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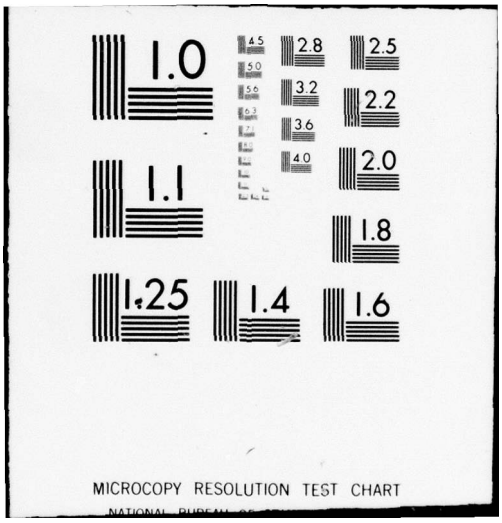
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INSTITUTE REPORT NO. 66

BEHAVIORAL EFFECTS IN RATS FOLLOWING MASSIVE TRANSFUSION WITH PLASMA PROTEIN SOLUTION

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JULY 1979

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Two groups of rats were exsanguinated and infused with Plasmanate (5 per cent solution of human plasma protein fraction) by cannulating the right jugular veins. Hematocrit levels were determined periodically during transfusion until replacement levels of 50 per cent in one group and 70 per cent in the other were obtained. A simulated transfusion was performed on a sham control group. Our purposes for this study were to (1) compare the mean baseline score with the recovery scores for each rat and (2) determine the patterns of recovery. Behavioral effects of transfusion were measured by two behavioral tests: the

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open-field test and the operant conditioning task. Results from our study indicate that significant treatment effects occurred on the first three days following transfusion and the effects of experimental treatment upon the subjects for all groups were dependent upon level of transfusion. The open-field test was not an effective means of measuring transfusion effects but the operant task appeared to be a reliable measure. Performance scores of the subjects were lowest immediately following transfusion; however, they gradually returned to baseline levels by the tenth post-treatment day.

ABSTRACT

Two groups of rats were exsanguinated and infused with Plasmanate (5 per cent solution of human plasma protein fraction) by cannulating the right jugular veins. Hematocrit levels were determined periodically during transfusion until replacement levels of 50 per cent in one group and 70 per cent in the other were obtained. A simulated transfusion was performed on a sham control group. Our purposes for this study were to (1) compare the mean baseline score with the recovery scores for each rat and (2) determine the patterns of recovery. Behavioral effects of transfusion were measured by two behavioral tests: the open-field test and the operant conditioning task. Results from our study indicate that significant treatment effects occurred on the first three days following transfusion and the effects of experimental treatment upon the subjects for all groups were dependent upon level of transfusion. The open-field test was not an effective means of measuring transfusion effects but the operant task appeared to be a reliable measure. Performance scores of the subjects were lowest immediately following transfusion; however, they gradually returned to baseline levels by the tenth post-treatment day.

PREFACE

We are grateful to Dr. Frank DeVenuto and Kenneth R. Busse for their advice and expertise, and Alan Hopkins for his statistical assistance.

John Surinchak is currently assigned to a medical technology program at Fort Sam Houston, Texas.

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INTRODUCTION

Blood with increased oxygen affinity produced a decrease in central venous oxygen tension in resting rats without apparent adverse effect (1). Results from this research (1) showed no change in physical performance when P_{50} was decreased by about 6 mm Hg as compared to a definite decrease in working ability of animals when P_{50} was decreased from 36 to 23 mm Hg. This reduction in working ability (about 10 per cent) was equivalent to that seen with hemoglobin concentration decrement of 10-15 per cent. When submaximal work performance in human subjects was compared before and after a 1,200 ml blood loss, physical performance capacity decreased by approximately 30 per cent and maximal oxygen uptake ($\dot{V}_{O_2}$ max) decreased 18 per cent (2).

