01	.07	.07	.09	.10	.09	.03	.03	
3	.18	.21							
3	.14	.20	.27	.26	.28	.28	.29	.28	.28
3	.21	.27	.29	.28	.28	.28	.28	.28	.28
3	.25	.32	.31	.32	.30	.30	.31	.29	.29
3	.19	.23	.23	.22	.23	.23	.22	.23	.22
3	.18	.18	.18	.18	.18	.18	.18	.18	.18
3	.06	.14	.15	.17					
3	.04	.10	.11	.12	.13	.13	.13	.12	.12
4	.16	.25	.31	.30	.29	.28	.29	.28	.30
4	.33	.56	.81	.47	.48	.47	.50	.62	.61
4	.06	.09	.25	.37	.31	.37	.39	.37	.37
4	.20	.24	.25	.26	.26	.25			
4	.21	.25							
4	.16	.21							
4	.06	.06	.06	.06	.06	.06	.06	.06	.06
4	.15	.21	.24	.26	.27	.25	.27	.27	.28
4	.32	.32	.32	.31	.32	.32	.32	.32	.32
4	.02	.10	.19	.21	.24	.23	.22	.16	.17
4	.18	.22	.23	.23	.23	.23	.23	.23	.23

Similarly, it was found for the second data set that

$$S_N^T = (15.21, 31.00, -38.64, -5.99) \text{ and}$$

$$V_N = \begin{bmatrix} 763.32 & -217.89 & -218.20 & -209.47 \\ -217.89 & 560.35 & -186.99 & -179.56 \\ -218.20 & -186.99 & 561.65 & -179.77 \\ -209.47 & -179.56 & -179.77 & 519.89 \end{bmatrix}.$$

With these, $M_N = 4.71$, yielding an approximate p-value of .32.

This analysis seems to indicate that the administration of halothane at different doses when rats have a heart attack has no effect on the values of DSTR measured by the ECG. Unlike blood pressure, DSTR does not seem to be affected by a 2% concentration of halothane.

For comparison with the two methods proposed in the preceeding section, the multivariate rank statistic was also computed for the blood pressure data. The results are summarized below.

data set #1

$M_N = 20.42$

p-value = .001

data set #2

$M_N = 6.89$

p-value = .141

In data set #1, the significant difference between treatments is less marked than using any of the other tests described earlier. On the other hand, for data set #2 the significance level of this test, which is comparable to the one of the approximate F-test, is lower than the significance level of the score test.

In conclusion, the score test should be used, for the analysis of the type of data we have at hand, when the assumption of multivariate normality is reasonable. However if the application of this test is not possible, one could estimate the missing values and perform an ordinary manova. This method is not rigorous so we must be cautious when interpreting the results. On the other hand, when the assumption of normality is not acceptable the multivariate rank test should be used. This test could also be performed with normal variates but the test would not be as sensitive as the score test.

5. LOG-LINEAR MODELS

An important objective of the experiments is to investigate the antiarrhythmic action of different drugs. The responses measured for this purpose are composed of the number and the duration of ventricular tachycardia and ventricular fibrilation (spontaneous or non-spontaneous), the total number of premature ventricular contractions and death by irreversible ventricular fibrilation. As shown in section 3.3 the observation of these variables is censored by the death of the rats. Hence comparison of means of these variables to study the effect of treatments on arrhythmia could be misleading.

Dr. Walker and his group partially solved this problem by constructing the arrhythmia score variable described in section 2.2. This variable combined the information given by the variables mentioned above, to measure the severity of the arrhythmia. The scores were defined such that the scale could be regarded as linear.

Considering the variable as continuous and analysing it using statistical methods developed for continuous variables is not very efficient although it is probably not harmful. These data can be set up in the form of a contingency table with the first dimension being the treatments and the second dimension the arrhythmia scores as shown in table 8. It is then more appropriate to use log-linear models to analyse this two dimensional contingency table.

Table 8. Distribution of the arrhythmia scores after 4 hrs by treatment groups.

treat	AS4									total
	0	1	2	3	4	5	6	7	8	
control	0	0	0	0	2	4	1	1	3	11
0.25%	1	1	0	1	1	4	1	1	1	11
0.50%	4	1	1	0	2	2	0	1	0	11
1.0%	4	0	0	4	1	1	0	1	0	11
2.0%	4	0	0	0	2	1	0	4	0	11
total	13	2	1	5	8	12	2	8	4	55

The homogeneity model (i.e. the model with no interaction) was fitted to these observed counts. The goodness of fit test (GOF) using the Pearson chi-square statistic yielded a p-value of .058. The adjusted residuals are given in table 9.

The GOF test seems to indicate that the distribution of arrhythmia scores is different among the treatment groups. In addition, the adjusted residuals show that the lack of fit is caused by the control group which suggests that halothane might have an antiarrhythmic action. However this conclusion should be treated with caution because of all the empty cells found in the table. In fact the results obtained when fitting a log-linear model to a table with so many empty cells are not very meaningful.

