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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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 μ. 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-

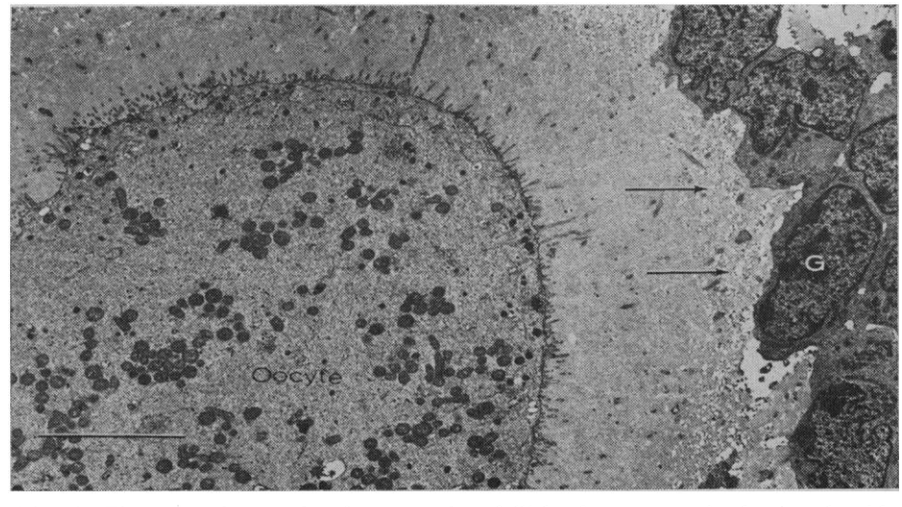

Fig. 1. Electron micrograph of an ovarian follicle from an animal treated with Pergonal. Vesiculation of the outer part of the zona pellucida (arrows) is present. *G*, granulosa cells. Line represents 3 μ. (× 2010)

tramuscularly. The animals were killed at various times after this treatment (9 to 14 days), and complete autopsies were performed. Half of each ovary was fixed in 4 percent buffered glutaraldehyde or in 1 percent buffered osmium tetroxide and embedded in araldite; thin sections were stained with uranyl acetate and lead citrate and examined in a Philips-200 electron microscope. The remaining portion of each ovary was fixed in 10 percent buffered formalin and embedded in paraffin; semiserial sections were stained with hematoxylin–eosin and periodic acid–Schiff reagent. Thick sections of material embedded in aradite were stained with 1 percent toluidine blue.

Under the light microscope there were signs of stimulation in both the follicular apparatus and the oocyte of all treated animals. Dissociation of follicular cells, early formation of the antrum, and hyperplasia of the theca cells were observed. No alterations of the zona pellucida were detected even in mature eggs examined shortly before ovulation.

When studied with the electron microscope, most of the oocytes (in secondary and tertiary follicles) of animals treated with gonadotropin showed various degrees of alteration of the zona pellucida. These changes consisted of a fine vesiculation of the outer parts of the zona, especially in areas where there was an early retraction of the granulosa cell prolongations (Fig. 1). The severity of the vesiculation was closely related to the stage of follicular and oocyte maturation; it spread throughout the zona just before ovulation. The granulosa cells had the appearance of active secretory cells. No alterations of the zona pellucida were observed in the oocytes of the control monkeys (Fig. 2).

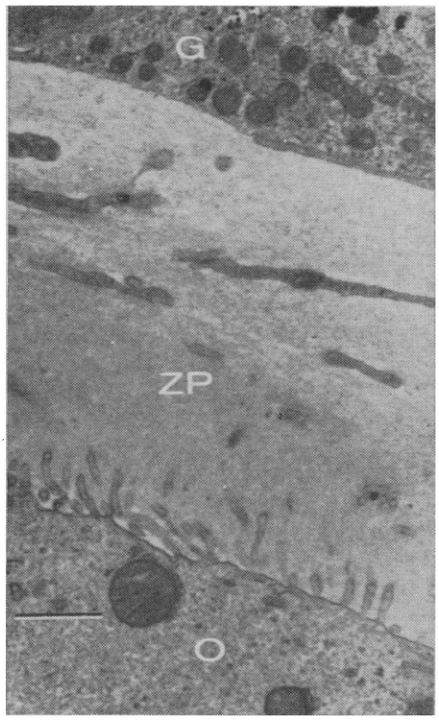

Fig. 2. Electron micrograph of an oocyte (*O*) of untreated animal with normal zona pellucida (*ZP*). *G*, granulosa cell. Line represents 1 μ. ($\times$ 11,520)

The increased response to the gonadotropin stimulation was evident in the ovaries as well as in other reproductive organs of the experimental animals. Signs of hyperstimulation, such as ovarian cysts, and a hyperplastic proliferative endometrium with increased ciliogenesis were common findings. These might have been responses to high concentrations of estrogen induced by the treatment, as the fine vesiculation of the zona pellucida might also be. On the other hand, the influence of estrogens on the loss of the zona pellucida has been demonstrated in the rat (*5*); in this animal the zona persists if the ovaries are removed on day 2 of pregnancy and, unless estrogen is supplied, remains undissolved in the uterine cavity.

Since the rhesus monkeys showed no changes in the zona pellucida observable with the light microscope, even in cases of gonadotropic hyperstimulation, it seems improbable that the "anomalies" reported in the baboon (*3*) are related to gonadotropin treatment. Since it is very difficult to fix the zona pellucida properly, it is possible that the changes in the zona pellucida of the baboon were caused by an acid fixative, especially when one analyzes the modifications present in the ova and granulosa cells of the reported pictures.

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Phenotypic Masking and Streptomycin Dependence

Abstract. *In attempting to define the role of ribosomes in the mechanism of streptomycin dependence, a new phenomenon has been discovered. Analysis of this phenomenon—called phenotypic masking—leads to the conclusion that "streptomycin dependent" mutants are actually "drug dependent" because their dependence is equally satisfied by several drugs. These drugs, some of which are totally unrelated chemically, act on the ribosome and induce misreading in vitro and suppression in vivo.*

Streptomycin induces suppression of a number of genetic mutations (*1*, *2*) and causes misreading of the genetic code (that is, ambiguity) in vitro (*3*, *4*). The site of this action is the 30*S* subunit of the ribosome (*3*). Mutations at the streptomycin locus (Sm) may reduce or eliminate ambiguity (*3*, *4*). They may also restrict or abolish the genetic suppression of nonsense mutations (*5*). The molecular basis of induction of ambiguity and of restriction of suppression is not known, but the two phenomena may be ascribed to two alterations of the ribosomal structure with opposite effects, namely that the ribosome in performing its function of translation, may become too flexible in the presence of streptomycin or too rigid upon genetic mutation. Specifically, streptomycin added to ribosomes from streptomycin-sensitive (Sm^S) strains induces misreading of certain codons, thereby introducing ambiguity into the process of translation. Ribosomes from streptomycin resistant (Sm^R) mutants are restricted in comparison to Sm^S in the sense that, if they permit ambiguity at all in the presence of streptomycin, it is only to a very limited extent. Streptomycin dependence (Sm^D), for which ribosomal involvement has been postulated (*6*) and confirmed (*7*), may be explained by the appearance of ribosomes so restricted that they do not allow the translation of certain or any codons at all. Streptomycin may permit or stimulate the normal reading of those codons which cannot be read in the absence of the antibiotic. Functionally this property of streptomycin may be analogous to its capacity to produce ambiguity with Sm^S ribosomes. However, streptomycin need not pro-