Clearly, the maintenance of life of the exsanguinating patient depends upon transfusion. Human albumin is presently used in clinical situations; however, the behavioral effects of its use during the recovery of the patient have not been evaluated. According to Mendelson (3), in the U.S. military experience in Vietnam during the period 1966 and 1968, 46 per cent of hospitalized casualties in U.S. military hospitals required transfusion. Army hospitals used a ratio of 2:1 (blood:fluid) per patient transfused, and the U.S. Naval hospital used 2 to 2.5 times more fluid than blood per patient infused.

The advantages of albumin infusion are many. Infused albumin reacts in a similar manner to albumin which is produced endogenously (4). In comparison to whole blood, which depletes 2,3-diphosphoglycerate (2,3-DPG) in storage and is more difficult to obtain, albumin is available and its ease of storage and handling is of further value. Woolf et al (5) have shown that storage of albumin for more than 10 years

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1. Woodson et al. J Clin Invest 52: 2717-2724, 1973
2. Ekblom, B. et al. J Appl Physiol 33: 175-180, 1972
3. Mendelson, J.A. J Trauma 15: 1-13, 1975
4. Davison, A.M. Clin Haematol 5: 135-148, 1976
5. Woolf, I.L. et al. Transfusion 12: 125-133, 1972

has little or no effect on its biological characteristics. Infusing with albumin poses few complications. Pasteurization is involved in the preparation method and, therefore, albumin does not transmit hepatitis. The most important complication is excessive volume expansion; however, careful monitoring should eliminate this possibility (4).

Unlike whole blood or hemoglobin solutions, albumin solutions do not bind oxygen specifically or avidly; hence, albumin solutions do not deliver significant amounts of oxygen to the tissues. A reduction in the amount of circulating oxygen, i.e. hypoxia, may occur during exsanguination and may persist during transfusion due to a reduction of hemoglobin, the major oxygen-carrier of the blood. However, physiological stress, resulting from trauma, can decrease the affinity of hemoglobin for oxygen which, in turn, permits the delivery of more oxygen to the tissues (6). Thus, the body's normal adaptive response compensates for the reduction of erythrocyte levels during exsanguination and transfusion.

During hemorrhage and massive transfusion with albumin, hemoglobin levels decrease. It was hypothesized that because the oxygen-delivery system of the organism is adversely affected by a reduction in the amount of hemoglobin in the blood, the organism's behavior may also be adversely affected. The present study was undertaken to determine the behavioral effects in rats of exsanguination followed by transfusion with albumin concentrate without focusing on the physiological and biological changes. Our purposes for this study were to (1) compare the mean baseline score with the recovery scores for each rat and (2) determine the patterns of recovery.

Subjects

Fifteen male outbred Sprague-Dawley rats (approximately 16 months of age at start of experiment, $\bar{x}$ wt = 450 g) were used. Each rat was randomly assigned to one of the following transfusion groups: (1) sham control, (2) 50 per cent replacement, and (3) 70 per cent replacement, the group denoting the amount of blood replaced. Each of the 3 experimental groups consisted of five animals. The subjects were maintained for over one year prior to the study on a 12:12-hour light-dark cycle with the dark phase coinciding in time with normal laboratory working hours. While in their home cages, all subjects were provided water ad libitum. Food was limited to the amount that was dispensed in the behavioral testing, plus a supplementary ration, which was given immediately following testing. This supplement was rationed to enable the rats to maintain a weight that was 10-20 per cent below rats that were provided food ad libitum. On weekends, an allotment equal to 38 g was given to each subject. All rats were weighee on a

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6. Collins, J.A. Clin Haematol 5: 201-222, 1971

weekly basis. Fluctuations in weight generally averaged less than 10 per cent of total body weight.

Procedure

Two test situations were used to measure psychomotor performance and behavior: (1) the open-field test which was to assess alterations in activity, and (2) the operant conditioning to measure performance and learning capacity of the subjects.