Table 9.   Adjusted   residuals   resulting   from   fitting   the
            homogeneity model to the data in table 8.

| treat | | AS4 | | | | | | | | |
		0	1	2	3	4	5	6	7	8
control		-2.1	-0.7	-0.5	-1.2	0.4	1.3	1.1	-0.6	2.9
0.25%		-1.3	1.1	-0.5	0.0	-0.6	1.3	1.1	-0.6	0.3
0.50%		1.1	1.1	2.0	-1.2	0.4	-0.3	-0.7	-0.6	-1.0
1.0%		1.1	-0.7	-0.5	3.5	-0.6	-1.1	-0.7	-0.6	-1.0
2.0%		1.1	-0.7	-0.5	-1.2	0.4	-1.1	-0.7	2.3	-1.0

To overcome this problem one might collect more data or, if
not possible, reduce the number of cells either by collapsing
them or classifying according to another variable with less
categories.

The following way to combine the scores was suggested by
Dr.   Walker :   0 and 1, 2 and 3, 4, 5, and 6 to 9. Apparently
this categorization is representative of the scoring system
originally constructed by Dr Walker.   The frequency table
associated with these new categories can be obtained from table
8.   In spite of the empty cells the homogeneity model was also
fitted to these data. It appeared that the homogeneity model
fits better than before.   The Pearson chi-square was equal to
23.49 with 16 degrees of freedom giving a p-value of .101.
However as in the first analysis, there was also a pattern in
the adjusted residuals of the control group.   More data would

certainly be necessary before reaching any conclusion.

Another solution proposed is to classify according to variables with few categories. For example, a two-way contingency table can be constructed with the first variable being the treatments and the second variable representing the incidence of irreversible VF. Table 10 shows the observed frequencies and the adjusted residuals obtained when fitting the homogeneity model to this frequency table.

Table 10.   Observed frequencies and adjusted residuals (in parenthesis) of the incidence of irreversible VF by treatment groups.

treat	irreversible VF	
	no	yes
control	5 (-2.7)	6 (2.7)
0.25%	8 (-0.3)	3 (0.3)
0.50%	11 (2.1)	0 (-2.1)
1.0%	10 (1.3)	1 (-1.3)
2.0%	8 (-0.3)	3 (0.3)
total	42	13

The Pearson chi-square obtained was 10.68 with 4 degrees of freedom giving a p-value of .03. This model does not fit the data very well suggesting that the administration of halothane

reduces the number of deaths by irreversible ventricular fibrilation.

Log-linear models also allow us to analyse multidimensional contingency tables. For example, as shown in table 11, a third variable representing the size of the occluded zone could be added. This would permit the study of the interaction among the three variables and maybe provide more information about the effect of treatments on irreversible VF.

Table 11. Observed frequencies of the incidence of irreversible VF by treatments and the size of the occluded zone.

treat	OZ	irreversible VF		
		no	yes	total
control	small	0	1	1
	medium	2	4	6
	large	3	1	4
0.25%	small	2	1	3
	medium	4	1	5
	large	2	1	3
0.50%	small	7	0	7
	medium	3	0	3
	large	1	0	1
1.0%	small	3	1	4
	medium	2	0	2
	large	5	0	5
2.0%	small	2	1	3
	medium	2	1	3
	large	4	1	5
	total	42	13	55

The analysis of this table using log-linear models was not

carried out since there are not enough data to get meaningful results.

In conclusion, log-linear models could be a useful tool to analyse arrhythmia if more data were available. It should be pointed out that this technique when used with the arrhythmia score variable does not consider the censoring problem caused by rats that died of heart failure.

6. CONCLUSION

The data set provided by Dr. Walker gave us the opportunity of applying various statistical techniques and being confronted with the common problem of missing data.

In a first step, it has been found that death of the rats could not be predicted by early measurements of the responses. This implied that the missing data could be considered as "missing at random" so that the process causing missing data could be ignored when making statistical inferences. It was also found that the size of the occluded zone could be a confounding variable when studying the effect of drugs on other responses. Furthermore we showed that the arrhythmia responses were censored by death of the rats.

In a second step, a score test and a multivariate rank test were proposed for the analysis of time-response variables with incomplete observations. The score test assumed multivariate normality of the observations while the multivariate rank test made no assumption on the distribution of the responses. An alternative to the score test consisting of replacing the missing values by an their regression estimates was also presented. This test lead to a p-value which appeared to be too small compared to the p-value of the score test and which could probably be explained by the loss of degrees of freedom due to estimating the missing data from the observed data. The application of these tests on the halothane data showed that only halothane at a concentration of 2% significantly lowered

the blood pressure while the value of DSTR did not seem to be affected by the administration of halothane.

In a third step, log-linear models were considered to analyse categorical variables such as the arrhythmia scores and the incidence of irreversible VF. Unfortunately the results obtained are not very reliable since there was not enough data for the application of this technique. An increase in the size of the treatment groups would be necessary to ascertain the effect of drugs on arrhythmias.

Finally it should be pointed out that when log-linear models are applied to the arrhythmia score variable, it does not consider the censoring caused by rats that died of heart failure. A more appropriate technique which would take into account this censoring problem is still to be developed.

