til they disappear altogether, leaving only the first deflection, the amplitude of which fluctuates markedly. This component also often disappears entirely for extended periods when the EEG shows the large slow waves characteristic of deeper sleep.

These data demonstrate that major changes occur in the evoked responses generated by activating a nonsensory, "central-central" system, and that these changes apparently correlate with changes in the background EEG and associated states of arousal. That the strength of stimulation was near maximal serves to emphasize the power of this central control phenomenon.

In contrast to the present results, most investigators report that under their experimental conditions evoked responses in sensory systems usually become larger in amplitude and duration in association with behavioral and EEG signs of reduced arousal. We have been able to reproduce this typical result under our experimental conditions in the primary cortical projection of the bulbar trigeminal nuclear complex (*5*). In this system the cortical response evoked by stimulating various points in the fifth nerve nucleus is enhanced in association with increased EEG synchrony and is attenuated when the background EEG is desynchronized. We have also demonstrated these opposite modifications of the cerebellocerebral and trigemino-cerebral projections taking place in the same cat simultaneously.

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1 November 1962

Reversible Agglomeration Used To Remove Dimethylsulfoxide from Large Volumes of Frozen Blood

Abstract. *The clumping that takes place when blood is mixed with isotonic glucose is well known to the clinician. This curious phenomenon has been characterized and used to prepare blood frozen in the presence of dimethylsulfoxide for transfusion. A hypothesis is advanced to explain the mechanism of reversible agglomeration of erythrocytes in nonelectrolyte solutions.*

Every surgeon has witnessed the clumping of erythrocytes that takes place within the intravenous set when a blood transfusion is followed by isotonic glucose in water. This commonly observed phenomenon is worrisome, but does no apparent harm to the patient.

In experiments undertaken to find a simple method for removing the preservative agent dimethylsulfoxide from thawed blood, we suspended 1 volume of packed human red cells in 10 volumes of isotonic sucrose. Within 3 minutes, progressively larger aggregates of cells formed, which settled to the bottom of the container. More amazing was the further discovery that resuspension of the agglomerated cells occurred after the addition of an equal volume of plasma.

Reversible agglomeration of blood cells was further studied to characterize the phenomenon. Aggregates were found to form in solutions of glucose or glycine as well as in sucrose. These diluents must range in concentration from slightly hypotonic (200 mosm/kg) to two and one-half times normal osmotic strength. At 5°C agglomeration takes place more slowly than at 20°C and occurs most rapidly at 40°C. The *p*H optimum for settling lies between 5.2 and 6.1. Between *p*H 6.1 and 6.5 clumping occurs, but settling is retarded. Above *p*H 6.5 no clumps form, and those previously formed at a lower *p*H resuspend. Dispersion also occurs and agglomeration is blocked by the presence of sodium chloride in concentrations greater than 0.02*M*.

These parameters, plus the known tendency of γ-globulins to form reversible complexes with plasma β-lipoproteins in the *p*H range 5.2 to 6.1 (*1*), permitted a tentative hypothesis to be advanced. At a *p*H between 5.2 and 6.1 γ-globulins in the plasma might form a reversible complex with the lipoprotein of the walls of red blood cells. Reduction of the ionic strength of the medium by dilution with a nonelectrolyte might then precipitate the γ-globulins with coprecipitation of the

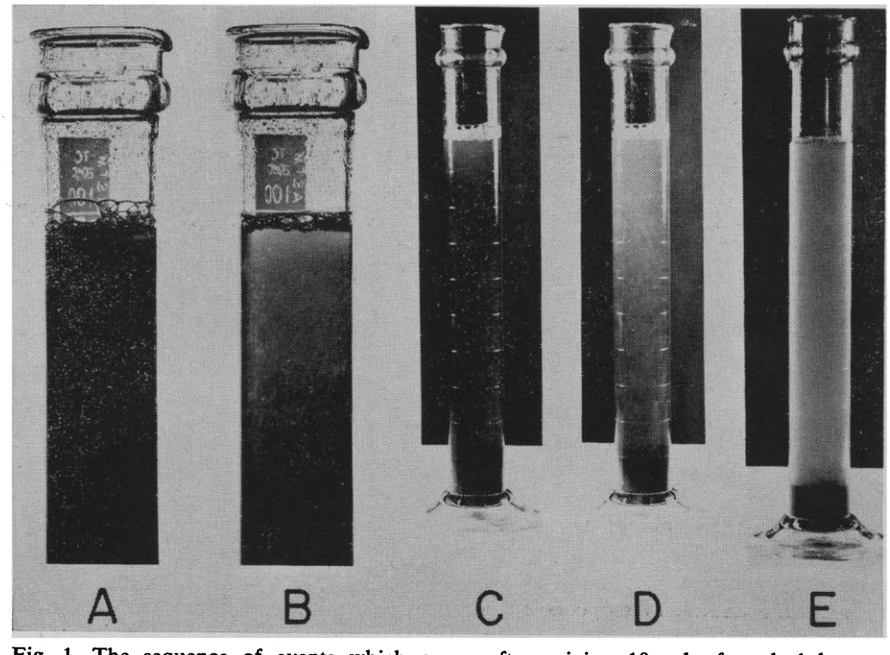

Fig. 1. The sequence of events which occur after mixing 10 ml of packed human blood with 90 ml of 10-percent glucose: *A*, immediately after mixing; *B*, 30 seconds; *C*, 80 seconds; *D*, 120 seconds (the fuzziness is due to motion of the clumps not stopped by exposure time of 1/25 second); *E*, 5 minutes. Note agglomeration to less than the initial volume of packed cells.

red blood cells. Resuspension of the cells would be effected either by raising the ionic strength, splitting the γ-globulin–γ-globulin bond, or by raising the *p*H, breaking the γ-globulin–lipoprotein bond.

