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BEHAVIORAL EFFECTS OF MASSIVE TRANSFUSION WITH CELL-FREE RESUSC--ETC(U)
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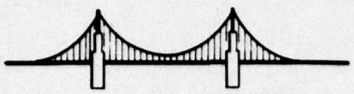

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BEHAVIORAL EFFECTS OF MASSIVE TRANSFUSION WITH CELL-FREE RESUSCITATING SOLUTIONS

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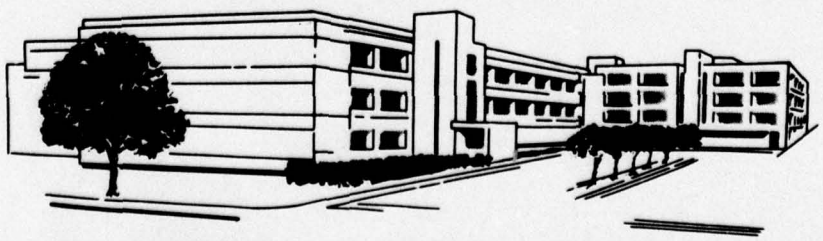
DEPARTMENT OF COMPARATIVE MEDICINE

JAN 1978



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Fixed ratio (FR) and DRL (differential reinforcement of low response rates) operant behavior of rats was examined following 65 percent exchange transfusion with bovine serum albumin (BSA), stroma-free hemoglobin (SFH), or a mixture (MIX) of these materials. Transient performance decrements were observed following transfusion with each solution. FR response rates of transfused animals were significantly depressed in comparison to surgical controls 24 hrs after exchange transfusion, but differences between transfused groups were not significant. There were no significant differences in FR response rates (cont)

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between the surgical control, hemoglobin-albumin, and albumin groups after the first daily test session. Response rates of the SFH group remained significantly lower in comparison to all other groups through the fourth day of testing. Transfusion related changes in DRL performance were similar to those observed with the FR task. Behavioral recovery appeared to be complete in all animals by the second week of testing. Behavioral testing was continued for six weeks. Animals were then sacrificed and tissue samples were obtained for histopathologic study. Light microscopic examination of eye, brain, lung, thymus, heart liver, spleen, pancreas, kidney, mesenteric lymph node, and bone marrow revealed no morphologic differences between control, MIX, BSA, and SFH groups. Further studies will be required to determine the mechanisms of behavioral changes observed during these preliminary experiments.

ABSTRACT

Fixed ratio (FR) and DRL (differential reinforcement of low response rates) operant behavior of rats was examined following 65 percent exchange transfusion with bovine serum albumin (BSA) stroma-free hemoglobin (SFH) or a mixture of these materials. Transient performance decrements were observed following transfusion with each solution. FR response rates of transfused animals were significantly depressed in comparison to surgical controls 24 hrs after exchange transfusion, but differences between transfused groups were not significant. There were no significant differences in FR response rates between the surgical control, hemoglobin-albumin, and albumin groups after the first daily test session. Response rates of the SFH group remained significantly lower in comparison to all other groups through the fourth day of testing. Transfusion related changes in DRL performance were similar to those observed with the FR task. Behavioral recovery appeared to be complete in all animals by the second week of testing. Behavioral testing was continued for six weeks. Animals were then sacrificed and tissue samples were obtained for histopathologic study. Light microscopic examination of eye, brain, lung, thymus, heart, liver, spleen, pancreas, kidney, mesenteric lymph node, and bone marrow revealed no morphologic differences between control, MIX, BSA, and SFH groups. Further studies will be required to determine the mechanisms of behavioral changes observed during these preliminary experiments.

PREFACE

In conducting the research described in this report, the investigators(s) adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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INTRODUCTION

Oxygen bearing cell-free resuscitating solutions and non-oxygen carrier plasma expanders which could replace or supplement whole blood as a transfusion material have been proposed but the total effects upon the central nervous system are not known. There is a growing amount of information concerning general physiological effects of these compounds (1,2); there is relatively little information concerning the effects of these materials on brain functioning or behavior. Casual observations made in this laboratory and elsewhere (3-5) suggest that animals may exhibit "normal" behavior following mass transfusion with various cell-free resuscitation solutions. However, on the basis of these observations, it cannot be concluded that behavioral changes did not occur, since more precise methods of evaluating behavior might have proved more sensitive to treatment effects.

In assessing the effects of blood replacement, behavioral measurements are important for several reasons. First, there is growing evidence that changes in behavior may occasionally precede other symptoms or occur in the absence of demonstrable pathological changes in the central nervous system (CNS) (6-9). Modifications of learned or phylogenetically determined behavior may, therefore, provide direct evidence of changes in the functional capacity of the CNS. Secondly, the identification of possible irreversible effects of transfusion on sensory, motor, cognitive, emotional or other capabilities of the individual are vital end points in themselves since such deficits will directly determine how the organism interacts with its environment following trauma. Finally, behavioral abnormalities might reflect changes in the general health of the individual which are not necessarily evident through observation of other biological variables. In such instances, properly quantified behavioral observations may be used to augment other physiological data which are used in evaluating the safety of experimental resuscitating solutions.