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APPENDIX

DERIVATION OF THE MAXIMUM LIKELIHOOD STATISTIC AND THE SCORE

STATISTIC IN THE MANOVA SETTING

In this appendix, the maximum likelihood (ML) statistic and the score statistic will be derived under the multivariate normal model. The case of complete data and the case of incomplete data will be considered separately.

I. COMPLETE DATA

In a one-way classification multivariate analysis of variance, the model assumes that there are k independent samples from a p-dimensional multivariate normal distributions with mean vectors μ_1 ,..., μ_k and a common unknown covariance matrix Ω . We first assume that there are no missing observations. The question of interest is whether or not the treatment means are all equal.

The notation for the model is the following:

$$Y_{i1} ,..., Y_{ini} \quad \text{i.i.d.} \quad N_p (\mu_i , \Omega) \qquad i=1,...,k$$

The null hypothesis is

$$H_0 : \mu_1 = \mu_2 = ... = \mu_k .$$

The alternative hypothesis is

$$H_1 : \mu_i \text{ not all equal.}$$

The likelihood function can be written

$$L(\mu_1,\ldots,\mu_k,\Omega;Y) =$$

$$\prod_{i=1}^{k} \prod_{j=1}^{ni} (2\pi)^{-p/2} |\Omega|^{-1/2} \exp\{-1/2(y_{ij}-\mu_i)^{\top}\Omega^{-1}(y_{ij}-\mu_i)\}$$

and the loglikelihood is

$$l = C-N/2(\log|\Omega|)-1/2\{ \sum_{i=1}^{k} \sum_{j=1}^{ni} (y_{ij}-\mu_i)^{\top}\Omega^{-1}(y_{ij}-\mu_i)\} \quad (1)$$

where $N = \sum_{i=1}^{k} n_i$.

The following notation for the vector score function and the information matrix will be used throughout this appendix.

$$U_{.\mu}(\mu,\Omega;Y) = E[-(\partial/\partial\mu)\ l]$$

$$i_{\mu\mu}(\mu,\Omega) = E[-(\partial^2/\partial^2\mu)\ l]$$

$$i_{\mu\Omega}(\mu,\Omega) = E[-(\partial^2/\partial\mu\partial\Omega)\ l]$$

$$i_{\Omega\Omega}(\mu,\Omega) = E[-(\partial^2/\partial^2\Omega)\ l]$$

A. Derivation of the maximum likelihood statistic

In general, we wish to test the composite hypothesis H_0: $\psi = \psi_0$ against H_1: $\psi \neq \psi_0$, without specifying any value for the nuisance parameter λ . The ML statistic defined in Cox and Hinkley (1974) and also proposed by Wald (1943) is

$$W_e = (\widehat{\psi}-\psi_0)^\top \; i.(\widehat{\psi}:\widehat{\lambda}) \; (\widehat{\psi}-\psi_0) \tag{2}$$

where

$$i.(\psi:\lambda) = i_{\psi\psi}(\psi,\lambda) - i_{\psi\lambda}(\psi,\lambda)i_{\lambda\lambda}^{-1}(\psi,\lambda)i_{\lambda\psi}(\psi,\lambda) \tag{3}$$

and $\widehat{\psi}$, $\widehat{\lambda}$ are the maximum likelihood estimates (mle) of ψ and λ.

This test is derived by Cox and Hinkley (1974, example 9.23) in the multivariate one-way analysis of variance case. The details of the derivation are given here. For this problem Cox and Hinkley define

$$\psi = (\mu_2-\mu_1,\ldots,\mu_k-\mu_1)^\top$$
$$\lambda = (\mu_1,\Omega)^\top \; .$$

The null hypothesis is then $\psi = 0$ with the alternative $\psi \neq 0$. The mle of ψ is

$$\widehat{\psi} = (\overline{Y}_{2.} - \overline{Y}_{1.},\ldots,\overline{Y}_{k.} - \overline{Y}_{1.})^\top \; .$$

Replacing μ_i by $\psi_i + \mu_1$ in equation (1) we find that

$$U_{\psi i}(\psi, \lambda; Y) = \Omega^{-1} \sum_{j=1}^{n_i} (y_{ij} - \psi_i - \mu_1) ;$$

hence

$$i_{\psi i \psi i}(\psi, \lambda) = n_i \Omega^{-1} ,$$

$$i_{\psi i \psi s}(\psi, \lambda) = 0 \qquad\qquad i \neq s ,$$

$$i_{\psi i \Omega}(\psi, \lambda) = 0 ,$$

$$i_{\psi i \mu 1}(\psi, \lambda) = n_i \Omega^{-1} , \quad \text{and}$$

$$i_{\mu 1 \mu 1}(\psi, \lambda) = N\Omega^{-1} .$$

Letting $i_{\Omega\Omega}(\psi, \lambda) = i(\Omega)$, then the information matrix is

