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EFFECTS OF LEAD AND CARBON MONOXIDE ON BLOOD ALA DEHYDRASE

This letter summarizes our discussion on the results of your lead-carbon monoxide study. The experiment was conducted primarily to determine the effects of lead and carbon monoxide on blood ALA dehydrase in rats. Five other measurements were also taken; DALA, osmolality, hematocrit, hemoglobin, and carboxy hemoglobin.

The analysis indicated that ALA dehydrase decreases in the presence of lead and increases in the presence of carbon monoxide. It further showed that each of the other quantities measured is affected by lead, carbon monoxide, or both. A description of these effects is contained in the discussion of the analysis, which is attached.

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Attach.

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N40749

1. EXPERIMENTAL DESIGN

Four groups of ten rats each were included in the experiment. The first group served as a control. The other three groups were exposed to lead and carbon monoxide as follows: group two: lead alone; group three: carbon monoxide alone; group four: lead and carbon monoxide. The test was conducted over a four-week period. Five complete sets of measurements were taken: one prior to treatment, and the other four at weekly intervals. The quantities measured, in addition to ALA dehydrase, were: DALA, osmolality, hematocrit, hemoglobin, and carboxy hemoglobin. In addition, the weight of each rat was recorded at regular intervals throughout the four weeks.

2. ANALYSIS

The data constituted a partially nested and crossed factorial design and were analyzed via analysis of variance. In the case of ALA dehydrase, data were transformed to logarithms prior to analysis to ensure that the variability would be independent of the response. These analysis of variance tables for each response are included in Appendix B.

In all cases, pretreatment data were analyzed separately, as a one variable design, with each lead-carbon monoxide combination considered one treatment.

3. RESULTS

A. Pretreatment Data

For ALA dehydrase, DALA, osmolality, and hematocrit, there is no difference among average response for the four treatment groups. Differences are apparent, however, in the other two responses: the average hemoglobin measurement for the control group is lower than for the other; the average carboxy hemoglobin measurement for the same group is higher than for the others. See Table A for a listing of the mean values and the least significant differences between those mean values.

B. Growth Data

Neither lead nor carbon monoxide had any consistent effect on the weights of the rats over the period of experimentation. On day 17, the lead/carbon monoxide group showed a slightly higher average weight than the others; on day 19, the control group was slightly lower than the others. The major effect seen was a significant weight gain over time. Table B1 gives average weights for each treatment group on the days when these measurements were taken; Table B2 gives the average weight of all forty rats on the days in question.

C. Treatment Data

Effects of lead and carbon monoxide upon each of the six responses are described below. Tables of means and graphs of significant interactions appear in Appendix A.

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1. ALA DEHYDRASE shows a significant decrease in the presence of lead, and a significant increase in the presence of carbon monoxide. There is also a time effect. The weekly responses, averaged over all groups, are different; however, here, as in the cases to follow, there is no trend apparent. The largest value occurs at week 1; the smallest, at week 3.

The lead x week interaction is significant: the non-lead groups show a fairly sharp drop in ALA dehydrase over the first three weeks, and an increase in the fourth week. The lead group measurements remain fairly stable throughout the period. (See Figure 1.)

2. HEMATOCRIT increases with carbon monoxide; there is no effect due to lead. There is a difference among weekly measurements: week 2 is significantly higher than the others. There is also a CO x week interaction: in the fourth week, the non-CO groups show a decrease in hematocrit, while the CO groups show an increase. (See Figure 2.)
3. DALA increases in the presence of lead and shows no change in the presence of carbon monoxide. Again, there is a time effect: the average measurement in the second week is higher than the others.
4. OSMOLALITY decreases with carbon monoxide; there is no effect due to lead.
5. HEMOGLOBIN increases in the presence of carbon monoxide and shows no change in the presence of lead. There is a time effect: the measurements in the second and fourth weeks are higher than the others. No significant interactions are evident in the data collected over the four-week period. However, the hemoglobin measurements taken prior to the experiment indicate that the control group averaged lower than the other three. A comparison of the pretreatment averages for the four groups with the fourth week averages shows that the hemoglobin increase for the lead group is substantially smaller than that for the other three. (See Figure 3.)

