Massive transfusions with a plasma protein fraction: effects on rat behavior[1]

G.E. Esgandarian, J.S. Surinchak and P.A. O'Mara

Division of Biorheology, Letterman Army Institute of Research, Presidio of San Francisco, San Francisco (CA 94129, USA), 30 September 1980

Summary. The behavioral effects of a plasma protein fraction used as a plasma expander were determined in rats at various blood replacement levels. Results of operant conditioning tests indicated that significant treatment effects occurred on the first 3 days following transfusion. Initial decrements in performance were directly related to the lowered hematocrit levels following transfusion.

The maintenance of life of the exsanguinating patient depends upon transfusion. Colloid solutions are presently used in clinical situations due to their many advantages[2,3]. However, the behavioral effects of their use during the recovery of the patient have not been evaluated.

During hemorrhage and massive transfusion with colloid solutions, 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 a plasma protein fraction without focusing on the physiological and biological changes. Our purposes for this study were to 1. compare the baseline score with the recovery scores for each rat and 2. determine the patterns of recovery.

Materials and methods. 15 male outbred Sprague-Dawley rats were used. Each rat was randomly assigned to one of the following transfusion groups: a) sham control, b) 50% replacement, and c) 70% replacement, the group denoting the amount of blood replaced. Each of the 3 experimental groups consisted of 5 animals.

2 test situations were used to measure psychomotor performance and behavior: 1. the open-field test which was to assess alterations in spontaneous activity and 2. the fixed ratio (FR) operant conditioning task to measure the performance of a simple food-reinforced motor task. The FR operant schedule required the rat to press a bar 20 times to obtain a 45-mg food pellet.

Each subject was given 4 runs in the open-field. The rats were tested 11 and 4 days prior to transfusion and 3 and 7 days after transfusion. FR behavioral conditioning was accomplished in a Skinner box 30 min per day, 5 days per week, over a 6-week period. This schedule produced stable performance in about 3 weeks. The averages of the 30-min response totals obtained during the week preceding transfusion were used as baseline scores. The baseline for each group was determined by averaging the baseline scores of all subject members in their respective groups. The statistical significance of the results was evaluated using analyses of covariance where the baseline performance was used as a covariate.

Transfusion was accomplished by inserting a catheter into the right jugular vein after heparinization of the animal and withdrawing blood that was replaced by a 5% solution of human plasma protein fraction (Plasmanate) in a series of 3-ml exchanges. The hematocrit for the animals was lowered to 20 and 12 (for 50- and 70% groups respectively) from the average normal of about 40. Controls were also heparinized and catheterized but no transfusion was performed. 24 h following transfusion, the subjects were tested daily on the FR task for 10 consecutive days to provide a profile of the 'recovery period' for each rat.

Results. Generally speaking, the anesthetized control group recovered from anesthesia more rapidly (approximately 45 min) than the transfusion groups and were the most active of all subjects. 3 control subjects who had undergone sham transfusions recovered from anesthesia within 30–45 min following treatment. 24 h following treatment, 1 rat in the control group made no response to the operant situation, and 2 subjects in each of the 2 transfused groups did not respond to the operant task. On the following day, all 5 subjects resumed responding.

The figure illustrates the post-transfusion effects for all 3 groups during the 10-day recovery period. Each point is the group mean adjusted for the effects of the covariate (baseline performance) with the standard error of the mean indicated by the vertical bar. During the 1st day after transfusion the control group performed the FR task at 78% of the baseline performance level. The corresponding levels for the other groups were 31% following the 50% transfusion and 8% following the 70% transfusion. The mean number of days each group of subjects required to return to its baseline performance level were: control group, 2 days; 50% group, 4 days; and 70% group, 10 days.

