Table 1. Induction of ovulation by synthetic LH-RH in golden hamsters pretreated with phenobarbital. Synthetic LH-RH was administered subcutaneously; LH, intraperitoneally.

Group	Additional pretreatment	Sample injected	Dose	Animals tested (No.)	Animals ovulated (No.)	Mean No. of ova found in the oviducts (± S.E.)
1		Saline		12	0	0
2		Synthetic LH-RH	0.357 nmole	5	5	13 ± 0.9
3		Synthetic LH-RH	0.179 nmole	4	4	9 ± 0.7
4		Synthetic LH-RH	0.089 nmole	5	3	4 ± 1.9
5	Hypophysectomy	Synthetic LH-RH	0.357 nmole	8	0	0
6	Hypophysectomy	LH	10 μg	6	6	11 ± 0.8

of body weight to block spontaneous ovulation (*7*). Experimental animals received various doses of synthetic LH-RH dissolved in 0.5 ml of acidified saline (0.01*M* acetic acid in 0.9 percent saline) in two subcutaneous injections at 3:00 and 4:00 p.m. to insure prolonged elevation of circulating LH. Control animals received 0.5 ml of acidified saline according to the same schedule. Some of the hamsters were hypophysectomized through the auditory canal soon after they were anesthetized. Some of these hypophysectomized animals received 0.357 nmole of synthetic LH-RH or 10 μg of ovine LH (NIH-LH-S 17) in 0.5 ml saline intravenously at 4:00 p.m.

Some of the experimental and control animals were bled from the jugular vein 20 minutes after the second injection of synthetic LH-RH or saline for determination of circulating LH levels. Luteinizing hormone was measured by radioimmunoassay as described by Goldman and Porter (*8*) and expressed as nanograms per milliliter of serum in terms of NIH-LH-S-18 standard.

The following morning the hamsters were killed and their oviducts were inspected under a microscope for the presence of ova. The results are shown in Table 1. All control hamsters injected with saline alone failed to ovulate, which indicates 100 percent blockade of spontaneous ovulation by phenobarbital, in agreement with the findings by Greenwald (*7*). On the other hand, subcutaneous injection of 0.357 nmole of synthetic LH-RH induced full ovulation in all hamsters. Figure 1 shows the ampulla of the oviduct from a control hamster and from an experimental hamster in which 15 ova were found in the oviducts. All animals that were given 0.179 nmole of synthetic LH-RH also ovulated, but the mean number of ova found in this group was smaller than the number found in the group that received 0.357 nmole. Administration of 0.089 nmole of LH-RH induced ovulation in three out of five hamsters. Injection of 0.357 nmole of synthetic LH-RH did not induce ovulation in hypophysectomized hamsters, excluding the possibility that synthetic LH-RH stimulates the ovaries directly. Intraperitoneal administration of 10 μg of LH to hypophysectomized hamsters induced ovulation in all the hamsters tested, which indicates that the ovaries of these hypophysectomized hamsters are capable of responding to LH.

Mean serum levels of LH 20 minutes after the second injection of saline and 0.179 nmole of synthetic LH-RH were 2.8 ± 0.72 (S.E.) and 38.4 ± 1.11 ng/ml, respectively. Although we could not determine circulating FSH levels, release of FSH may also have occurred following injection of synthetic LH-RH, as it does after the administration of natural LH-RH (*4*). It is likely that both the LH and the FSH released played a synergistic role in inducing ovulation (*7*).

The amount of peptide was estimated by basing it upon the content of tryptophan. The amount of active decapeptide may have been smaller than was estimated. This indicates that a very small dose of synthetic LH-RH was able to induce ovulation. We also observed that quick intravenous injection of 0.357 nmole of synthetic LH-RH did not affect blood pressure and the electrocardiogram as tested in the anesthetized rat, which indicates lack of undesirable side-effects on the cardiovascular system. Because of the ease of administration (subcutaneous injection), the synthetic LH-RH should become a very useful tool in clinical and veterinary fields.