The open-field test was carried out in a 3 x 3 ft (91.44 cm) field with a grid floor ruled into 16 nine-inch (22.5 cm) squares. The only source of illumination was provided by fluroscent lamps 15 ft (4.57 m) away, which resulted in a dimly lighted room. All subjects were placed individually for three minutes in the open-field, during which time their scores on ambulation were recorded. Data on ambulation were used as an index of rat exploration. Ambulation scores were determined by the number of grid lines each rat crossed during the three-minute interval.

Each subject was given four runs in the open field. The rats were first exposed to the test 11 days before transfusion and were retested seven days later. The first two runs were spaced seven days apart to discourage habituation yet provide a dependable pre-treatment baseline. Four days after the animal's second run, each animal was transfused. Recovery from the experimental treatment was expected to last approximately seven days. Accordingly, the last two runs, scheduled to occur during the course of recovery, were designed to measure two variables: (1) rate of recovery and (2) the behavioral effects of experimental treatments. Therefore, three days following transfusion, each subject was run in the open-field for the third time; seven days following transfusion, each was tested for the fourth and final time. As each rat completed the open-field test, it was immediately placed in the Skinner box for the operant testing.

Operant conditioning was carried out in modular test chambers (Skinner Box, Coulbourn Instruments, Inc.) which were located in sound attenuating cubicles. Operant schedules were under digital logic control. All subjects were trained in the operant conditioning for 30 minutes a day, five days a week on a Fixed Ratio (FR) schedule of 20 responses per reinforcement. The reinforcement was one pellet of food (a 45 mg Noyes pellet) after pressing a bar 20 times in succession. When each rat achieved a minimum score of 2000 responses during one 30-minute interval, behavioral training was then maintained for an additional six-week period. Following this period, behavioral training was considered complete and behavioral testing and transfusion procedures began. Twenty-four hours following transfusion, the subjects were tested daily for ten consecutive days to provide a profile of the "recovery period" for each rat. After recovery, they were returned to the original five-day week schedule. During the week immediately

preceding transfusion, a baseline score that represented the mean response rate for each rat was recorded. The baseline for each group was determined by averaging the baseline scores of all subject members in their respective groups.

Approximately 3 ml of methoxyflurane were poured onto gauze sponges placed on the floor of an air-tight animal jar. Rats were placed in the jar for approximately 90 seconds, or until anesthetized, prepared for surgery, and maintained under halothane. The total maintenance time under halothane for the subjects transfused to 70 per cent was approximately 90 minutes; subjects in the 50 per cent group required about 50 minutes, and sham control subjects about 30 minutes. A polyethylene catheter (PE 90) was inserted into the right jugular vein and advanced approximately halfway to the heart. Blood was replaced with a 5 per cent solution of human plasma protein fraction (Plasmanate(R)) in a series of 3 ml exchanges. Hematocrits were taken at every third exchange until the desired transfusion level was obtained. The catheter was then removed and the vein ligated. This technique is in accordance with the procedure performed by Friedman et al (7). Rats in the surgical control group were anesthetized, incised, and catheters inserted into the jugular vein, but they were not transfused.

Data Analysis

Total responses and reinforcements were obtained in the Skinner box from all subjects. The baseline score was the independent variable and was used as a covariate in selected subsequent data analyses. The dependent variables were the daily response totals and the number of days each rat required to return to its baseline performance. Before applying the analyses of covariance to the data, we determined if the mean pre-treatment baseline scores differed among groups. These data were analyzed by the analysis of variance and group differences were statistically insignificant ($P > 0.05$). The analysis of covariance allowed for an adjustment in the post-treatment operant response scores based on the pre-treatment baseline scores. One-way analysis of covariance (BMDP1V) (8) was performed on the response rates and recovery data by using the baseline response scores for each group as the covariates. In all statistical analyses, a probability level of $P < 0.05$ was chosen to be indicative of a statistically significant experimental effect.