The purpose of the present study was to evaluate the effects of transfusion by using readily quantified measures of behavior obtained under controlled conditions. The methods of behavioral evaluation were based upon operant techniques in which animal subjects were required to perform specific tasks in order to obtain food reinforcement. Operant conditioning methods were selected because these procedures permit precise manipulation and quantification of numerous features behavior.

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Moreover, such techniques are commonly employed in the evaluation of behavioral effects of various classes of drugs (10,11) and non-therapeutic chemical agents (7).

Two operant schedules were selected for preliminary study. These were the fixed ratio (FR) and the DRL (differential reinforcement of low response rates) task. The FR schedule provided food reinforcement in direct proportion (1:20) to the number of bar-press responses emitted by the animal. FR performance is characterized by relatively high and stable response rates (12). The stability of FR responding has led to the use of this type of operant behavior as a baseline against which changes in performance occurring under other operant schedules can be compared (13). The test was selected for detailed investigation because the basic skills required for FR performance are similar to those required to perform the more elaborate operant schedules proposed for future study. The DRL task (14) required that subjects develop precise temporal spacing of responses in order to obtain food reinforcement. In contrast to the FR schedule, successful DRL performance produces low rates of responding with responses spaced at critical time intervals. In the present experiment rats were required to space their bar-presses exactly 10-14 seconds apart in order to obtain reinforcement. This schedule will be referred to as "DRL10, LH4" indicating the occurrence of a 4 sec (LH4 or "limited hold" of 4 sec) critical time window ten seconds after the preceding response. The DRL10, LH4 schedule was selected as a means of determining whether relatively complex timing behavior is preserved following massive exchange transfusion.

Solutions of human stroma-free hemoglobin (SFH), bovine serum albumin (BSA), or a mixture of those materials were selected for study. The formulations of BSA and SFH solutions used have been described previously (15) and were comparable to those used by other investigators with respect to protein content, electrolytes, and pH. The clinical uses and research investigations of serum albumin have recently been reviewed elsewhere (2,16). One reason for selecting BSA for use in the present studies is the ability of albumin solutions to maintain colloid osmotic pressure within normal limits following transfusion. Circulating albumin is also involved in the transport of hormones, enzymes, fatty acids, ions, metals, metabolites and bilirubin (16). With a half-life of several days, substantial amounts of infused albumin remain the circulatory system during the period in which components of normal blood are being regenerated.

One obvious limitation to the use of albumin infusions for blood replacement is the inability of this material to oxygenate tissues. However, SFH solutions are capable of providing oxygen to perfused tissues and massive exchange transfusions of this material have been accom-

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plished by a number of investigators (17-20) using such diverse species as dogs, mice, non-human primates, pigs, rats, and rabbits. It has also been reported that hemoglobin solutions have been used successfully in human patients in the Soviet Union (17). Stroma-free hemoglobin, unlike albumin, is rapidly removed from the circulatory system following exchange transfusion. During the present experiments, loss of infused SFH was essentially complete at the time of behavioral testing.

METHODS

Subjects

Subjects were male outbred Sprague-Dawley rats, 90 days old at beginning of the experiments. All animals were maintained under constant temperature and humidity conditions and under 12 hr light-dark cycle adjusted so that the dark phase corresponded in time to normal laboratory working hours. The reversed light cycle permitted behavioral testing during periods in which rats were normally most active. Throughout the experiments, subjects were provided water ad libitum while in their home cages. Total food consumption was limited to the amount of food obtained during operant sessions plus a supplementary food ration given immediately after each operant session. Extra food rations were provided over weekends. Rats continued to gain weight during the several weeks in which the studies were conducted but weights remained approximately 10-20 percent below rats maintained on food rations ad libitum. None of the animals used in the present experiments had been used for previous studies.

Procedure

Training and behavioral testing was accomplished in Coulbourn Instrument, Inc. modular test chambers which were located in sound attenuating cubicles. All operant schedules were under digital logic control. Subjects assigned to FR and DRL groups were allowed one five-day week for preliminary adaptation and training to bar press for food (45 ng Noyes pellets) reinforcement. All animals were operating on continuous reinforcement (CRF, one response per pellet) by the end of their first week of training. During the second week of training FR animals were brought to 20 responses/reinforcement (FR20) level and were maintained on this schedule for a minimum of one month before experimental treatments were administered. DRL subjects were switched from CRF to DRL10, LH4 schedule for two months before treatment. Subjects in all groups were run 30 min/day, 5 days/wk, but were run daily for the 10 days preceding ("baseline") and following ("recovery") treatment. Post-treatment sessions were then continued using the 5 day/wk schedule for a period of one month.

