identical to LDH-1 from adult mouse tissues, or was a different molecular form having the mobility of adult LDH-1. A total of 50 to 100 eggs of the same developmental stage were divided into two equal groups, and the LDH activity was assayed in low ($3.25 \times 10^{-4}M$) and in high ($1.6 \times 10^{-2}M$) concentrations of pyruvate. The ratio of LDH activity in the low concentration to that in the high concentration of pyruvate fell in the range of 4.25 to 4.50 in several assays. The same ratio for mouse LDH-5 was 1.9, while that for LDH-1 was 4.50.

The subunit structure of egg LDH was studied by a dissociation-recombination experiment described by Markert (*12*). Egg LDH and LDH-5 were mixed in $0.1M$ sodium phosphate with $0.5M$ NaCl (*p*H 7.0), frozen, and then thawed; and the material was subjected to electrophoresis. Three new bands of LDH activity appeared in the electrophoretic pattern. The recombination under these conditions seemed to favor a combination of two A subunits from the LDH-5 and two subunits from the egg LDH at the expense of the other two heteropolymeric isozymes (Fig. 2).

Our results on the electrophoretic nature of mouse egg LDH agree with those of Auerbach and Brinster (*5*), except that no trace of isozymes other than that with the mobility of LDH-1 was detected. According to our data the mouse egg LDH isozyme is identical with adult mouse LDH-1 in electrophoretic mobility, substrate inhibition, and subunit structure. The exclusive presence of B subunits in the mouse ova before implantation, on the one hand, and the general knowledge that implanted young embryos contain LDH with an overwhelming amount of A subunits, on the other suggest an abrupt change in the activity of the corresponding genes at the time of or shortly after the implantation. Regulation of the differential gene activity is not known, but it has been proposed that tissues undergoing aerobic metabolism contain LDH isozymes with mainly B subunits, whereas tissues capable of anaerobic metabolism exhibit A subunits (*3, 13*). Our results agree with this, since the oxygen uptake of rat and rabbit eggs is significantly higher than that of implanted embryos (*14*). It could be thought that the change from aerobic metabolism of eggs before implantation to the less aerobic metabolism of implanted embryos promotes the initiation of A subunits synthesis, which lasts until implantation is inhibited. Mouse eggs and embryos before implantation are unusual in that they contain only a single LDH isozyme which, to our knowledge, does not exist in any other mammalian cell type, with the possible exception of spermatozoa (*15*).

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16. Supported by grant from the Sigrid Jusélius Stiftelse, the Finnish Medical Research Council and Damon Runyon Memorial Foundation (DRG-938).

5 June 1967

## Hydroxyethyl Starch: Extracellular Cryophylactic Agent for Erythrocytes

Abstract. *Studies in vitro indicate that hydroxyethyl starch is an effective extracellular cryoprotective agent for erythrocytes. It is as effective as polyvinylpyrrolidone in cryophylactic ability. It is degraded to glucose units in the circulation, is not retained in tissues, and is inexpensive to produce. Changes in the size and degree of hydroxyethylation of the branched-chain starch may result in a more effective cryoprotective agent.*

A 6-percent solution of hydroxyethyl starch (HES) has been shown to be effective without serious side effects as an expander of plasma in laboratory animals and in man (*1*). Physiologically, it is degraded slowly in the bloodstream, and initial studies suggest a very low degree of antigenicity (*2*). It has been compared to dextran for its plasma-expander properties; however, it is more stable on long-term storage and freezing and thawing than dextran is (*3*), and it is inexpensive to produce (*4*).

In the bloodstream, starch is progressively hydrolyzed by contact with $\alpha$-amylase. Unaltered starch will not persist in the circulation, but substitution of the starch hydroxyl groups by hydroxyethyl ether groups interferes with the breakdown of the starch by $\alpha$-amylase.

Other expanders of plasma, such as dextran and polyvinylpyrrolidone (PVP), have been studied for their extracellular cryoprotective activity. Because HES is slowly degraded in the circulation, it seemed reasonable to explore its ability to act as a cryoprotective agent in the freezing of erythrocytes.

Intracellular protective agents, such as glycerol, are now being effectively used for the long-term preservation of erythrocytes. To utilize an intracellular protective agent, it is necessary to remove the material from the erythrocytes prior to administration. An extracellular agent which can be metabolized and could be administered with the cells would have a definite advantage in reducing to a minimum the processing after thawing.

We previously used PVP as an extracellular cryophylactic agent for long-term preservation of erythrocytes by the Linde liquid-nitrogen technique (*5*). Although PVP offers excellent protection to the erythrocyte during the freezing and thawing process,

there is the possibility of long-term retention of the larger-molecular-weight PVP in the recipient.

Like HES, a 15-percent concentration of dextran was found to give optimum protection to the erythrocytes. Small doses given to rabbits and dogs produced no adverse reactions, although lack of this material limited investigation.