The product-moment correlation analysis was performed on the open-field and operant conditioning data to determine if a correlation

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7. Friedman, H.I. et al. Lab Invest 39: 167-177, 1978

8. Dixon, W.J., and M.B. Brown (Ed), Berkeley, University of California Press, 1977

existed. Data from the operant task and extinction runs were also subjected to this analysis. The product-moment correlation analysis was also used to determine if a common factor was measured by the open-field and operant conditioning tests.

RESULTS

Behavioral Changes

Immediately following transfusion, most rats in the 50 per cent and 70 per cent groups appeared lethargic until full recovery from anesthesia, which took approximately 45-90 minutes. Two animals in the 50 per cent and 70 per cent groups were alert within a few seconds following transfusion and displayed much activity in the next 45 minutes. All subjects in the transfused groups appeared fairly alert to sights and sounds and displayed some activity after recovery from anesthesia.

Generally speaking, the anesthetized control group recovered from anesthesia more rapidly (approximately 45 minutes) than the transfusion groups and were the most active of all subjects. Three control subjects who had undergone simulated transfusions recovered from anesthesia within 30-45 minutes following treatment. Twenty-four hours following treatment, one rat in the control group made no response to the operant situation, and two subjects in each of the two transfused groups did not respond to the operant task. On the following day, all five subjects resumed responding. The mean number of days each group of subjects required to return to its baseline performance level were: control group, two days; 50 per cent group, four days; and 70 per cent group, ten days or more. The response rate for each subject 24 hours after transfusion was used to determine its per cent change relative to each subject's individual baseline. The mean per cent change for each group was then determined; it was as follows: control group, 78 per cent; 50 per cent group, 31 per cent; 70 per cent group, 8 per cent.

Analysis of covariance showed significant changes in the subjects' performance scores during the first three days following transfusion. These data provided information on the two most significant aspects of the study: (1) changes in performance from pre-treatment baseline levels to post-treatment recovery scores and (2) patterns of recovery. The figure illustrates the post-transfusion recovery profiles of all three groups during the ten-day recovery period. The standard error of adjusted means for each point in the figure appears in Table 1. The probability levels indicated that the response rates were not significantly different among the groups after the third day of recovery. For the first post-treatment day, the P value for differences among groups was 0.0041; for Day 2, the P value for all three groups was 0.0138; for Day 3, the P value for all three groups was 0.0581.

The adjustment performed by the analysis of covariance on the first day's post-treatment performance level for each group was achieved by including the pre-treatment baselines so that the adjusted group means could be computed. The data revealed that, by chance, the subjects in the group transfused to 70 per cent exchange had the highest pre-treatment baselines; subjects in the control group had the second highest; subjects in the 50 per cent group had the lowest baselines of the three groups. According to the analysis of covariance, the general trend of performance varied in relation to group levels of transfusion. As shown in Figure, slight inconsistencies to this trend were observed on Days 4, 6, 7, and 9 of the ten post-treatment days. The data indicated that the pre-treatment performance standing of the groups did not become reinstated during the ten-day recovery period. With the subjects unassigned to groups, there was no correlation greater than 0.6 on the product-moment correlation analysis between response rates on the FR task and level of activity in the open-field (Table 2).

At the completion of the study, the subjects were run for five days on an extinction schedule during which all reinforcement was withheld in the operant task. This schedule was designed to test the speed at which the subjects, without reinforcement, would terminate their bar-pressing activity. The rats were not assigned to groups. There was not a strong correlation between pretreatment baseline levels and overall per cent of retained responses. A correlation coefficient of 0.897 between the pretreatment baselines and the first day of extinction indicated that rats with high baseline levels also had high response levels, and rats with low baseline levels had low response levels. However, by the second day of extinction, the trend reversed (correlation coefficient of -0.637). The correlation remained negative the third, fourth, and fifth days (-0.491, -0.376, -0.349, respectively).