$$I(\psi, \lambda) = \left[\begin{array}{ccccc|cccc}
n_2\Omega^{-1} & 0 & \cdots & & 0 & n_2\Omega^{-1} & 0 & \cdots & 0 \\
0 & n_3\Omega^{-1} & \cdots & & 0 & n_3\Omega^{-1} & 0 & \cdots & 0 \\
\cdot & \cdot & & & \cdot & \cdot & & & \cdot \\
\cdot & \cdot & \cdots & & \cdot & \cdot & & \cdots & \cdot \\
0 & & \cdots & & n_k\Omega^{-1} & n_k\Omega^{-1} & 0 & \cdots & 0 \\
\hline
n_2\Omega^{-1} & & \cdots & & n_k\Omega^{-1} & N\Omega^{-1} & 0 & \cdots & 0 \\
0 & & \cdots & & 0 & 0 & & & \\
\cdot & & \cdots & & \cdot & \cdot & & i(\Omega) & \\
\cdot & & \cdots & & \cdot & \cdot & & & \\
0 & & \cdots & & 0 & 0 & & &
\end{array}\right]$$

From this matrix and using (3) , we can calculate the matrix

$i.(\psi:\lambda)$;

$$
i.(\psi:\lambda) = \begin{bmatrix} (n_2 - n_2{}^2/N)\Omega^{-1} & -(n_2 n_3/N)\Omega^{-1} & \cdots & -(n_2 n_k/N)\Omega^{-1} \\ \bullet & \bullet & & \bullet \\ \bullet & \bullet & & \bullet \\ \bullet & \bullet & & \bullet \\ -(n_2 n_k/N)\Omega^{-1} & -(n_3 n_k/N)\Omega^{-1} & \cdots & (n_k - n_k{}^2/N)\Omega^{-1} \end{bmatrix} \quad .
$$

Then substituting this matrix in (2) we get

$$
W_e = \sum_{i=2}^{k} (n_i - n_i{}^2/N)(\bar{Y}_{i.} - \bar{Y}_{1.})^\top \hat{\Omega}^{-1}(\bar{Y}_{i.} - \bar{Y}_{1.})
$$

$$
- \sum_{i=2}^{k} \{(\bar{Y}_{i.} - \bar{Y}_{1.})^\top (n_i/N)\hat{\Omega}^{-1} \sum_{j\neq i} n_j (\bar{Y}_{j.} - \bar{Y}_{1.})\}
$$

where $\hat{\Omega}$ is the mle of Ω.

But

$$
\sum_{j\neq i} n_j (\bar{Y}_{j.} - \bar{Y}_{1.}) = N(\bar{Y}_{..} - \bar{Y}_{1.}) - n_i (\bar{Y}_{i.} - \bar{Y}_{1.}) .
$$

Hence

$$
W_e = \sum_{i=2}^{k} n_i (\bar{Y}_{i.} - \bar{Y}_{1.})^\top \hat{\Omega}^{-1}(\bar{Y}_{i.} - \bar{Y}_{1.})
$$

$$
- N(\bar{Y}_{1.} - \bar{Y}_{..})^\top \hat{\Omega}^{-1}(\bar{Y}_{1.} - \bar{Y}_{..}) .
$$

$$i^{\psi\psi}_{} = i^{-1}_{\psi\psi} + i^{-1}_{\psi\psi} i_{\psi\lambda} (i_{\lambda\lambda} - i_{\lambda\psi} i^{-1}_{\psi\psi} i_{\psi\lambda})^{-1} i_{\lambda\psi} i^{-1}_{\psi\psi}. \qquad (7)$$

This test was also proposed by Rao (1947). Using the score function and the information matrices determined in the preceeding section it can be shown that

$$i^{\psi\psi}(\psi_0, \widehat{\lambda}_0) = \begin{bmatrix} \widehat{\Omega}(1/n_1 + 1/n_2) & \widehat{\Omega}/n_1 & \cdots & \widehat{\Omega}/n_1 \\ \widehat{\Omega}/n_1 & \ddots & \cdots & \widehat{\Omega}/n_1 \\ \vdots & & \ddots & \vdots \\ \widehat{\Omega}/n_1 & \cdots\cdots & & \widehat{\Omega}(1/n_1 + 1/n_k) \end{bmatrix}$$

and

$$U_{.\psi}(\psi_0, \widehat{\lambda}_0) = (\widehat{\Omega}^{-1} n_2 (\overline{Y}_{2.} - \overline{Y}_{..}), \ldots, \widehat{\Omega}^{-1} n_k (\overline{Y}_{k.} - \overline{Y}_{..}))^{\mathsf{T}}.$$

Using these results we therefore can calculate W_u:

$$W_u = \sum_{i=2}^{k} (n_i/n_1)(n_1 + n_i)(\overline{Y}_{i.} - \overline{Y}_{..})^{\mathsf{T}} \widehat{\Omega}^{-1} (\overline{Y}_{i.} - \overline{Y}_{..})$$

$$+ \sum_{i=2}^{k} [(n_i/n_1)(\overline{Y}_{i.} - \overline{Y}_{..})^{\mathsf{T}} \widehat{\Omega}^{-1} \sum_{j \neq i} n_j (\overline{Y}_{j.} - \overline{Y}_{..})].$$

Going through the same kind of algebra as for the ML statistic we find that the score statistic is the same as the ML statistic i.e.

$$W_u = \sum_{i=1}^{k} n_i (\bar{Y}_{i.} - \bar{Y}_{..})^\top \hat{\Omega}^{-1} (\bar{Y}_{i.} - \bar{Y}_{..}) .$$

Again we should point out that the same result would be obtained using the parametrization defined in (5). In fact, under this parametrization we would find that $(i_{\lambda\lambda} - i_{\lambda\psi} i_{\psi\psi}^{-1} i_{\psi\lambda})^{-1} i_{\lambda\psi} i_{\psi\psi}^{-1} = 0$ hence from (7) $i^{\psi\psi}(\psi,\lambda) = i_{\psi\psi}^{-1}(\psi,\lambda)$. This makes the calculations to find W_u much easier.