<u>GROUP</u>	<u>PRETREATMENT</u>	<u>FOURTH WEEK</u>	<u>DIFFERENCE</u>
Control	14.07	15.51	1.44
Lead	15.04	15.39	.35
CO	14.96	16.33	1.37
Lead/CO	14.82	16.06	1.24

The average difference for the lead group is significantly different from that for the control; those for the CO and lead/CO groups are not different. This indicates that an interaction is present after all: lead alone suppresses hemoglobin; lead in combination with carbon monoxide does not.

6. CARBOXY HEMOGLOBIN decreases in the presence of lead and increases in the presence of carbon monoxide. There is an effect due to

time. Average measurements in weeks 2 and 3 are lower than those in weeks 1 and 4.

The lead x CO interaction is significant: lead decreases carboxy hemoglobin in the presence of carbon monoxide. Differences in the initial values of carboxy hemoglobin (high values in the control group) do not affect the interaction appreciably. (See Figure 4.) A CO x week interaction is also present, but small. (See Figure 5.)

APPENDIX A

1. TABLES OF MEANS
2. GRAPHS OF SIGNIFICANT INTERACTIONS

TABLE A: AVERAGE PRETREATMENT RESPONSES BY GROUP

RESPONSE	CONTROL	LEAD	CO	LEAD/CO	LSD*
1. ALA Dehydrase	30.80	31.30	31.70	35.70	3.74
2. DALA	0.14	0.14	0.14	0.14	0.03
3. Hematocrit	41.10	42.50	42.60	41.00	2.40
4. Osmolality	1,845.30	1,806.10	1,846.60	1,805.10	348.60
5. Hemoglobin	14.07	15.04	14.96	14.82	0.33
6. Carboxy Hemoglobin	3.00	1.10	0.40	0.30	0.79

* The difference between a pair of means is significant at the .05 level if it exceeds the given least significant difference.

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TABLE B1: AVERAGE WEIGHTS BY TREATMENT GROUP

MEASUREMENT DAY	CONTROL	LEAD	CO	LEAD/CO	LSD
4	252.30	250.90	251.10	253.30	6.66
7	290.00	291.50	291.00	294.50	6.66
9	306.50	310.00	305.90	310.70	6.66
12	316.80	317.10	318.90	323.20	6.66
14	329.10	328.40	329.00	335.60	6.66
17	349.60	353.20	353.20	360.60	6.66
19	362.70	370.80	374.30	380.50	6.66

TABLE B2: AVERAGE WEIGHT BY DAYS

MEASUREMENT DAY	AVERAGE WEIGHT
4	251.90
7	291.75
9	308.28
12	319.00
14	330.53
17	354.15
19	372.08
LSD	3.33

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TABLE C1: LEAD MEANS*

RESPONSE	WITH LEAD	WITHOUT LEAD	LSD
ALA Dehydrase	4.61**	31.93	1.85
Hematocrit	46.09	45.91	1.09
DALA	.20**	.15	.02
Osmolality	1,845.85	1,871.53	189.82
Hemoglobin	15.26	15.49	.32
Carboxy Hemoglobin	3.59**	4.51	.82

TABLE C2: CARBON MONOXIDE MEANS*

RESPONSE	WITH CO	WITHOUT CO	LSD
ALA Dehydrase	21.56**	14.78	1.85
Hematocrit	47.08**	44.93	1.09
DALA	.18	.16	.02
Osmolality	1,750.38**	1,967.00	189.82
Hemoglobin	15.71**	15.05	.32
Carboxy Hemoglobin	5.20**	2.90	.82

* Each figure is the average measurement for twenty rats over the four-week period.

** Significant difference between means at the .05 level

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TABLE C3: WEEK MEANS*

RESPONSE	WEEK 1	2	3	4	LSD
ALA Dehydrase	25.76**	18.53	12.90	15.87	1.44
Hematocrit	44.98**	47.20	45.78	46.05	.89
DALA	.17**	.20	.17	.16	.02
Osmolality	1,871.08	1,889.35	1,828.95	1,845.38	119.99
Hemoglobin	15.20**	15.89	14.60	15.82	.22
Carboxy Hemoglobin	4.50**	3.70	3.35	4.65	.75

* Each figure is the average measurement over all forty rats.