To examine this hypothesis, two red cell lots of the same blood were washed centrifugally with saline solutions buffered at *p*H's of 5.5 and 7.2. Under my hypothesis, the γ-globulins responsible for reversible agglomeration would be expected to remain complexed to the cells washed at *p*H 5.5 and to be removed from those washed at *p*H 7.2. Those cells washed at *p*H 5.5 agglomerated and settled after dilution with 10-percent glucose, while those washed at *p*H 7.2 did not. Addition of minute amounts of human immune γ-globulin to the cells washed at *p*H 7.2 restored their ability to clump together and settle. Human serum albumin, fibrinogen, and egg white were ineffective in restoring the ability to agglomerate.

Collateral evidence to support my hypothesis was obtained when I found a patient with severe hypogammaglobulinemia (E.S. MGH No. 88-80-75). Her packed blood cells in acid citrate dextrose solution (ACD) anticoagulant settled poorly; however, another 1-ml sample to which had been added 20 mg of human immune γ-globulin settled normally when diluted with 10-percent dextrose in water. Neither the *p*H nor the electrical conductivity of the supernatant was altered by the addition of the γ-globulin.

The mechanism of action of reversible agglomeration of human erythrocytes may well prove more complex than my simple hypothesis would suggest. It does, however, explain observed facts and has been found useful in the development of a simple technique for washing dimethylsulfoxide from thawed human blood.

Clinical units of blood (500 ± 100 ml) from healthy donors were drawn into standard plastic bags containing ACD anticoagulant. The blood was separated centrifugally. The packed cells thus obtained were diluted with an equal volume of either autoclaved 5-percent glucose or 8-percent sucrose made up in 8.6*M* dimethylsulfoxide (*2*). The treated cells were reconcentrated by centrifugation and their plastic container placed horizontally in an electrical deepfreeze at −88°C. The frozen cells were completely thawed in 5 to 8 minutes by placing their plastic bags in a vigorously stirred water bath at 40° ± 0.5°C. Hemolysis was less than 5 percent, and generally less than 2 percent, for a consecutive series of 67 units stored for periods up to 42 days (*3*).

The thawed blood was transferred from its plastic bag to the bottom of a glass cylinder (48.5 cm long by 8.5 cm in diameter), and stirred with a magnetic stirrer. Sterile 10-percent glucose at a temperature of 20°C was added slowly to effect dilution from 300 to 2000 ml. The clumped cells settled, the supernatant was discarded, and the washing by dilution repeated twice. No attempt was made to resuspend the cells between dilutions. The supernatant-free agglomerated cells were finally resuspended by the addition of the thawed plasma from the original donor. The reconstituted blood was drawn off into a sterile plastic bag and stored at 5°C.

Recovery of 75 to 85 percent of the originally donated cells may be easily accomplished in 45 to 60 minutes after thawing. It is fortuitous that the *p*H of ACD anticoagulated blood and 10-percent glucose are optimal for reversible agglomeration of the cells. Dimethylsulfoxide concentration of the reconstituted blood, as measured by gas chromatography, is less than 0.2 percent (*4*).

Clinical units of human blood have been treated with dimethylsulfoxide, stored at −88°C, and administered without apparent ill effect. Reversible agglomeration of the red blood cells, possibly mediated by erythrocyte-γ-globulin coprecipitation, has proved a simple, effective, and inexpensive method for rapidly removing dimethylsulfoxide from thawed blood. Further studies to perfect this method for clinical preservation of blood by freezing are in progress.

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10 December 1962

Disinhibition after Prefrontal Lesions as a Function of Duration of Intertrial Intervals

Abstract. *Dogs were trained preoperatively in both positive and inhibitory conditioned food reflexes on a schedule of either a 15-second or 1-minute intertrial interval. After lesions had been made in the medial surface of the prefrontal cortex, errors of disinhibition occurred in both schedules and in association with an increased "drive" for food. In contrast, lesions of the dorsolateral prefrontal cortex produced the disinhibition syndrome only in the group which was tested at short intertrial intervals, and no increase in food-directed activity was noticed. In each instance the postoperative recovery was very rapid. It is suggested that the quality of disinhibition in prefrontal animals is different, depending on the placement of the lesion.*

This study was undertaken to delineate further the role of the prefrontal cortex in inhibitory activity (*1*) by investigating the effect of partial lesions on conditioned reflexes (CR's) in dogs.

The animals were prepared as previously described (*2*). Briefly, the procedure consisted of training the dogs to place their right forelegs on a food tray to obtain food reinforcement when a 1000-cy/sec tone (positive conditioned stimulus) was presented, and to refrain from this response when a 700-cy/sec tone (inhibitory conditioned stimulus) was presented. No food was given on the inhibitory trials. Errors

Table 1. Scores of pre- and postoperative inhibitory trials and errors, including the criterion, in group 1 animals (15-second intertrial interval). *T*, trials; *E*, errors.

Dog No.	Preoperative		Postoperative	
	T	E	T	E
Dorsolateral prefrontal lesions				
30	360	195	270	92
31	520	346	270	64
32	830	395	140	22
33	200	120	155	41
Medial prefrontal lesions				
34	520	263	125	60
35	360	172	115	32
36	605	451	215	94
37	280	143	245	85
Posterior cingulate lesions				
26	420	291	50	5
27	295	171	55	7
28	435	273	50	3
Unoperated controls				
47	440	230	50	4
48	290	120	50	5