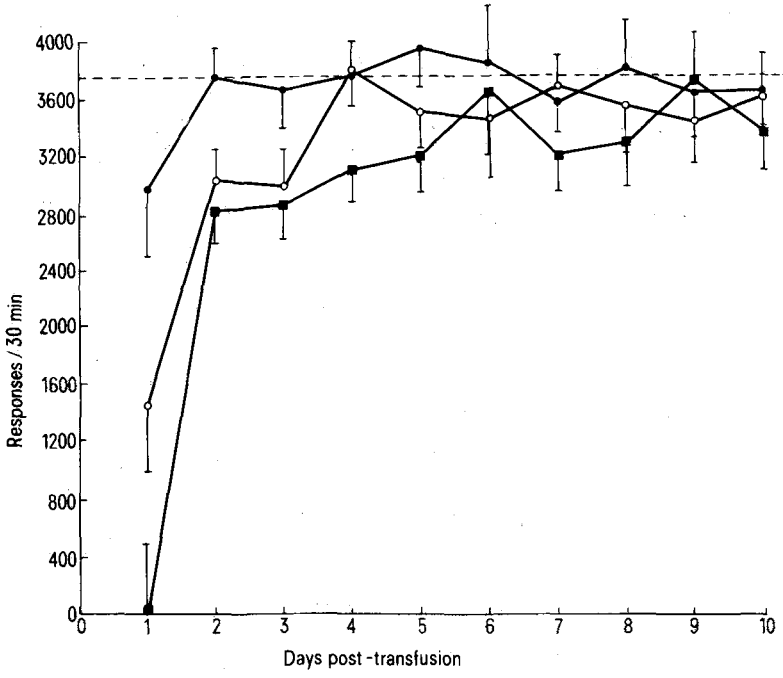
The analysis of covariance showed significant changes in the subjects' performance scores during the first 3 days following transfusion (table). These data provided information on the 2 most significant aspects of the study: a) changes in performance from pre-treatment baseline levels to post-treatment recovery scores and b) patterns of recovery. The response rates were not significantly different among the groups after the 3rd day of recovery.

No significant difference was observed in the open-field test between pre- and post-treatment measurements. There was no correlation greater than 0.6 on the product-moment correlation analysis between response rates on the operant conditioning task and the level of activity in the open-field.

Discussion. The data obtained from the analysis of covariance showed a significant treatment effect only during the first 3 days following transfusion. The effect of the experimental treatment upon the subjects for all groups was dependent upon their level of transfusion. Performance scores for the subjects were lowest immediately following transfusion. From the 4th to the 10th 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[4–6] in which hypoxia, produced by blood loss and other causes, produces

Analysis of covariance results for the (FR) operant conditioning task 10 days following transfusion

Day	d.f.	F-value	P-value
1	2,11	9.44	0.0041
2	2,11	6.47	0.0138
3	2,11	3.72	0.0581
4	2,11	3.50	0.0667
5	2,11	2.53	0.1245
6	2,11	0.24	0.7919
7	2,11	1.26	0.3228
8	2,11	0.78	0.4818
9	2,11	0.25	0.7868
10	2,11	0.42	0.6648



Recovery of fixed ratio operant performance in rats following exchange transfusion with Plasmanate. Each point shows the mean ± SE of 5 rats. The solid circles show the control group. The open circles represent a 50% transfusion and the solid squares a 70% transfusion. Each point has been adjusted for differences in baseline performance. The average baseline response rate for all groups was 3768 ± 84.17.

a decrease in exercise tolerance and work performance in human and animal subjects.

In the present study it was not possible to assess the recovery of the hematocrit or other blood factors due to the possibility of the sampling techniques affecting the behavioral results. However, other studies performed in this laboratory have examined the recovery of blood factors following 70-76% exchange transfusion of rats with albumin[7]. Those studies employed the same surgical and anesthetic procedures as used in this study. The results showed that the hematocrit and oxygen capacity of the blood recovered in about 5-8 days. The course of recovery was similar to the behavioral recovery observed in the present investigation.

1 In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Institute of Laboratory Animal Resources, National Academy of Science, National Research Council. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Intracellular free calcium as a pathogen in cell damage initiated by the immune system[1]

A.K. Campbell and J.P. Luzio[2]

Department of Medical Biochemistry, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (Great Britain), and Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR (Great Britain), 16 February 1981

Summary. It is proposed that the earliest intracellular event induced by the action of complement is an increase in cytosolic free calcium, which can occur in the absence of lysis. This increase causes morphological and chemical changes in the cell and also results in modified responses to physiological stimuli.

In many diseases interactions of the immune system occur with tissue antigens involving antibody, complement or cell-mediated responses. Circulating antibodies to constituents of the cell membrane have been identified in several pathological conditions and have effects on the tissues primarily responsible for the clinical manifestations of the disease (table)[3-12]. Although antibodies may interfere with cell function as a direct result of binding to cell surface receptors[4-7], in many cases cell damage is mediated by activation of the complement pathway. A major problem is that it is not yet clear which chemical changes in the cell lead to modifications of tissue function after attack by the immune system.

Using the calcium activated photoprotein oblein trapped in