** Significant difference among means at the .05 level.

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0 ——— WITHOUT Pb
1 - - - - WITH Pb

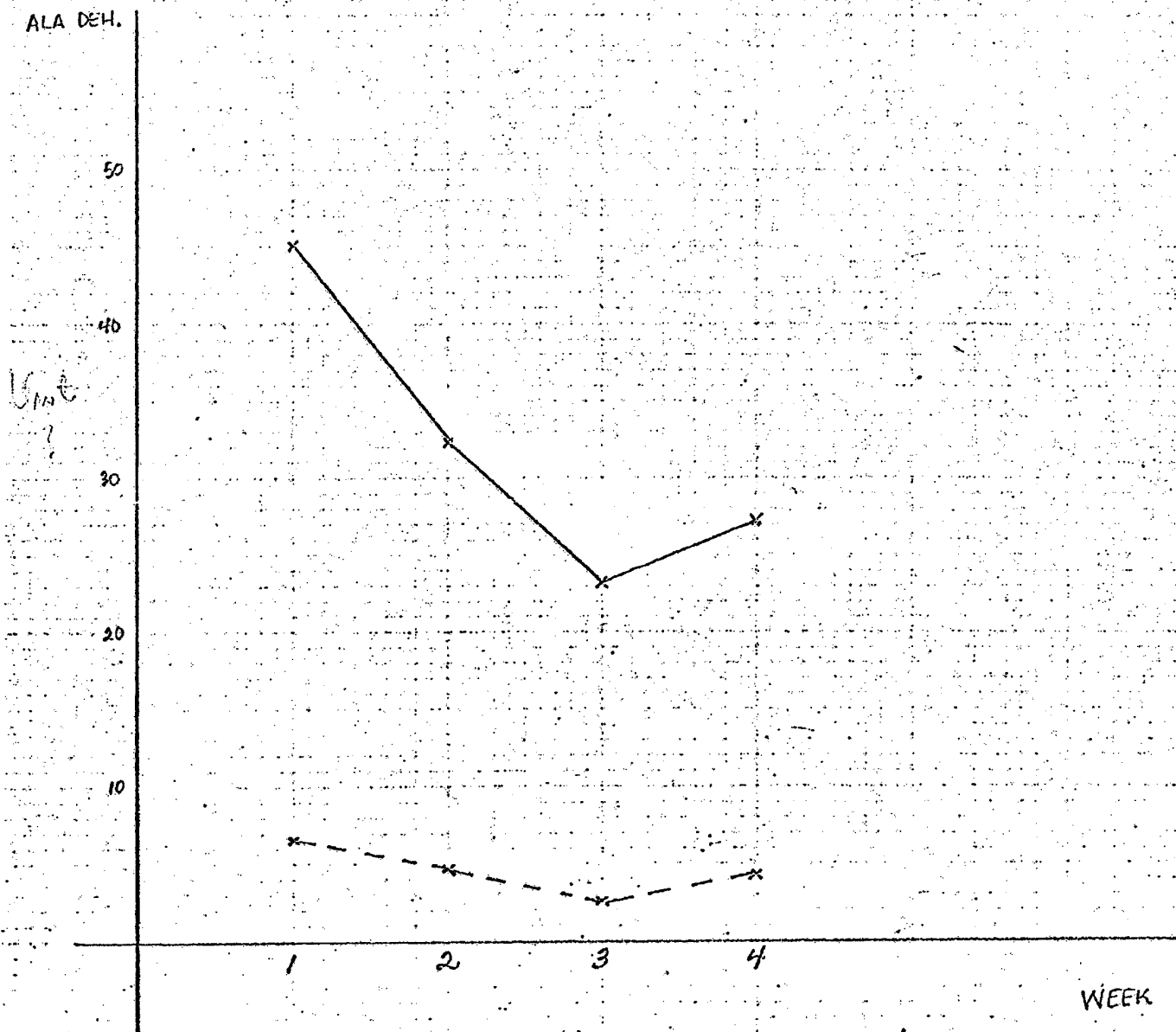


FIGURE 1

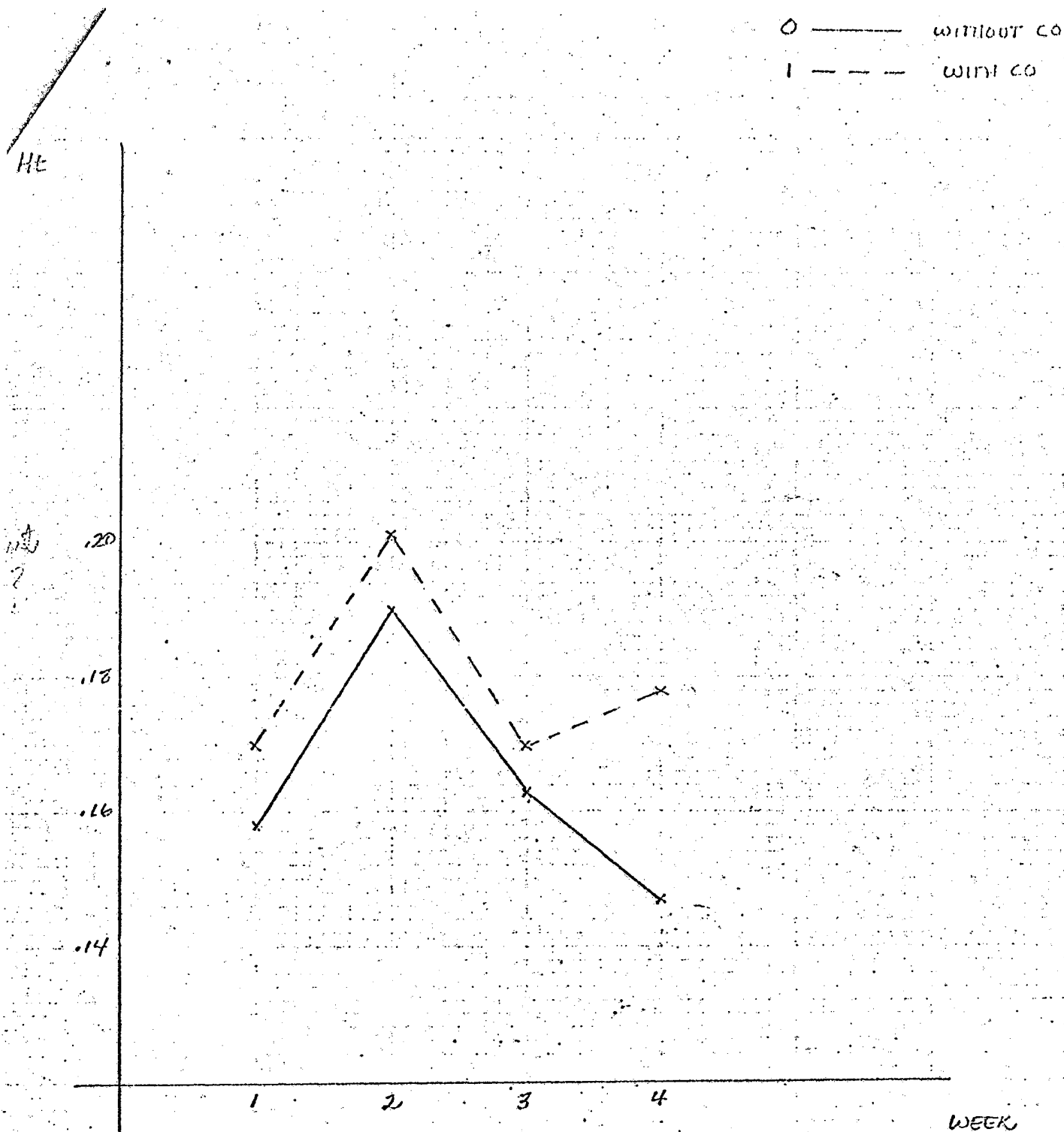


FIGURE 2

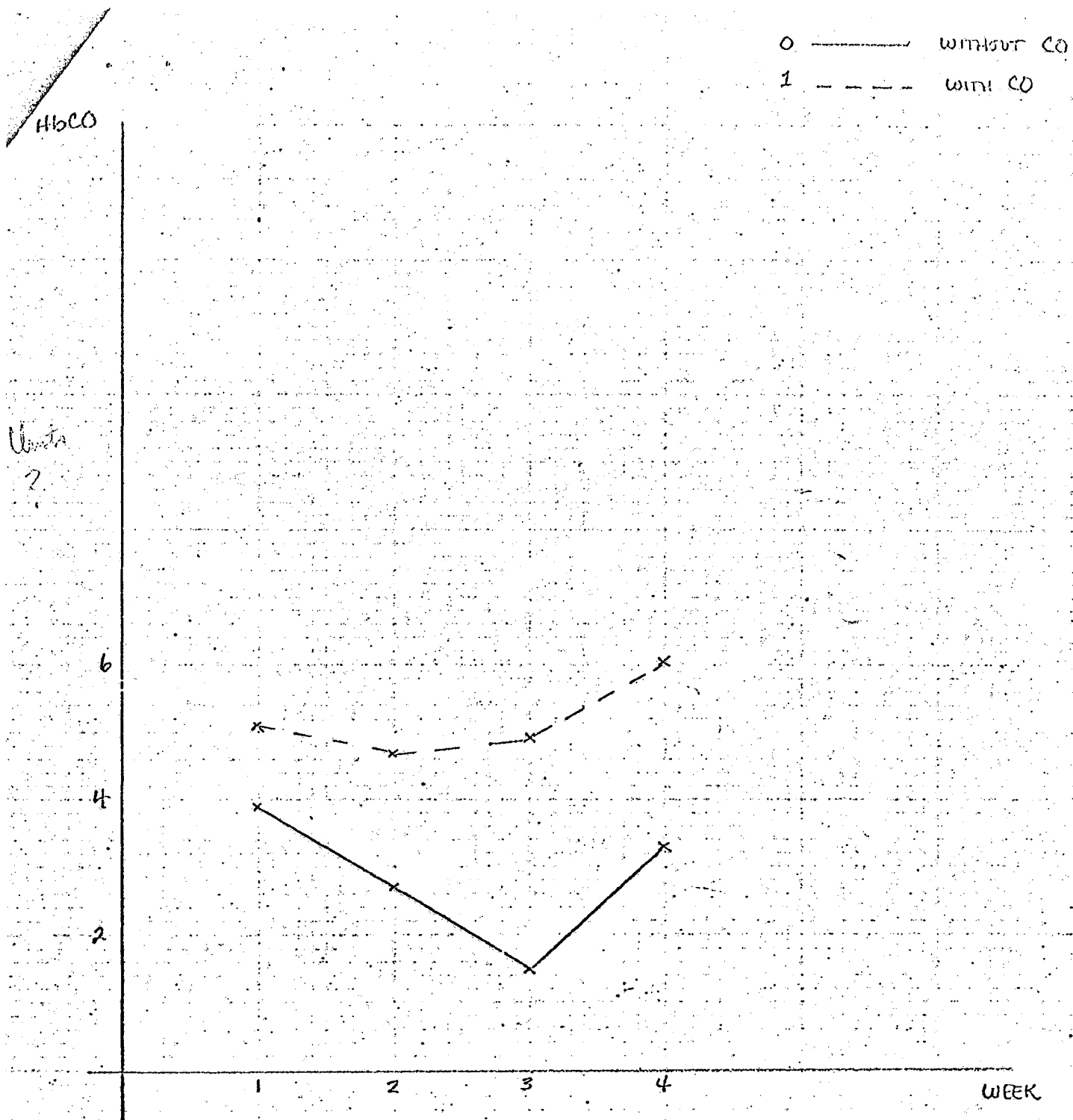


FIGURE 5

APPENDIX B

ANALYSIS OF VARIANCE TABLES:

1. ALA Dehydrase
2. DALA
3. Hematocrit
4. Osmolality
5. Hemoglobin
6. Carboxy Hemoglobin

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RESPONSE NUMBER 1 LOG ALA DEHYD

A.N.O.V.A. TABLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F RATIO
TOTAL	159	4207.30206	3313.14355	0.000000
A = LEAD	1	3313.14355	3313.14355	23.27
B = CD	1	71.70167	71.70167	0.85
AB	1	2.62579	2.62579	0.05
C = RATS	36	110.80842	3.07801	31.66
D = WEEK	3	242.51309	114.17103	3.94
AD	3	42.63301	14.21100	1.00
BD	3	10.86565	3.62188	0.33
ABD	3	3.53478	1.17826	
CD	108	389.47560	3.60626	

.. SIGNIFICANT AT THE .05 PROBABILITY LEVEL (ONE-TAIL TEST)
 .. SIGNIFICANT AT THE .01 PROBABILITY LEVEL (ONE-TAIL TEST)