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Human Red Blood Cells: Prostaglandin E_2, Epinephrine, and Isoproterenol Alter Deformability

Abstract. *The human red blood cell responds to prostaglandin E_2, epinephrine, and isoproterenol with a decrease in deformability. The maximum decrease is brought about by 10^{-10}M prostaglandin E_2, 10^{-9}M epinephrine, or 10^{-7}M isoproterenol. The dose response curve is biphasic. The sensitivity of the red cell to prostaglandin suggests that this cell may be a primary target for prostaglandin action. These changes in response to vasoactive substances indicate that the red cell must be considered an active element in circulatory control.*

Control of blood flow through the microcirculation has been thought to reside somewhere in the vascular bed. Changes, therefore, in the relations between pressure and flow of the circulation in an intact animal or in a perfused organ after various treatments have been interpreted as indications solely of

an alteration of vascular tone. In studies based on this model, the fact that major components of shock and essential hypertension have not been accounted for suggests that an important factor in the control of peripheral circulation has been overlooked. We now present evidence that the red cell is itself capable of undergoing structural change in response to the vasoactive compounds, prostaglandin E_2, epinephrine, and isoproterenol. These changes suggest that the red cell has an important role in circulatory control.

A sensitive index of the red cell's deformability may be obtained in vitro by passing suspensions of these cells at high hematocrit through analytical filters whose pores are small. Such a technique was devised by Teitel (*1*), modified by Weed, LaCelle, and Merrill (*2*), and has been used to evaluate the deformability of the red cell in various hemolytic disorders and also following a variety of in vitro conditions (*1*). These workers have demonstrated that changes in filterability are a sensitive index of red cell deformability; Weed *et al.* (*2*) have correlated the filterability changes with changes in both viscosity and deformability of the cells, as measured by a microcapillary technique.

In our studies, blood from young healthy male and female laboratory personnel and volunteer donors was drawn into tubes or syringes containing heparin (10 units per milliliter of blood). The blood was immediately centrifuged at top speed (IEC centrifuge, model CL) for 7 minutes at room temperature, and the plasma and buffy coat were removed. A platelet-free preparation was obtained by washing three times and then removing the top 1 mm of red cells after each washing. The buffer used for washing and in the incubation consisted of (mmole/liter): 145 m*M* NaCl, 5 m*M* KCl, 1 m*M* $CaCl_2$, 1 m*M* $MgSO_4$, 5m*M* NaH_2PO_4 + Na_2HPO_4; and glucose 200 mg per 100 ml. The *p*H of the buffer was 7.0. Incubation with hormone was carried out at either *p*H 7.0 or 7.4, as indicated; 1.4 ml of the washed red cells was added to 0.6 ml of buffer. Hormone was either present initially or added after a 10-minute preliminary incubation period. After incubation for 10 minutes in the presence of hormone at 37°C in a shaker bath, the blood was added to filters (Schleicher and Schuell 589 white ribbon) which had been wetted with buffer. Just before it was added to the filters, the blood was agitated on a Vortex mixer. Hematocrits on the filtrate showed that there was no separation of the components of the suspension to account for the differences observed in the flow.

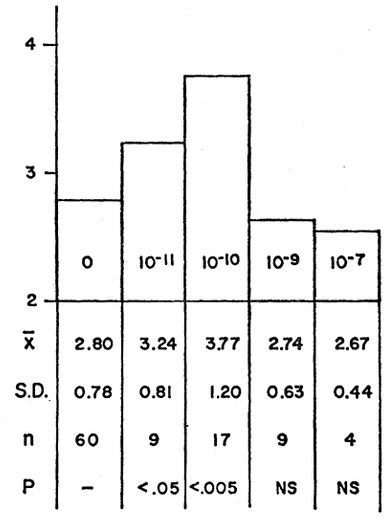

Fig. 1. The effects of PGE_2 on filterability. Prostaglandins were added to washed red cell suspensions in buffer, *p*H 7.0, to the final concentration indicated within the bars, and then passed through analytical filters at high hematocrit levels. $t_{1/2}$, half time for filtration; $\bar{X}$, mean $t_{1/2}$; S.D., standard deviation of the mean; *P*, probability, two-tailed Student's *t*-test, matched pairs; *n*, number of observations.

The time for half the volume added to pass the filters was obtained and used as the index of the filterability. Decreased deformability resulted in an increase in the half time for filtration ($t_{1/2}$).