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Following training FR rats were assigned at random to treatment groups and were exchange transfused with 7 percent solutions (in kidney dialysis fluid) of BSA, SFH, or a mixture of 7 percent SFH and 7 percent BSA (14 percent total concentration). DRL rats received only BSA or SFH transfusions due to the small number of animals trained to perform this task. Prior to blood replacement rats were anesthetized with diethyl ether. A sterile PE90 polythylene catheter was then inserted into the heart via the exposed jugular vein. A serial 1 ml exchange of blood with one of the test solutions was then accomplished until approximately 65 percent reduction in hematocrit was obtained. The entire transfusion process required about 15 min. The catheter was then removed and the jugular vein ligated. Rats not assigned to BSA, SFH, or MIX groups were subjected to identical surgical and anesthetic conditions but were not transfused. All animals were allowed to recover for 24 hrs and were then returned to the laboratory for routine daily behavioral testing.

Behavioral testing was continued for one month following transfusion. Following completion of behavioral studies, 19 rats were sacrificed for pathological study. Five rats were available from each of the SFH, BSA, and surgical control groups and four rats from the group transfused with the SFH and BSA mixture. Each rat was anesthetized with pentobarbital sodium by intraperitoneal injection. As much blood as possible was collected by cardiac puncture. Complete blood counts were performed on blood collected in EDTA. Serum was subjected to automated analysis (SMA 6/12) of the following components: creatinine, urea nitrogen (BUN), uric acid, total protein, glucose, cholesterol, calcium phosphorous, serum glumatic oxalic transaminase (SGOT), lactic dehydrogenase (LDH), and total bilirubin. Tissues were collected for histopathologic examination from the eye, brain, lung, thymus, heart, liver, spleen, pancreas, kidney, mesenteric lymph node, and bone marrow.

Data Analysis

Total responses and reinforcements were obtained for each 30 min session from all subjects. For FR subjects the response totals for the 10 days preceding treatment were averaged to obtain the base response rates. The dependent variables for the FR groups were the daily response totals and the number of days required to reattain baseline response rates. Additional data were obtained from each DRL animal. Specifically, frequency distributions of inter-response times were generated by measuring the elapsed time between successive responses and categorizing each observation into one of fifteen 2-sec time intervals. Two indices of performance efficiency were also derived. The first, ER1, was the ratio of total reinforcements to total responses expressed as a percent; the second, ER2, was the the percent of possible reinforcements obtained during a 30 min session (a maximum of 180 reinforcements could be obtained in a 30 min session if subjects spaced their responses exactly 10 sec apart). Each of these efficiency measures and total responses were selected as dependent variables for the DRL task.

One-way analysis of covariance (BMDPIV) (21) was performed on the FR response rate and recovery data. Pre-transfusion baseline response rates

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were the covariates of primary interest in the present study. In addition, for groups subjected to exchange transfusion, the percent change of hematocrit following blood replacement was also used as a covariate. The analysis of covariance permitted the adjustment of post transfusion FR performance scores for the effects of slight differences in the pre-transfusion baseline performance levels and amounts of blood replaced. Prior to the application of the analyses of covariance several statistical assumptions of these analyses were tested. All variables selected as covariates were first analyzed by using analyses of variance to be sure that pre-treatment differences between group means were not significant. At that time tests for homogeneity of variance were also applied. Statistical assumptions dealing with the similarity of within group linear regression coefficients were tested at the time that the analyses of covariance were performed. In addition to the analyses of covariance, two tailed t-tests for correlated data were used to determine whether or not within group changes between baseline and initial (24 hr) post-treatment test sessions were significant. Data collected from DRL animals were summarized for comparison with results of the FR analyses. However, between group statistical analyses were not used for these data due to the small number of DRL trained animals. The $P \leq 0.05$ level was used for all tests of statistical significants.

RESULTS

Behavioral Changes

A total of 27 FR animals were available for post-treatment behavioral testing: seven surgical controls, eight subjects in each BSA and SFH group, and four in the MIX group. Four additional rats were trained to perform the DRL task. Two DRL animals were transfused with SFH and one with BSA. The remaining DRL subject was used as a surgical control.

There was considerable individual variation in the general behavior of transfused subjects. Two SFH transfused animals in the FR study were anorexic and stuporous following transfusion. One of the subjects had received a 72% blood replacement and remained generally unresponsive for several days. The second subject, who exhibited similar although less dramatic symptoms, had received a 54% transfusion with SFH. These two animals were among the first subjects transfused during the present series of experiments. Subsequent transfusions of other rats with either SFH or BSA mixtures did not produce such obvious behavioral aftereffects. In general, rats were active within a few hours of transfusion or surgery and appeared to be alert immediately before their first (+24 hr) post-treatment operant test sessions.