Cox and Hinkley argue that tests based on W_e and W_u are asymptotically equivalent in regular problems. We have shown that for the multivariate analysis of variance they are also equivalent for finite samples.

II. INCOMPLETE DATA

In this section, the ML statistic and the score statistic will be derived under the same model as before but now assuming that we have incomplete data. The pattern of missing data is assumed to be nested so that explicit equations for the mle of the mean and the variance-covariance matrix are available. Indeed, explicit equations for the estimates are extensions of Anderson's (1957) methodology.

Taking into account the missing observations the j^{th} observation from the i^{th} sample proceeds as follows:

$$Y_j^i = \begin{pmatrix} Y_{jo}^i \\ Y_{jm}^i \end{pmatrix} \qquad Z_{jo}^i = \begin{pmatrix} Y_{jo}^i \\ 0 \end{pmatrix} \qquad Z_{jm}^i = \begin{pmatrix} 0 \\ Y_{jm}^i \end{pmatrix} \qquad \begin{aligned} j &= 1,\ldots,n_i \\ i &= 1,\ldots,k \end{aligned}$$

where Y_j^i has a p-dimensional multi-normal distribution with mean μ_i and covariance Ω. Y_{jo}^i is the observed portion of Y_j^i and Y_{jm}^i, the missing portion. For purposes of notation and derivation the superscript i will be dropped temporarily. We then have

$$Y_j = Z_{jo} + Z_{jm} \sim N_p(\mu, \Omega) \qquad j = 1,\ldots n.$$

For each Y_j, μ and Ω can be partitioned appropriately:

$$E[Z_{jo}] = \theta_{jo} = \begin{pmatrix} \mu_{jo} \\ 0 \end{pmatrix},$$

$$E[Z_{jm}] = \theta_{jm} = \begin{pmatrix} 0 \\ \mu_{jm} \end{pmatrix},$$

$$\mu = \theta_{jo} + \theta_{jm},$$

$$\operatorname{cov}(Y_{jo}) = \Omega_{joo} \qquad \operatorname{cov}(Y_{jm}) = \Omega_{jmm} \quad, \text{ and}$$

$$\Omega = \begin{pmatrix} \Omega_{joo} & \Omega_{jom} \\ \Omega_{jmo} & \Omega_{jmm} \end{pmatrix}.$$

Note that

$$Y_{jm} \mid Y_{jo} \sim N(\mu_{jm} + \Omega_{jmo}\Omega_{joo}^{-1}(Y_{jo} - \mu_{jo}), V_j) \qquad (8)$$

where

$$V_j = \Omega_{jmm} - \Omega_{jmo}\Omega_{joo}^{-1}\Omega_{jom}.$$

The score functions and the information matrices will be derived with incomplete data using equation (2.13) of Orchard and Woodbury (1972):

$$U_{.\mu}(\mu,\Omega;Y_o) = E[U_{.\mu}(\mu,\Omega;Y)\mid Y_o].$$

In the case of complete data we know that

$$U_{.\mu}(\mu,\Omega;Y) = \Omega^{-1}\sum_{j=1}^{n}(Y_j - \mu)$$

hence

$$U_{.\mu}(\mu,\Omega;Y_o) = \Omega^{-1}\sum_{j=1}^{n}E(Y_j - \mu \mid Y_o)$$

$$= \Omega^{-1} \sum_{j=1}^{n} \begin{pmatrix} Y_{jo} - \mu_{jo} \\ \\ 0 \end{pmatrix} + \begin{pmatrix} 0 \\ \\ \Omega_{jmo} \Omega_{joo}^{-1} (Y_{jo} - \mu_{jo}) \end{pmatrix}$$

$$= \sum_{j=1}^{n} \Omega^{-1} \begin{pmatrix} I \\ \\ \Omega_{jmo} \Omega_{joo}^{-1} \end{pmatrix} (Y_{jo} - \mu_{jo}) \quad . \qquad (9)$$