Prostaglandin E_2 (PGE_2) (*3*), $10^{-10}M$, brought about maximum decrease in deformability (Fig. 1). Further increase in the concentration of hormone decreased this response. Thus, there was a biphasic dose response curve with a significant response to hormone obtained at $10^{-11}M$. This effect of PGE_2 was greater at *p*H 7.0 than at *p*H 7.4. Synthetic PGE_2 (*4*) gave results identical to those obtained with the Upjohn compound isolated from biological material.

Figure 2 presents the data obtained from experiments with epinephrine or DL-isoproterenol. These experiments were performed at *p*H 7.4. Epinephrine brought about a maximum decrease in filterability at $10^{-9}M$ and, as with PGE_2, the dose response was biphasic. The effect of DL-isoproterenol, the prototype β-adrenergic compound, is similar, although the change in filterability is greater at $10^{-7}M$ than the maximum response to epinephrine. Care was taken to ensure no autoxidation of these compounds; solutions were prepared immediately before they were added to the red cells, and for the minute or so preceding the addition the solutions were kept in light-shielded containers on ice.

Although Teitel (*5*) has shown that a change in cell shape does not of itself

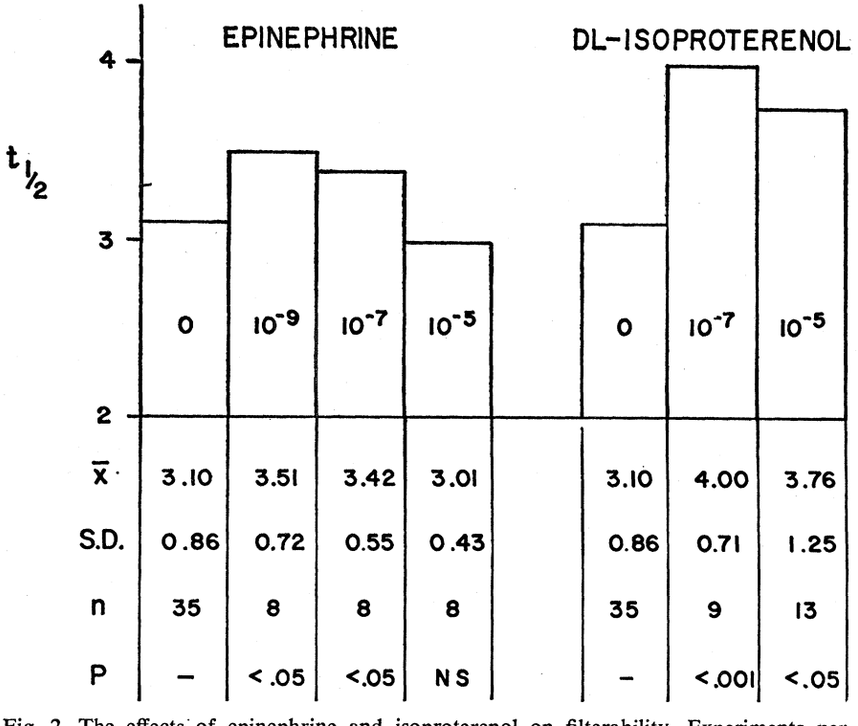

Fig. 2. The effects of epinephrine and isoproterenol on filterability. Experiments performed as in Fig. 1, but at *p*H 7.4.

alter filterability, it seemed possible that a shape or volume change might be occurring in parallel with the alterations in deformability. Hematocrit and microscopic observation showed no change in mean cell volume or cell shape under conditions that alter filterability.

These data show that red cell suspensions are responsive to several vasoactive substances known to have effects on the circulation in vivo at similar concentrations. The response of red cells to PGE_2 is of interest in that it is the most sensitive response to this compound reported to date; this suggests that red cells may be one of the primary receptors for prostaglandin stimuli.

Burton and Weed (*6*, *7*), among others, have pointed out the importance of deformability in the microcirculation; and Weed (*7*) has proposed that deformability governs the destruction of erythrocytes by the spleen. Leblond *et al.* (*8*) have recently proposed that release of young erythrocytes by the marrow is also a function of deformability. It is becoming clear that deformability is a functional characteristic of the red cell which varies with cell age and metabolic condition. Our report shows that deformability is subject to alteration by hormonal stimuli. This in turn indicates that the red cell must be considered for a role in the moment-to-moment control of the circulation, and that hormonal stimuli may play a role in destruction of red blood cells.