FR response rates were significantly depressed 24 hrs after blood replacement with each of the three transfusion solutions. Bar press responding was not significantly depressed following surgical control procedures. The averaged 30 min response rates before and 24 hrs after exchange transfusion or surgical control procedures were as follows: surgery, 2434 and 2224 ($t = -1.23$, $P < 0.05$), BSA 2620 and 1564 ($t = -4.41$, $P < 0.01$), SFH 2914 and 1419 ($t = -5.52$, $P < 0.01$), MIX 2875 and 1525 ($t = -3.63$, $P < 0.05$). The results of the analysis of covariance showed

that response rates of all transfusion groups were significantly lower than the surgical control group, but there were no significant differences between transfusion groups 24 hrs after blood replacement. Rapid recovery of response rates were observed in groups transfused with the hemoglobin-albumin mixture or with BSA alone. There were no significant differences between surgical control, MIX, and BSA groups on the second day of testing and thereafter. However, response rates of SFH transfused rats were significantly lower in comparison to each of the other groups during the 2nd, 3rd, and 4th post-transfusion sessions. Since there were no significant differences (after the 1st recovery session) between BSA, MIX, and surgical control groups, it was possible to combine observations from these groups for comparison with the SFH group. Two specific contrast analyses were performed. The first compared the performance of the SFH group with that of the combined BSA and MIX groups, the second contrasted SFH group with all other groups. The results of these analyses of variance showed that response rates of the SFH rats were significantly lower than all other groups during each of the seven recovery sessions which were analyzed. Recovery times (in days) did not differ significantly between surgical control (2.35 $\pm$ .78 S.E.), MIX (2.86 $\pm$ 1.13 S.E.) and BSA (4.14 $\pm$ .73 S.E.) conditions. The recovery of the SFH transfused groups (6.42 $\pm$ .72 S.E.) was significantly delayed in comparison to all other groups.

Linear regression analyses, performed in conjunction with the analyses of covariance, showed that the effects of surgical control and exchange transfusion procedures were strongly dependent upon baseline performance levels. Rats having the highest pre-treatment FR response rates were least affected by experimental manipulations, regardless of their group assignments. The effect of this covariate was most evident in the daily total response scores. There were no statistically significant relationships between the baseline response covariates and recovery of performance. The percent change in hematocrit during exchange transfusion influenced recovery and post-transfusion response rates. Decreases in hematocrit were directly related to increased delays in recovery times and to reductions in response rates. The group means for response rate scores corrected for the effects of both performance and blood replacement covariates are illustrated in Figure 1.

The effects of transfusion or surgical control procedures on DRL performance were similar to those obtained in rats trained to perform under the FR schedule. Baseline (average of 10 days pre-treatment data) and post-treatment values of response rates and efficiency ratios are summarized in Table 1 for all rats performing under the DRL schedule. Gross response rates for the 30 min sessions were moderately affected 24 hrs after exposure to surgical procedures (-40%) or BSA (-26%) transfusion. Response rates of the two rats transfused with SFH alone were relatively more depressed 24 hrs after exchange transfusion (-95% and -82%). Recoveries of response rates to baseline values were similar for all four rats. The surgical control and BSA rats required four days to return to baseline response levels, and the two SFH rats recovered in five and six days.

Although gross response rates may have been transiently depressed following SFH transfusion, it was also evident from the DRL data that timing behavior was maintained even during low response rate sessions.

In Figure 2, the distributions of inter-response times (IRTs) are illustrated. In this figure the uppermost control or "baseline" plot represents the average of the IRT distributions obtained during the 10 days prior to transfusion or surgery. The distributions immediately below the baseline averages are the IRT distributions obtained during successive daily sessions. Each of the points of the IRT distributions has been converted to percent of the total response rate in order to emphasize the temporal distribution of responses rather than absolute numbers of responses per interval. Data presented in Figure 2 show that responses falling within the critical time period are maintained under all experimental conditions although some relative changes in the amplitude and dispersion of the IRT distributions are evident.

Pathology

The results of serum chemistry, hematologic, and histopathologic analyses of tissue samples were unremarkable. Sufficient serum was available from 19 animals for complete blood counts, BUN creatinine determinations. There were no differences in the outcomes of those analyses which were related to transfusion or surgical control conditions. Insufficient serum was available from seven of the 19 rats for total protein, SGOT, total bilirubin, LDH, Ca++, cholesterol, uric acid, alkaline phosphotase, and phosphorous determinations. Results of those analyses which were completed generally revealed no obvious differences between groups. Serum samples from two SFH transfused animals were analyzed for SGOT and LDH. The SGOT values of 430 mu/ml and 348 mu/ml fell outside the range of all other animals in the control, BSA, and MIX groups ($x = 238$ mu/ml, range 193-268, $N = 10$). Similarly, LDH values (760 mu/ml, 808 mu/ml) were elevated in comparison to values obtained from all other animals ($x = 529$ mu/ml, range 333-600, $N = 10$). Hematological studies of the blood samples showed no obvious group differences in complete blood counts, hemoglobin, hematocrit, mean corpuscular volume or red blood cell volumes. No microscopic lesions attributed to transfusion or surgical control procedures were observed in any of the organs or tissue samples described in "methods."

DISCUSSION

Fixed ratio (FR) response rates were significantly depressed following exchange transfusion with cell-free solutions. The magnitude and duration of the effects depended on pre-transfusion performance proficiency, amounts of blood replaced, and on the transfusion solutions employed.

