of *Paramecium* caused by starvation in equilibration medium.

Cells maintained in RR-culture medium were not prestarved and they represented an array of stages within the cell cycle. This fact probably accounts for the individual variability of cell resistance to RR and to Ba^{++}.

The cell membrane itself and the membrane potential changes during cell cycle[27,28], and this may influence timing of effects of RR in a particular cell.

The behavioral reaction of wild type cells during their immersion into RR solutions displayed some variability: short lasting reversions, inhibition of reversions and/or fast forwards swimming. All these reactions are known to be under membrane potential control[29], since hyperpolarization decreases the frequency of action potentials and increases ciliary beat frequency while depolarization increases frequency of action potentials and decreases ciliary beat frequency. Therefore it is expected that, at least in the majority of cells, RR solution brings about the temporal hyperpolarization and inhibits depolarization. However, this prediction requires an electrophysiological confirmation.

Cells immersed into RR solution and then washed out are able to reverse; however, after long exposure to RR they gradually lose excitability. These cells become resistant to the toxic action of Ba^{++} and become capable of spontaneous shape transformation. They also behave like pawn cells in the presence of chlorpromazine. All these facts are taken as evidence that RR acts on the Ca^{++} gating mechanism through its gradual inactivation. Since this reaction depends on periods of exposure and concentration of RR, and since its kinetics depends on temperature, some conformational changes in cell membrane are probably involved in this inactivation. This gradual inactivation of Ca^{++} gating mechanism is specific to RR and not to all polycationic dyes, since it does not occur in the presenc of AB.

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Hematocrit as an index of changes in plasma volume in conscious dogs[1]

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Summary. Hematocrit (HCT) measurements were made in intact and splenectomized conscious dogs to determine if observed decreases in HCT were produced by plasma volume expansion or splenic sequestration of erythrocytes. We found that in conscious dogs HCT is a poor indicator of changes in plasma volume.

During the course of experiments on conscious dogs it was observed that hematocrit values obtained from animals immediately after being brought from the kennel area were considerably higher than those obtained subsequently from the same animals after lying unrestrained for 15–30 min in the laboratory. The experiments being conducted required the administration of various amounts of water and it was our desire to use hematocrit as an indication or confirmation of changes in plasma volume. Although such correlations are commonly used with anesthetized animal preparations it was unlikely that the volumes of water used in our experiments would have increased plasma volume by more than 2% and probably could not alone bring about the observed reductions in hematocrit. It is well documented that large changes in hematocrit can occur in dogs during exercise[5–7] due to an increase in sympathetic stimulation and contraction of the splenic reservoir. It would then seem reasonable that a trained dog brought from the kennel environment where the level of exercise and excitement is presumably high might show a reduction in hematocrit when placed in the more controlled and less stimulating laboratory environment. This reduction in hematocrit would most likely be a gradual process accomplished by a decrease in sympathetic tone and splenic dilatation over a period of minutes or perhaps hours. It might also be possible to observe an increase in hematocrit during the course of an experiment if the animal were to become emotionally aroused or if sympathomimetic agents are administered. This posed an interesting question concerning the acceptability of using hematocrit as an index of

The effect of splenectomy on hematocrit in the excited, resting and norepinephrine stimulated dog (means ± SE)

Kennel		Recumbency		Recumbency (pre-norepinephrine)		Post-norepinephrine
Pre-splenectomy		15–30 min		1.5–2 h		
49.4 ± 0.7%	($p < 0.001$)	41.6 ± 0.9%	($p < 0.025$)	39.0 ± 0.4%	($p < 0.001$)	50.8 ± 1.0%
Post-splenectomy		15–20 min		1.5 h		
41.6 ± 2.0	(NS)	40.3 ± 2.0%	(NS)	41.1 ± 1.7%	(NS)	41.6 ± 1.9%

changes in plasma volume in conscious dogs. Therefore, it was necessary to ascertain what part splenic sequestration of erythrocytes played in the observed reductions in hematocrit.

