Introduction. Synthetic erythrocytes (SE) are a new type of blood substitute, consisting of stroma-free hemoglobin (SFH) solution encapsulated within a phospholipid-cholesterol membrane (liposome) and suspended in a plasma expander. Characteristics include: $P_{50}$ and $O_2$-dissociation curve similar to those of erythrocytes; absence of blood groups ("universal donor") and immune reactions; non-toxic, naturally metabolized; negative trans potential; mechanically more stable than RBC (1).

Current studies determined the cause of rat death after total blood exchange transfusions using SE, and histopathologic findings in tissues of vital organs, caused by transfusion.

Methods. Controls consisted of 6 Sprague-Dawley female rats, anesthetized with pentobarbital, and slowly transfused isovolemically with Eri-Lite kidney dialysis solution, with 10% human plasma albumin added, until the rat died of hypoxia. From this, the hematocrit at death (lethal HCT) was determined.

In 6 experimental rat transfusion was done the same way, using Eri-Lite albumin solution until HCT reached 10%, after which point transfusion was continued with the SE suspension in the Eri-Lite - 10% albumin solution containing 50% SE by volume. Transfusion was ended when at least 90% of original blood was removed. After closure of the incision, rats were left to live breathing normal air without life-support equipment, until death. Rats were necropsied immediately after death. For light microscopic examination tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with H&E and PAS.

Results. In controls, the mean value of lethal HCT was 6.1% (SEM±0.1%), mean value of $O_2$ content of 3.2 vol% (SEM±0.04%).

In the experimental group at the end of transfusion the mean value of HCT was 4.0% (SEM±0.3); $O_2$ content, 4.6 vol% (SEM±0.1); mean concentration of SE, 39% (SEM±5%). At death, mean HCT was 4.1% (SEM±0.3); mean $O_2$ content, 4 vol% (SEM±0.2); mean SE concentration 47% (SEM±5%). Mean survival time after the end of transfusion using SE was 15 hr (SEM±5). Blood plasma and urine samples taken during transfusion and after death did not show detectable presence of Hb.

The cause of death in all rats in the experimental group was hypovolemic shock, with histopathologic findings consistent with those of acute hypovolemic shock: heavy and wet lungs, intraseptal edema, and accumulation of edema fluid and exuded plasma within the alveolar spaces, alveolar walls perhaps more thick, many bleeding membranes; branches of pulmonary artery often obstructed with fibrin-cholesterol thrombi; kidneys showing multifocal tubular necrosis, most prominent in straight portion of proximal tubules. Eosinophilic, PAS-positive casts common in distal tubules and collecting ducts. Paracellular interstitial edema and scattered accumulations of polymorphonuclear leukocytes within dilated vasa recta. Liver displayed severe centriflobular necrosis and edema. Peripherobular hepatocytes accumulated fat. Adrenals showed depletion of lipid from cortical cells, transforming them from the hyaline state to non-vacuolated or compact cells. Moderate to severe edema characteristic of all other organs and tissues.

Discussion. The prolonged post-transfusion survival time clearly demonstrates the ability of SE to transport $O_2$ and $CO_2$ sufficient to maintain life even at HCT levels which are more than 30% below the lethal. Death is not caused by hypoxia since the $O_2$-carrying capacity of blood at death is sufficient to maintain life (Devenuto et al. (2)). The cause of death, hypovolemic shock is, in this setting, a function of both the quantity of blood plasma lost and the rate of loss. Although the withdrawn plasma is immediately replaced with an equal volume of the SE suspension, much of the fluid is lost into interstitial tissues, due to the lack of high molecular weight proteins in the SE suspension. The addition of 10% human plasma albumin to the isotonic Eri-Lite did not prevent extravasation of fluids and hypovolemia, although it may have slowed it down. These experiments point out that to achieve unlimited survival after total transfusion with SE, a more effective oncotic agent must be added to the suspending medium. It will also help to encapsulate more concentrated SFH (around 35 gm%) instead of the present 12-14 gm% solution. This would enable SE to double their $O_2$-carrying capacity.

References.