Animals with high response rates were least affected by treatment conditions while rats having moderate or low rates were disproportionately influenced by transfusion or surgical procedures. Such rate-dependent effects are known to occur with FR schedules following administration of

various drugs (22,23). Typically, however, it is the high response rates which are most depressed. Performance of schedules which engender relatively low response rates are usually least influenced by drugs, or in some instances, responding on low rate schedules may actually increase. Rate-dependent effects are usually demonstrated by training animals to perform tasks which include both low and high response rate components. Thus, rate comparisons are usually made within subjects. In the present study, rate-dependent effects were demonstrated by a between-subjects analysis. It is unlikely, however, that this difference in procedure could account for the different outcome, since more recent studies (24) on between-subjects analysis of operant response data support of the typical rate-dependent phenomenon. The results of the present study are most likely due to fundamental differences in the effects of the experimental procedures. The existence of strong rate-dependent effects has practical significance with respect to the design and conduct of future operant studies of transfusion. Specifically, provisions should be made to control statistically or experimentally pre-treatment differences in group performance levels. Adoption of schedules which contain both high and low rate features might also be desirable.

In the present studies, groups were closely matched with respect to amounts of blood replaced. The greatest variations in exchange transfusion levels were observed between subjects within each group. Regression analyses of blood replacement and performance variables showed that levels of transfusion predicted response decrements and recovery times. However, the most severe replacements (approximately 70-75% reduction in hematocrit) used in the present experiments were close to levels which were found to be inconsistent with long term survival. The strong relationships between transfusion and performance may, therefore, be peculiar to a critical region of the dose-response curve. Provisions for systematic dose-response manipulations would be a desirable feature of future behavioral studies of blood transfusion.

Performance of each of the operant task was affected by the type of transfusion materials used. In general, rats transfused with a mixture of hemoglobin and albumin were superior to those transfused with either albumin or hemoglobin alone, although the relative difference between control, albumin, and MIX groups was not statistically significant in these studies. The most dramatic effects in both FR and DRL experiments were observed following SFH transfusion. Several factors must be considered when interpreting this result. First, SFH, with half-life of only 3½ hrs (20), was almost entirely excreted prior to initial behavioral testing 24 hrs following transfusion. It is unlikely, therefore, that the observed behavioral effects could be attributed to factors associated with the presence of large amounts of extracellular hemoglobin at the time of testing. It might be argued that the physio-

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logical changes underlying the observed behavioral changes were initiated shortly after transfusion while SFH levels were relatively high. However, the MIX group which received comparable levels of hemoglobin performed better and recovered significantly faster than rats transfused with SFH alone.

It is possible that the excretion of SFH and the attendant changes in osmolarity may be related in some way to performance changes in the SFH group. This hypothesis could also explain the relatively better performance of the BSA and MIX groups since albumin, with a half-life of several days, would maintain colloid osmotic pressure within normal limits. In contrast, the hypoproteinaemic state of SFH subjects may produce concomitant modulation of hormonal activity, catecholamines, and divalent cations such as calcium (16). Hypovolemia, which occurs following SFH excretion would also stimulate catecholamine production and could exacerbate existing hypoxic conditions. These biochemical changes, in addition to possible hypoxic, edematous, and circulatory effects, could directly influence CNS functions which mediate appetitive operant behavior. Further studies will be required to evaluate these and any alternative hypotheses which might be advanced to account for the behavioral effects observed in the present studies.

The absence of demonstrable histopathological and hemotological changes following massive exchange transfusion with cell-free solutions was not expected considering the long delay between transfusion and tissue sampling. Previous studies (17,25) using shorter temporal delays also failed to demonstrate morphologic changes in the kidney, liver, or lungs of dogs transfused with a SFH solution similar to that employed in this study. The absence of morphological sequelae in the brains (or other tissues) of rats previously exhibiting behavioral abnormalities might be explained in several ways. First, there may have been no histopathologic consequences of transfusion. Functional changes might have been the result of short term neurochemical or metabolic abnormalities without morphological manifestations. Secondly, lesions may have been present following perfusion with subsequent recovery leaving no trace of damage. Finally, existing lesions or evidence of past morphologic change may have been overlooked due to the limitations of light microscopy and examination of a limited number of tissue samples.

Apparent elevations in SGOT and LDH values of the two SFH transfused animals should be interpreted with caution. Such increases are typically associated with active tissue necrosis. In the present study there was no evidence of differential histopathological changes associated with transfusion materials. It is possible, therefore, that the relatively high SGOT and LDH values represent a spurious association between group membership and the pathological processes of individual rats. Error variability in enzyme determinations may also have contributed to a chance elevation within this group. Possible group differences in SGOT and LDH levels should be reinvestigated with larger number of animals in future studies.

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CONCLUSIONS AND RECOMMENDATIONS

FR and DRL operant behavior was transiently changed following exchange transfusion with cell-free solutions under the limited conditions of the present studies. Behavioral effects were observed in the absence of obvious long term morphological changes. Additional studies should be conducted with these tests to examine parametrically the behavioral consequences of transfusion with a wider variety of materials. Appropriate baseline data must also be obtained with standard transfusion materials such as banked blood in order to evaluate properly experimental resuscitating solutions or techniques. A continued effort should be made to increase the sensivity of behavioral tests through experimental or statistical control of variability in behavior. Other types of behavioral testing should also be considered for possible inclusion in a test battery which would permit rapid and comprehensive evaluation of treatment effects under a wide variety of treatment conditions.