Materials and methods. Hematocrit determinations were carried out in 2 female mongrel dogs (19.1 and 30.8 kg) during the course of another series of experiments already in progress. The animals were previously trained to lie on a laboratory table and were accustomed to the various manipulations. During the first 15–20 min of recumbency a foley catheter was placed in the bladder and an intercath was placed in a leg vein for venous blood sampling and infusions. At 30–45 min from the beginning of the experiments, the animals received 15 cm^3/kg of warm tap water via stomach tube or an i.v. or intracarotid infusion of distilled water at 3 cm^3/min for 30 min. Blood samples were drawn from the 2 dogs 1. in the kennel area, 2. after walking from the kennel to the laboratory and lying unrestrained for 15–30 min on a table, and 3. after 1.5–2 h of recumbency. At the end of this time l-norepinephrine (Levophed, Winthrop) was infused i.v. at 10 µg/min for 10 min and a final blood sample was taken. The infusion of norepinephrine was given to simulate pharmacologically the level of exercise and excitement encountered in the kennel environment. Following completion of this series of experiments the 2 animals were splenectomized and after a recovery period of 10 days hematocrit measurements were obtained following the same protocol.

Hematocrit determinations were made in duplicate using capillary tubes and a microcentrifuge. Blood samples were spun at 11,500 rpm for 5 min. The values obtained included the buffy coat with no correction for plasma trapping.

10 such experiments were conducted before and after splenectomy, 6 in one dog and 4 in the other. Since the number of animals was small, each experiment was taken as a separate individual for statistical purposes. The results were compared with an analysis of variance and Newman-Keuls test for comparisons within groups and unpaired t-test for comparisons between the groups.

Results. The results of the experiments are summarized in the table. The animals remained calm throughout the experiments with heart rates always below 90 beats/min and usually below 70 beats/min. Heart rates occasionally fell below 55 beats/min when the animals were sleeping. Although blood pressure was not measured, the infusion of norepinephrine always produced a bradycardia which was presumably reflex in nature.

Before splenectomy, hematocrit was significantly decreased from the mean kennel value following 15–30 min of recumbency and was further decreased after 1.5–2 h of recumbency. Following the infusion of norepinephrine hematocrit was significantly higher than the pre-norepinephrine value but was not different from the mean kennel value.

Following splenectomy the mean kennel hematocrit was significantly lower than before splenectomy ($p < 0.02$) but was no longer changed following 15–20 min of recumbency, 1.5 h of recumbency, or following the infusion of norepinephrine.

Discussion. The results clearly show that when the trained dog is taken from the kennel environment and brought into the laboratory hematocrit decreases and this decrease appears to be, at least in part, a function of the time at rest. This is apparently due to a decrease in sympathetic tone and dilation of the splenic reservoir since no significant changes in hematocrit were observed during the course of experiments after splenectomy. In addition, the infusion of norepinephrine given to stimulate pharmacologically an increase in sympathetic tone seen during exercise or emotional arousal produced an increase in hematocrit only in the intact animals. Following removal of the spleen, the mean kennel hematocrit decreased to a level similar to that of the calm resting intact animals.

These results are consistent with others[5–7] who have shown that hematocrit is higher in the exercising dog than in the calm resting dog. Vatner et al.[7] have also demonstrated that removal of the spleen results in a red blood cell volume which is similar to the intact resting dog. Kraan et al.[6] found that exercise is associated with an increase in hematocrit in beagles due to an increase in circulating erythrocytes and also a decrease in plasma volume. Although we did not measure plasma volume directly, we find no difference between the hematocrits of splenectomized dogs in the kennel environment and their hematocrits in the resting state indicating that plasma volume is probably unchanged. Experiments in humans[8] have shown that infusions of norepinephrine do not alter hematocrit or measured plasma volume. It is not surprising that our results with norepinephrine infusions in splenectomized dogs are consistent with these human experiments since studies of the isolated perfused human spleen[4] have demonstrated that nerve stimulation and norepinephrine infusion produce vasoconstriction but unlike the spleen of the dog shows very little change in splenic volume.

Although the various solutions administered may have increased plasma volume and decreased hematocrit, these changes were not detectable within the error of our measurements. We conclude that in the conscious dog changes in hematocrit are a poor indicator of experimental changes in plasma volume and do not reflect the volume of solutions administered. Hematocrit in the conscious intact dog may also change during the course of an experiment with changes in the level of excitement of the animals.

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