Multiple 55-ml units of whole human blood were prepared with hydroxyethyl starch in final concentration of 15 percent as the protective material. The blood was frozen in metal containers in liquid nitrogen at −196°C with the use of the Linde blood-processing apparatus. The blood was agitated during freezing at a rate of 200 cycle/min. The frozen blood was stored for periods of up to 1 week in liquid-nitrogen vapor at approximately − 140°C. Thawing was accomplished by immersion and agitation for 60 seconds at 160 cycle/min in a water bath at 47°C. The temperature of the thawed blood at the time of removal from the water bath was approximately 37°C.

Studies in vitro of the blood specimens after thawing included measuring the total recovery of the erythrocytes, the efficiency of the process or saline stability, and concentration of supernatant hemoglobin. Fifty-one units of whole blood were frozen and thawed as described. The average recovery of erythrocytes in vitro was 97.4 percent, ranging as high as 99 percent. The saline stability averaged 83.4 percent. Amounts of hemoglobin in the plasma averaged 283.3 mg per 100 ml upon thawing. Our observations in vitro indicated that hydroxyethyl starch has cryophylactic properties for erythrocytes comparable to those of PVP. This starch offers the advantage (over PVP) of being metabolized and therefore not retained in the recipient. This feature would eliminate the need for extensive processing of blood after thawing prior to transfusion.

The average molecular weight of the hydroxyethyl starch used was 450,000. As it is possible to vary the size of the branching starch molecule, a smaller size which may have even better cryophylactic properties is easily produced.

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6. Supported in part by ONR government order NA onr-8-67. We thank Mrs. Shirley Block for technical assistance. Hydroxyethyl starch was supplied by the Don Baxter Corporation and by Dr. T. J. Schoch of the research department of the Corn Products Company.

28 July 1967

## Zona Pellucida of Rhesus Monkey Ovum after Gonadotropin Stimulation

Abstract. *The outer part of the zona pellucida of ovarian ova from rhesus monkeys* (Macaca mulatta) *treated with Pergonal and human chorionic gonadotropin appears diffusely vesiculated in the electron microscope but not in the light microscope.*

The pattern of development of the zona pellucida in the oocyte of the rhesus monkey (*Macaca mulatta*) closely resembles that previously observed in other mammalian oocytes (*1*). In very young follicles, the oocyte is surrounded by a single layer of elongated follicle cells which, in some areas, are as thin as 0.05 $\mu$. At a slightly later stage, a space forms between the oocyte and the follicle cells; as this space increases, a relatively electron-opaque material is deposited into it, forming the zona pellucida (*2*). Recently, it was reported that the formation and constitution of the zona pellucida in the baboon ovum are affected by treatment with Pergonal (trade name for human postmenopausal gonadotropin) and human chorionic gonadotropin (*3*). These changes consisted of vesiculation, hypotrophy, and absence of the zona. The authors did not study these tissues with the electron microscope.

I have studied the ovaries of 20 rhesus monkeys that were part of the breeding colony of the Oregon Regional Primate Research Center and that had a well-known menstrual record for at least 1 year before the experiment began. Ten animals were used as controls, and ten were treated as follows: starting on day 1 of the menstrual cycle, 75 international units of Pergonal (*4*) were injected intramuscularly for 6 days. On days 7 and 8 of the cycle, human chorionic gonadotropin (2000 units) was administered in-

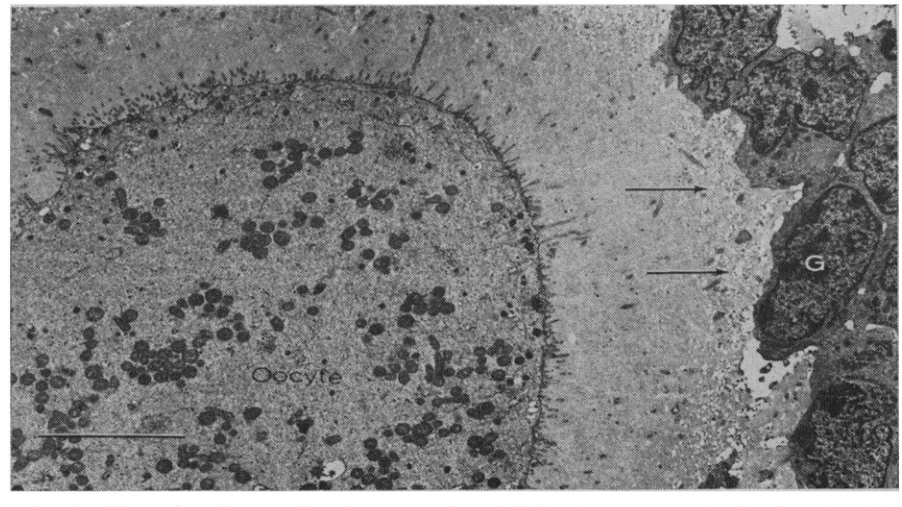


Fig. 1. Electron micrograph of an ovarian follicle from an animal treated with Pergonal. Vesiculation of the outer part of the zona pellucida (arrows) is present. *G*, granulosa cells. Line represents 3 $\mu$. (× 2010)