Future pathological studies should include evaluations of tissue samples obtained at shorter post-transfusion intervals. Electron microscopy should be used in an attempt to detect subtle changes in tissue. Biochemical analyses should be expanded to include neurochemical assays.

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APPENDIX A
FIGURE CAPTIONS

Fig 1 Recovery of fixed ratio response rates. Average response rates for each group were obtained during successive daily test sessions following exchange transfusion or surgical control procedures. The first session occurred approximately 24 hours after transfusion or surgery. The location of each data point has been corrected for the effects of pretreatment differences in response rates and for group differences in the amounts of blood replaced.

Fig 2 DRL interresponse time distributions. Each distribution was derived by measuring the times between pairs of bar press responses and accumulating observations into one of 15 consecutive 2-sec wide intervals. Total counts within each interval of the resulting frequency distributions were divided by the total number of observations. The resulting distributions of proportions illustrate the relative frequencies of response categories independent of absolute response rates. The left-most point of each plot corresponds to the midpoint of the first interval (i.e., 1 sec). The uppermost plot (baseline) of each series of histograms represents the average of the distributions obtained during the ten days preceeding exchange transfusion or surgical control procedures. Plots located below the baseline distributions were obtained during successive recovery days. The prominent peaks of the distributions fall within a critical 10-14 sec time window which produced food reinforcement. Each series of distributions is identified by subject number, percent drop in hematocrit, and type of transfusion solutions.

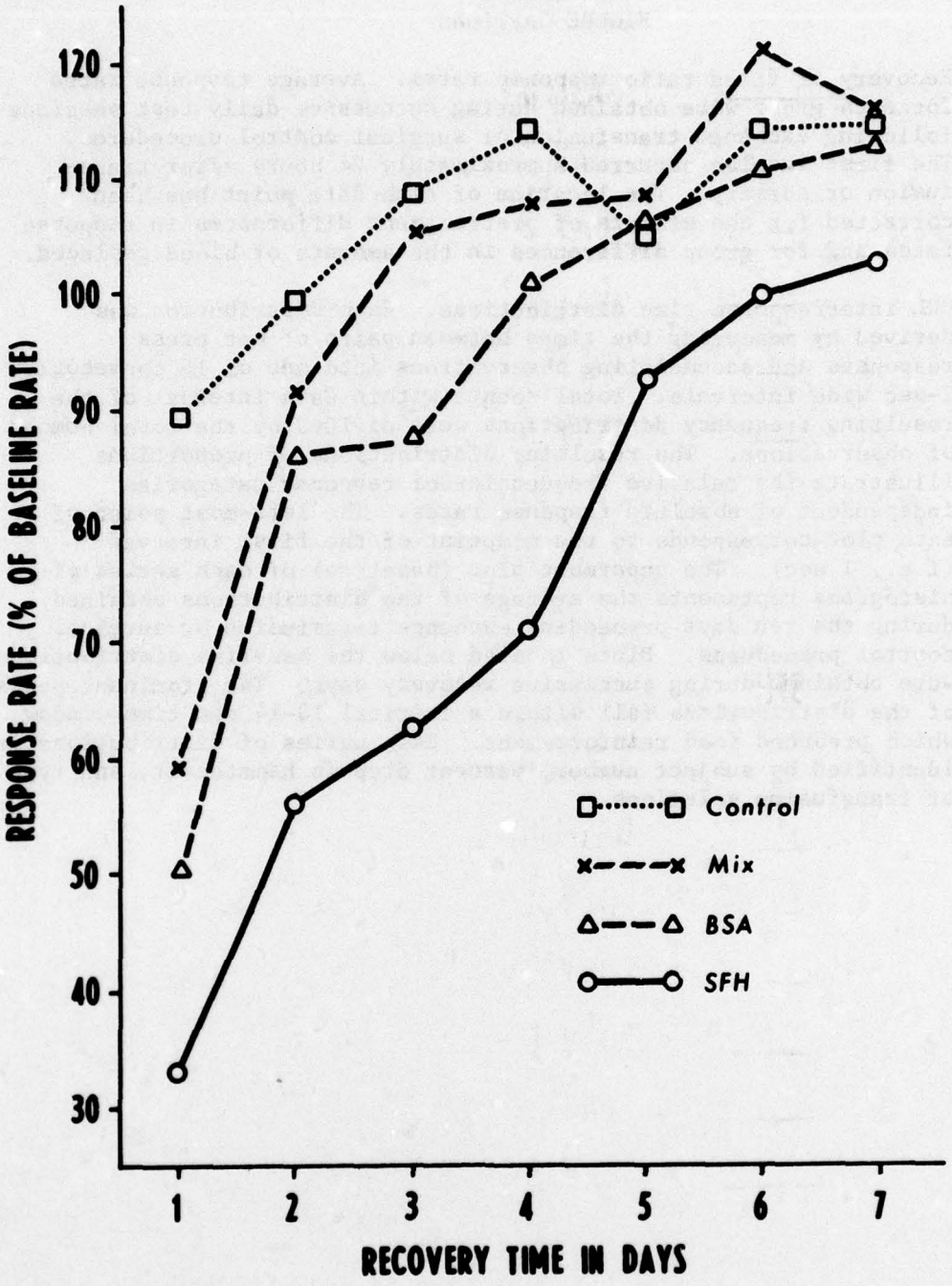

RESPONSE RATE (% OF BASELINE RATE)
120
110
100
90
80
70
60
50
40
30
1
2
3
4
5
6
7
RECOVERY TIME IN DAYS
Control
Mix
BSA
SFH