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Interchangeability of Phosphorylation Coupling Factors in Photosynthetic and Respiratory Energy Conversion

Abstract. *The nonsulfur purple photosynthetic bacterium* Rhodopseudomonas capsulata *can obtain energy for growth either by anaerobic photophosphorylation or dark oxidative (aerobic) phosphorylation. Successful resolution of phosphorylation coupling factors from energy-converting membranes of this bacterium permitted tests for reciprocal function of such protein factors in oxidative- and photophosphorylation processes. Evidence was obtained for the interchangeability of coupling factor preparations from dark-grown and photosynthetically grown cells in both kinds of energy conversion.*

In eucaryotic cells, photosynthetic and respiratory phosphorylation processes are generally catalyzed by different kinds of membrane systems that are spatially separated in the form of discrete organelles (chloroplasts and mitochondria) which are believed to be semiautonomous in character. On the other hand, the procaryotic nonsulfur purple bacteria, which can obtain the energy for growth by either photosynthetic or (dark) respiratory phosphorylation, do not contain subcellular organelles, and a considerable body of evidence indicates that in these organisms the cytoplasmic membrane or its extensions, or both, must be the locus of both kinds of energy conversion (*1*). When typical bacteria with these capacities are transferred from photosynthetic to heterotrophic conditions, or vice versa, appropriate differentiation of the energy-converting membrane system ensues (*2*). The possibility that common components may function in the photosynthetic and respiratory phosphorylation processes of such organisms has been discussed [for example, see (*3*) and Thore *et al.* (*1*)], but until now there has been no compelling evidence in support of this notion. In this report, we present results showing that phosphorylation coupling factor preparations derived from heterotrophically and photosynthetically grown cells of *Rhodopseudomonas capsulata* (strain St. Louis; American Type Culture Collection No. 23782) can link either oxidative or light-dependent electron transport to phosphorylation of adenosine diphosphate (ADP).

Phosphorylating membrane preparations were obtained from cells grown either photosynthetically (anaerobically) or aerobically in darkness, in a synthetic medium (*4*) containing DL-malate and $(NH_4)_2SO_4$ as the respective carbon and nitrogen sources. In the following, the designation P is used for membrane vesicles, or derived components, from cells grown photosynthetically, and A for corresponding preparations from bacteria cultivated aerobically in darkness. Membrane vesicles, or fragments, will be referred to as particles. The P particles were prepared as described previously (*4*). The P coupling factor was extracted from acetone powders of such particles, and purified by procedures described elsewhere (*5*). For certain experiments, P particles depleted of P coupling factor (that is, "uncoupled" P particles) were required, and these were prepared by sonication of the particles in the presence of 1 m*M* ethylenediaminetetraacetate [EDTA; for details, see (*4*)]. The procedure used for obtaining A particles was very similar to that employed for isolation of P particles. In brief, cells grown aerobically in darkness were suspended in 0.05*M* glycylglycine buffer (*p*H 7.2), containing 0.4*M* sucrose, and disrupted in a French pressure cell. Residual intact cells and large debris were removed from the extracts by a low-speed centrifugation and A particles then were collected by centrifugation at 250,000*g* for 1 hour. These were ordinarily resuspended in the glycylglycine plus sucrose buffer, supplemented with 10 m*M* $MgCl_2$ and bovine serum albumin (2 mg/ml). Alternatively, to prepare uncoupled A particles, the sedimented particles were resuspended in 1 m*M* EDTA and the cold suspension was subjected to three short bursts (30 seconds each) of sonic energy from a Biosonik oscillator (Bronwill Scientific); the treated particles were collected by centrifugation at 250,000*g* for 1 hour and resuspended in the buffer of glycylglycine plus sucrose plus bovine serum albumin ($MgCl_2$ omitted). For measurement of phosphorylation, a hexokinase plus glucose "trap" was employed to convert [^{32}P]ATP, produced