Dropping the subscript j temporarily,

$$\Omega^{-1} \begin{pmatrix} I \\ \\ \Omega_{mo} \Omega_{oo}^{-1} \end{pmatrix} =$$

$$= \begin{pmatrix} \Omega_{oo}^{-1} + \Omega_{oo}^{-1} \Omega_{om} V^{-1} \Omega_{mo} \Omega_{oo}^{-1} & -\Omega_{oo}^{-1} \Omega_{om} V^{-1} \\ \\ -V^{-1} \Omega_{mo} \Omega_{oo}^{-1} & V^{-1} \end{pmatrix} \begin{pmatrix} I \\ \\ \Omega_{mo} \Omega_{oo}^{-1} \end{pmatrix}$$

$$= \begin{pmatrix} \Omega_{oo}^{-1} \\ \\ 0 \end{pmatrix} \quad .$$

Then

$$U_{.\mu}(\mu,\Omega;Y_o) = \sum_{j=1}^{n} \begin{pmatrix} \Omega^{-1}_{joo}(Y_{jo}-\mu_{jo}) \\ \\ 0 \end{pmatrix} . \tag{10}$$

Next

$$i_{\mu\mu}(\mu,\Omega) = E[U_{.\mu}(\mu,\Omega;Y_o)U_{.\mu}(\mu,\Omega;Y_o)^{\mathsf{T}}]$$

$$= E\{[\sum_{j=1}^{n} \begin{pmatrix} \Omega^{-1}_{joo}(Y_{jo}-\mu_{jo}) \\ \\ 0 \end{pmatrix}][\sum_{j=1}^{n} \begin{pmatrix} \Omega^{-1}_{joo}(Y_{jo}-\mu_{jo}) \\ \\ 0 \end{pmatrix}]^{\mathsf{T}}\}$$

$$= \sum_{j=1}^{n} \begin{pmatrix} \Omega^{-1}_{joo} & 0 \\ \\ 0 & 0 \end{pmatrix} . \tag{11}$$

Further

$$i_{\mu\Omega}(\mu,\Omega) = E[-\partial/\partial\Omega \; U_{.\mu}(\mu,\Omega;Y_o)]$$

Replacing $U_{.\mu}(\mu,\Omega;Y_o)$ by expression (9) we get

$$i_{\mu\Omega}(\mu,\Omega) = \sum_{j=1}^{n} -[((\partial/\partial\Omega) \; \Omega^{-1} \begin{pmatrix} I \\ \\ \Omega_{jmo}\Omega^{-1}_{joo} \end{pmatrix})E(Y_{jo}-\mu_{jo})]$$

$$= 0 \; .$$

These results are sufficient to derive the ML and score statistics.

A. Derivation of the ML statistic

The ML statistic is given by (2) and (3). The parametrization defined in (5) is easier to work with and therefore will be used.

Going through the same derivations as above with $\psi_i = \mu_i - \mu$ and $\lambda = (\mu, \Omega)$, we get the following results.

$$U_{\psi i}(\psi, \lambda; Y_o) = \Omega^{-1} \sum_{j=1}^{ni} \begin{pmatrix} Y_{jo}^i - \psi_{jo}^i - \mu_{jo} \\ 0 \end{pmatrix} \quad ,$$

$$i_{\psi i \psi i}(\psi, \lambda) = \sum_{j=1}^{ni} \begin{pmatrix} \Omega_{joo}^{i^{-1}} & 0 \\ 0 & 0 \end{pmatrix} \quad ,$$

$$i_{\psi i \psi s}(\psi, \lambda) = 0 \qquad\qquad i \neq s \quad ,$$

$$i_{\psi i \mu 1}(\psi, \lambda) = \sum_{j=1}^{ni} \begin{pmatrix} \Omega_{joo}^{i^{-1}} & 0 \\ 0 & 0 \end{pmatrix} \quad ,$$

$$i_{\mu\mu}(\psi, \lambda) = \sum_{i=1}^{k} \sum_{j=1}^{ni} \begin{pmatrix} \Omega_{joo}^{i^{-1}} & 0 \\ 0 & 0 \end{pmatrix} \quad , \quad \text{and}$$

$$i_{\psi i \Omega}(\psi, \lambda) = i_{\mu \Omega}(\psi, \lambda) = 0 \quad .$$

Using equation (3) we can show that

$$i.(\psi:\lambda) = \begin{bmatrix} A_{n_1} - A_{n_1} B A_{n_1} & -A_{n_1} B A_{n_2} & \cdots & -A_{n_1} B A_{nk} \\ -A_{n_2} B A_{n_1} & & \ddots & \vdots \\ \vdots & & & \vdots \\ -A_{nk} B A_{n_1} & \cdots\cdots & & A_{nk} - A_{nk} B A_{nk} \end{bmatrix}$$

where

$$A_{ni} = \sum_{j=1}^{ni} \begin{pmatrix} \Omega_{joo}^{i^{-1}} & 0 \\ 0 & 0 \end{pmatrix}$$

and

$$B = \begin{bmatrix} \sum_{i=1}^{k} \sum_{j=1}^{ni} \begin{pmatrix} \Omega_{joo}^{i^{-1}} & 0 \\ 0 & 0 \end{pmatrix} \end{bmatrix}^{-1} .$$

Note the similarity of this expression to the expression for $i(\psi:\lambda)$ with no missing data (page 58). The extra notation is necessary because the observations (indexed by j) are allowed to have differing numbers of missing components in each treatment.