Figure 1

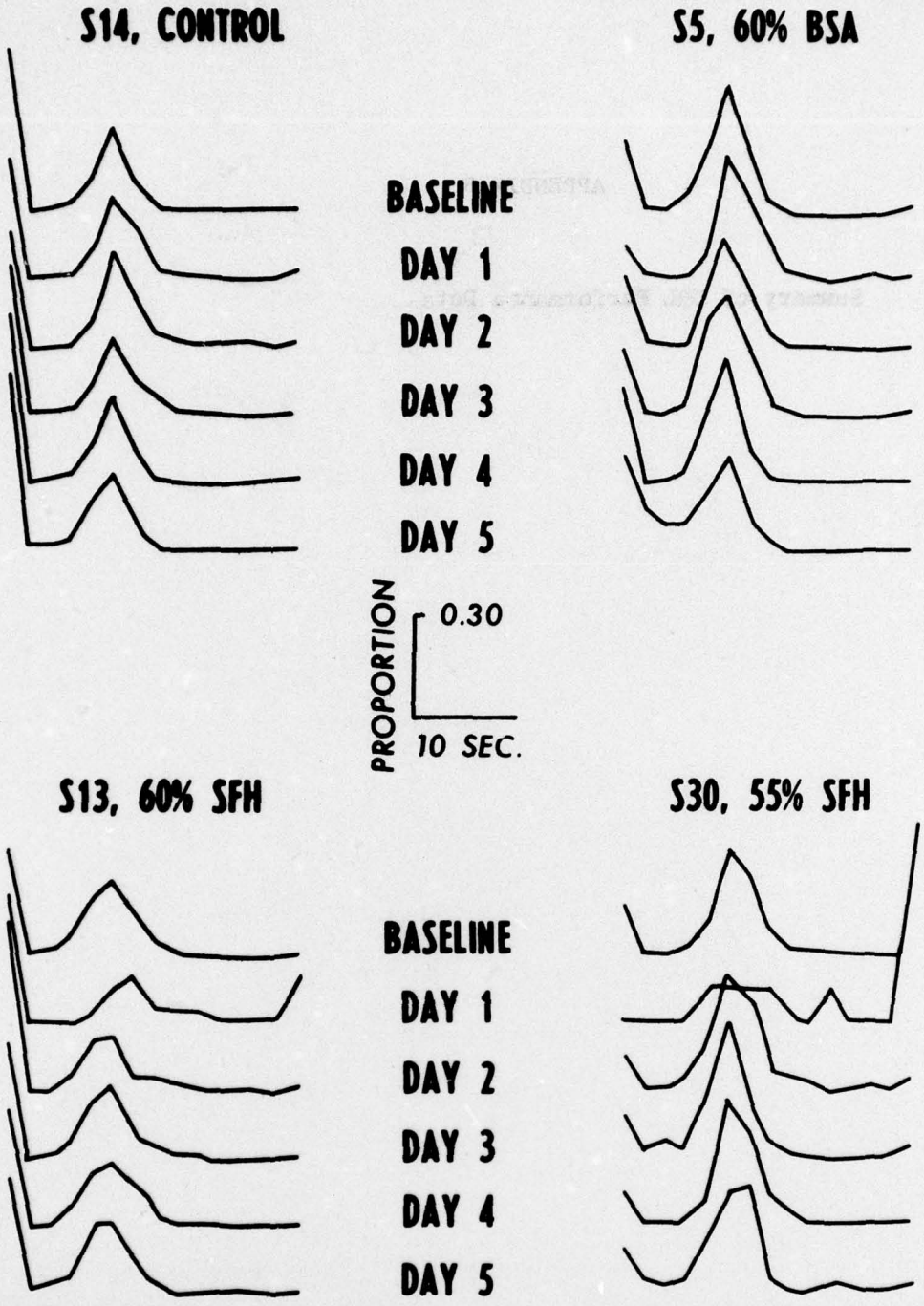
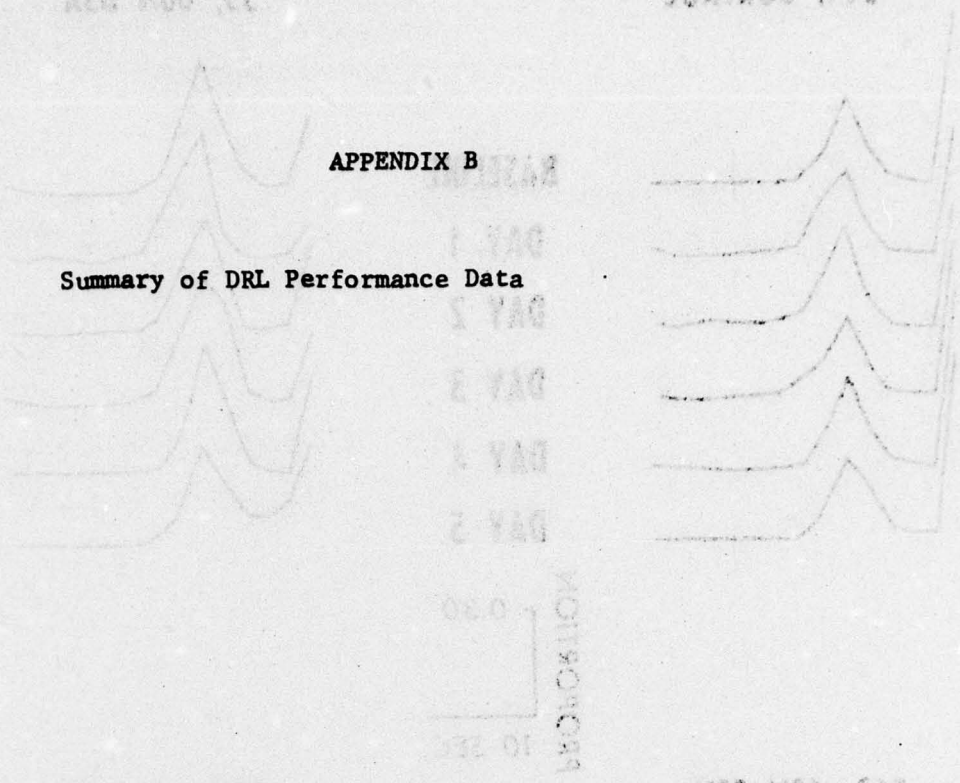