DISCUSSION

The data obtained from the analysis of covariance showed a significant treatment effect only during the first three days following transfusion. The effect of the experimental treatment upon the subjects for all groups was dependent upon their level of transfusion. From the fourth to the tenth days, the groups reverted to their normal baseline levels and significant between-group differences decreased. These results suggest that the hypoxic effects resulting from blood loss are consistent with the findings of other researchers (1,2,9) in which hypoxia, produced by blood loss and other causes, produces a decrease in exercise tolerance and work performance in human and animal subjects.

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9. Oski, F.A. et al. Ann Intern Med 74: 44-46, 1971

The results showed no significant correlation between open-field activity and high FR performance levels in the operant conditioning task. These data have theoretical implications regarding the nature of exploration and food acquisition. However, our data indicated that the level of exploratory drive in the rat was not related to its drive in the acquisition of food but the two drives appear to be independent factors.

It was speculated that rats with high baseline levels on the FR task would demonstrate greater resistance to extinction in comparison to rats with low baseline levels. This expectation was validated by data obtained on the first extinction day only. However, considering that there was a negative correlation from the second to the fifth day of extinction, it seems plausible to attribute considerable importance to the possibility that rats with the highest FR response rates also quickly learned the extinction task.

Results from our study indicate that significant treatment effects occurred on the first three days following transfusion and the effects of experimental treatment upon the subjects for all groups were dependent upon level of transfusion. Performance scores of the subjects were lowest immediately following transfusion; however, they gradually returned to baseline levels by the tenth post-treatment day.

CONCLUSIONS AND RECOMMENDATIONS

Dose-response studies should be done. Dose-response studies would be valuable to perform from the standpoint that most clinical transfusions are performed below the levels of 50 per cent and 70 per cent. A further consideration would be to study the effects of transfusion upon the subjects transfused with various other materials (such as hemoglobin).

Studies using banked blood as the transfusion medium would be useful to perform in order to obtain baseline data from a standard transfusion medium. The comparison of these data with data obtained from studies using other transfusion materials would be useful in the development and refinement of transfusion solutions. It could also be used to evaluate the sensitivity of certain behavioral and biochemical measures, and to assess their value and suitability for future studies. In addition, pathological studies may be performed on each experimental subject using tissue samples to corroborate with any data of physiological and cerebral damage.