Now we have to compute $\hat{\mu}$ and $\widehat{\Omega}$ from the pooled data. The approach suggested here is an extension of Anderson's (1957) methodology and is described in Morrison (1976) .

The data are pooled and rearranged such that the matrix has the following pattern:

$$
X = \begin{bmatrix}
X_{11} & X_{12} & \cdots & X_{1,q-1} & X_{1,q} \\
X_{21} & X_{22} & \cdots & X_{2,q-2} & \text{---} \\
\vdots & \vdots & \cdots & & \text{---} \\
X_{l-1,1} & X_{l-1,2} & \cdots & \text{---} & \text{---} \\
X_{l1} & \text{---} & \cdots & \text{---} & \text{---}
\end{bmatrix}
$$

where the dashes indicate blocks of missing observations. In the following notation X_{uv} represents an $r_u \times w_v$ submatrix of observations with $\sum\limits_{u=1}^{l} r_u = N$ the total number of experimental units

and $\sum\limits_{v=1}^{q} w_v = p$ the total number of responses.

The mean vector is correspondingly partitioned as $\mu^T = (\mu_1{}^T, \mu_2{}^T, \ldots, \mu_q{}^T)$ and the covariance matrix consists of $w_u \times w_v$ submatrices Ω_{uv}, $u,v = 1,2,\ldots,q$. The likelihood can be written as

$$
L(\mu,\Omega) = \left\{ \prod_{u=1}^{l} f(X_{u1}) \right\} \left\{ \prod_{u=1}^{l-1} f(X_{u2} | X_{u1}) \right\} \left\{ \prod_{u=1}^{l-2} f(X_{u3} | X_{u1}, X_{u2}) \right\} \ldots
$$

$$
\{ f(X_{1,q} | X_{11}, \ldots, X_{1,q-1}) \}
$$

where the right-hand side has been written in term of conditional densities instead of likelihood to avoid unnecessary complicated notation.

To maximize $L(\mu, \Omega)$, we have to maximize each factor in the brackets. The first factor is the "likelihood" of a normal distribution with mean μ_1 and variance-covariance matrix Ω_{11}. Therefore the mle $\hat{\mu}_1$ and $\widehat{\Omega}_{11}$ based on all N sampling units will maximize this factor. The second factor is the conditional "likelihood" of a normal distribution with mean $\mu_2 + \Omega_{21}\Omega_{11}^{-1}(X_1 - \mu_1)$ and variance-covariance matrix $\Omega_{22} - \Omega_{21}\Omega_{11}^{-1}\Omega_{12}$. Regressing X_1 on X_2, will give estimates of $\mu_2 - \Omega_{21}\Omega_{11}^{-1}\mu_1$, the regression parameters $\Omega_{21}\Omega_{11}^{-1}$ and the conditional covariance matrix $\Omega_{22} - \Omega_{21}\Omega_{11}^{-1}\Omega_{12}$ that will maximize the second term. With these and using the first estimates $\hat{\mu}_1$ and $\widehat{\Omega}_{11}$ we can easily find the maximum likelihood estimates of μ_2, Ω_{12} and Ω_{22}. We continue in this fashion until the unconditional parameters of the q^{th} set have been computed.

Now that we have $\hat{\mu}$ and $\widehat{\Omega}$ we then form

$$\hat{Y}^i_j = \begin{pmatrix} Y^i_{jo} \\ \\ \hat{Y}^i_{jm} \end{pmatrix}$$

where

$$\hat{Y}_j = Z_{jo} + \hat{Z}_{jm}$$

and

$$\hat{Z}_{jm} = \begin{pmatrix} 0 \\ \\ \hat{\mu}_{jm} + \widehat{\Omega}_{jmo} \widehat{\Omega}^{-1}_{joo} (Y_{jo} - \hat{\mu}_{jo}) \end{pmatrix} .$$

The corresponding estimated parameters, for the j[th] observation in the i[th] sample are obtained by partitioning $\hat{\mu}$ and $\widehat{\Omega}$ as

$$\hat{\mu} = \begin{pmatrix} \hat{\mu}^i_{jo} \\ \\ \hat{\mu}^i_{jm} \end{pmatrix}$$

and

$$\widehat{\Omega} = \begin{pmatrix} \widehat{\Omega}^i_{joo} & \widehat{\Omega}^i_{jom} \\ \\ \widehat{\Omega}^i_{jmo} & \widehat{\Omega}^i_{jmm} \end{pmatrix} .$$

For each group i, calculate

$$\bar{Y}^i_i = (n_i)^{-1} \sum_{j=1}^{ni} \bar{Y}^i_j .$$

From (8) it can be shown that $\bar{Y}^i$ is the unrestricted mle of μ_i.