S14, CONTROL
S5, 60% BSA
BASELINE
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5
PROPORTION
0.30
10 SEC.
S13, 60% SFH
S30, 55% SFH
BASELINE
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5

Figure 2

APPENDIX B

TABLE 1 Summary of DRL Performance Data



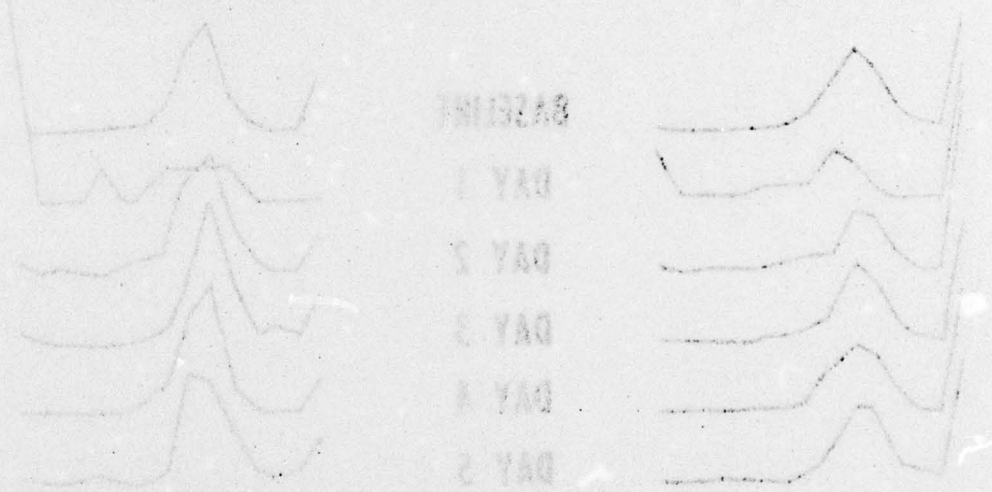


TABLE 1

SUMMARY OF DRL PERFORMANCE DATA

	S14, CONTROL			S5, 60% BSA			S13, 60% SFH			S30, 55% SFH		
	R	ER1	ER2	R	ER1	ER2	R	ER1	ER2	R	ER1	ER2
BASELINE:	348	0.35	0.66	232	0.43	0.55	272	0.32	0.48	183	0.60	0.52
RECOVERY:												
DAY 1	208	0.39	0.44	171	0.63	0.60	47	0.17	0.04	10	0.30	0.17
DAY 2	243	0.49	0.67	228	0.46	0.58	234	0.20	0.26	172	0.56	0.54
DAY 3	292	0.36	0.58	228	0.50	0.64	152	0.29	0.24	179	0.48	0.48
DAY 4	350	0.37	0.72	258	0.40	0.57	190	0.29	0.30	171	0.66	0.62
DAY 5	341	0.31	0.59	214	0.50	0.59	279	0.30	0.46	171	0.57	0.54
DAY 6	313	0.41	0.72	217	0.49	0.59	233	0.32	0.41	185	0.63	0.65
DAY 7	336	0.30	0.56	232	0.47	0.61	221	0.19	0.23	178	0.62	0.62

NOTE: R = Total number of bar presses/30 min session
ER1 = Proportion of food reinforced responses
ER2 = Proportion of the maximum number of reinforcements which were actually obtained

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