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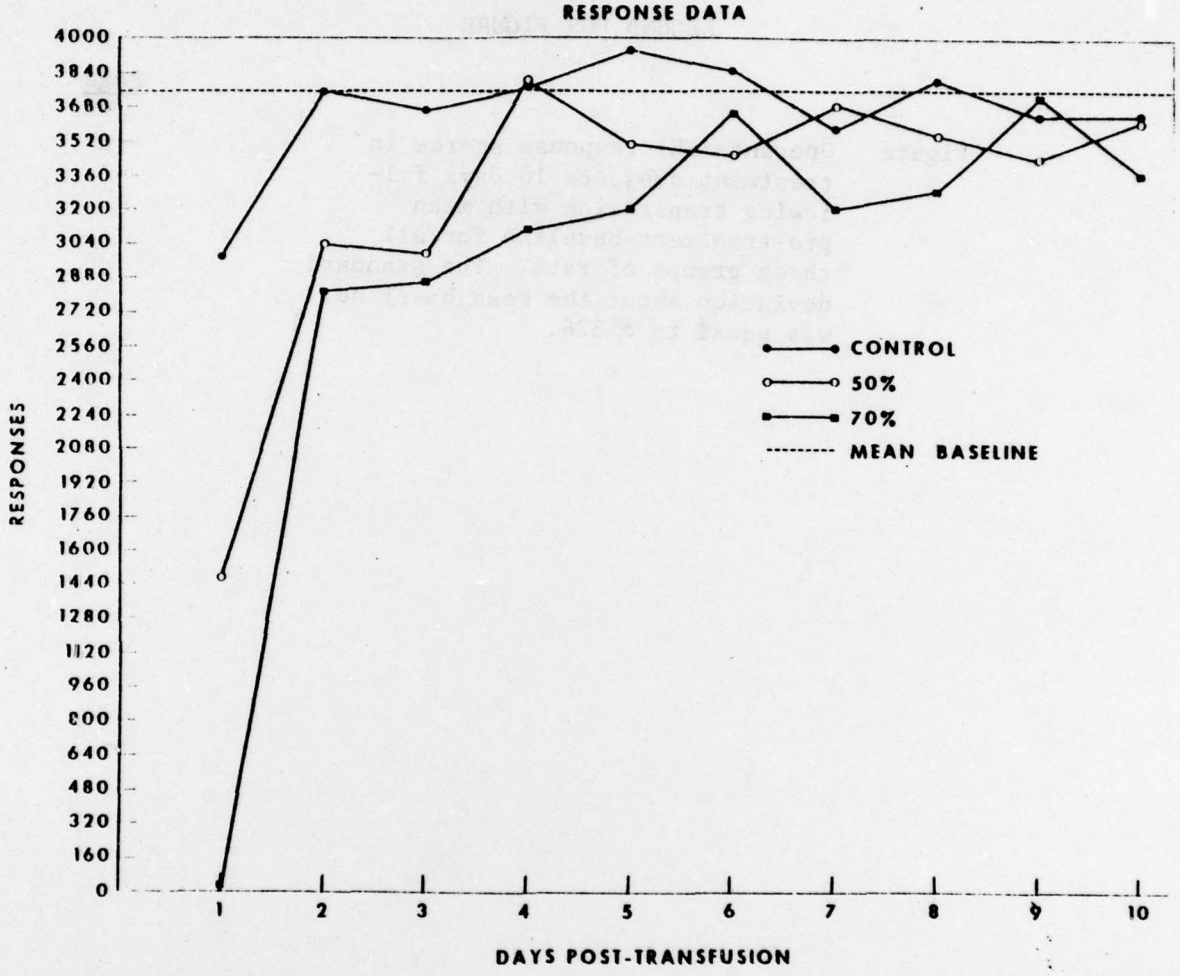
Figure	Operant (FR) response scores in treatment subjects 10 days following transfusion with mean pre-treatment baseline for all three groups of rats. The standard deviation about the mean baseline was equal to ± 326.	10

APPENDIX A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4
5
6
7
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10
DAYS POST-TRANSFUSION
CONTROL
50%
70%
MEAN BASELINE

Figure

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APPENDIX B

Table 1. Operant response scores in treatment subjects 10 days following transfusion. Standard error of adjusted means.

	CONTROL		50%		70%	
Day	Adj Grp Mean	Std Error	Adj Grp Mean	Std Error	Adj Grp Mean	Std Error
1	2972	±464	1469	±476	31	±485
2	3767	±192	3039	±197	2821	±201
3	3669	±220	2999	±226	2868	±230
4	3773	±196	3791	±201	3102	±205
5	3946	±228	3512	±234	3205	±238
6	3846	±399	3455	±409	3656	±417
7	3581	±215	3702	±221	3205	±455
8	3802	±276	3559	±283	3298	±289
9	3636	±277	3445	±284	3730	±289
10	3641	±221	3624	±226	3367	±230

Table 2. Correlation matrix between fixed ratio and open-field data.

	Baselines Fixed Ratio	Baselines Ambulation	First Day* Fixed Ratio+	First Day* Open-Field	Seventh Day* Fixed Ratio	Seventh Day* Open-Field
Baselines Ambulation	-.673					
First Day* Fixed Ratio	.231	-.435				
First Day* Open-Field	-.069	.181	.569			
Seventh Day* Fixed Ratio	.856	-.548	.567	.238		
Seventh Day* Open-Field	-.243	.413	-.003	.325	-.116	
Difference Between First Day FR* & Baselines FR	.305	.087	-.856	-.594	-.100	-.127
Difference Between Seventh Day FR* & Baselines FR	-.077	.053	-.731	-.569	-.580	-.160

Significance level, based on 12 df:r = $P \leq 0.05$. *Post-Transfusion. + Differences between First Day FR* and Baselines FR = 0.675

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