Substituting i.$(\psi : \lambda)$ and the mle into equation (2) we obtain

$$W_e = \sum_{i=1}^{k} (\bar{Y}^i - \bar{Y})^T (\hat{A}_{ni} - \hat{A}_{ni} \hat{B} \hat{A}_{ni})(\bar{Y}^i - \bar{Y}) \tag{12}$$

$$- \sum_{i=1}^{k} [(\bar{Y}^i - \bar{Y})^T \hat{A}_{ni} \hat{B} \sum_{j \neq i} \hat{A}_{nj} (\bar{Y}^j - \bar{Y})]$$

where

$$\hat{A}_{ni} = \sum_{j=1}^{ni} \begin{pmatrix} \hat{\Omega}^{i-1}_{joo} & 0 \\ 0 & 0 \end{pmatrix} ,$$

$$\hat{B} = \left[\sum_{i=1}^{k} \sum_{j=1}^{ni} \begin{pmatrix} \hat{\Omega}^{i-1}_{joo} & 0 \\ 0 & 0 \end{pmatrix} \right]^{-1} .$$

B. Derivation of the score statistic

The score statistic will be derived using the parametrization in (5) and equations (6) and (7).

As in the case of complete data it can be shown that

$$(i_{\lambda\lambda} - i_{\lambda\psi} i^{-1}_{\psi\psi} i_{\psi\lambda})^{-1} i_{\lambda\psi} i^{-1}_{\psi\psi} = 0 ,$$

Hence

$$i^{\psi\psi}(\psi,\lambda) = i^{-1}_{\psi\psi}(\psi,\lambda) \quad .$$

Substituting the mle we have

$$U_{\cdot\psi}(\psi_0,\widehat{\lambda}_0) = \left(\sum_{j=1}^{n1}\left(\begin{array}{c}\widehat{\Omega}^{1^{-1}}_{joo}(Y^1_{jo}-\widehat{\mu}^1_{jo})\\ 0\end{array}\right),\ldots,\sum_{j=1}^{nk}\left(\begin{array}{c}\widehat{\Omega}^{k^{-1}}_{joo}(Y^k_{jo}-\widehat{\mu}^k_{jo})\\ 0\end{array}\right)\right)^\top$$

and

$$i^{\psi\psi}(\psi_0:\widehat{\lambda}_0) = \text{diag}\left[\sum_{j=1}^{ni}\left(\begin{array}{cc}\widehat{\Omega}^{i^{-1}}_{joo} & 0\\ 0 & 0\end{array}\right)\right]^{-1}.$$

Therefore

$$W_u = \sum_{i=1}^{k}\left[\sum_{j=1}^{ni}\left(\begin{array}{c}\widehat{\Omega}^{i^{-1}}_{joo}(Y^i_{jo}-\widehat{\mu}^i_{jo})\\ 0\end{array}\right)\right]^\top\left[\sum_{j=1}^{ni}\left(\begin{array}{cc}\widehat{\Omega}^{i^{-1}}_{joo} & 0\\ 0 & 0\end{array}\right)\right]^{-1}\left[\sum_{j=1}^{ni}\left(\begin{array}{c}\widehat{\Omega}^{i^{-1}}_{joo}(Y^i_{jo}-\widehat{\mu}^i_{jo})\\ 0\end{array}\right)\right].$$

But

$$\widehat{Y}_j - \widehat{\mu} = \left(\begin{array}{c}Y_{jo}-\widehat{\mu}_{jo}\\ 0\end{array}\right) + \left(\begin{array}{c}0\\ \widehat{\Omega}_{jmo}\widehat{\Omega}^{-1}_{joo}(Y_{jo}-\widehat{\mu}_{jo})\end{array}\right)$$

$$= \begin{pmatrix} I \\ \widehat{\Omega}_{jmo}\widehat{\Omega}^{-1}_{joo} \end{pmatrix} (Y_{jo} - \hat{\mu}_{jo})$$

$$= \widehat{\Omega} \begin{pmatrix} \widehat{\Omega}^{-1}_{joo} & 0 \\ 0 & 0 \end{pmatrix} \begin{pmatrix} Y_{jo} - \hat{\mu}_{jo} \\ 0 \end{pmatrix}$$

$$= \widehat{\Omega} \begin{pmatrix} \widehat{\Omega}^{-1}_{joo}(Y_{jo} - \hat{\mu}_{jo}) \\ 0 \end{pmatrix} \quad .$$

Thus

$$\begin{pmatrix} \widehat{\Omega}^{-1}_{joo}(Y_{jo} - \hat{\mu}_{jo}) \\ 0 \end{pmatrix} = \widehat{\Omega}^{-1}(Y_j - \hat{\mu}) \quad .$$

Substituting this equation in W_u we finally obtain

$$W_u = \sum_{i=1}^{k} (\overline{Y}^i - \hat{\mu})^\top \widehat{\Omega}^{-1} S_i^{*-1} \widehat{\Omega}^{-1} (\overline{Y}^i - \hat{\mu}) \tag{13}$$

where

$$S_i^* = (n_i)^{-2} \sum_{j=1}^{ni} \begin{pmatrix} \widehat{\Omega}^{i-1}_{joo} & 0 \\ 0 & 0 \end{pmatrix} \quad .$$

Unlike the complete data case, there does not seem to be a

simplification of W_e which would make possible a comparison between W_u and W_e. One way to compare them would be to use a real data set and compute both statistics using equations (12) and (13). This was not done here since the programming of the two equations would have taken a considerable amount of time and was beyond the scope of this thesis.