volving changes in length of daily light-darkness intervals suggest to us that the critical period is more closely related to the time of "lights-off" than to when the lights come in, *i.e.*, starts 5 to 6 hours before "lights-off" rather than a definite interval after "lights-on"(11). The control of reproductive function in the rat by light-dark cycles serves as an example of the many functions which appear to be light regulated and have a circadian nature in this species. Thus a general brain response to "lights-off" may play an important regulatory function for control of cyclic phenomena in this species.

Preliminary observations on the rabbit, a species which does not have a spontaneous ovulation cycle and does not appear to employ light cycles to regulate function to the same extent as the rat, indicate no marked influence of a "lights-off" signal for regulation of brain function in relation to sleep-wakefulness intervals.

Summary. Paradoxical sleep occurred in rats in response to a 5-minute period of "lights-off" stimulation. Usually the paradoxical sleep episode had ended before the lights came on again. The "lights-on" stimulus did not appear to produce any changes in the sleep-wakefulness cycle.

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Effect of Polyvinyl Pyrrolidone on Plasma Coagulation Factors.* (31572)

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Polyvinyl pyrrolidone (PVP) provides effective protection for human red blood cells during frozen storage in liquid nitrogen. Hemolysis during processing and loss of ability to survive on subsequent transfusion remain at an acceptably low level(1,2). PVP does not enter the red cells; thus there is no need to wash it out of the cells by the series of complicated maneuvers required for the endocellular protective agents, glycerol and dimethyl sulfoxide(3,4). The principal obstacle to the use of PVP has been evidence that the transfused polymer is stored in the reticuloendothelial tissues of the recipients for prolonged periods(5). It seemed possible that this obstacle might be largely overcome by centrifugation of the thawed blood, discarding the plasma with its contained PVP before transfusion of the red blood cells. In the course of experiments to evaluate this approach, it became obvious that gross precipitate formation occurred in the plasma of blood to which PVP had been added, associated with a marked effect on plasma coagulation factors. The mechanism of the interaction and quantitative effects were therefore explored.

Materials and methods. 1. Blood for the

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freezing experiments was collected at the blood bank in plastic bags one to two days prior to processing. 500 ml blood were added to 75 ml N.I.H. Formula A acid-citrate-dextrose (ACD) solution. For tests on small quantities of blood, nine volumes of blood were collected into one volume of acid citrate (3 parts 0.1 M sodium citrate, 2 parts 0.1 M citric acid). In both instances the plasma pH averages 6.9. 2. 450 ml of the ACD blood were transferred to a 1000 ml aluminum container(6). The inside of this container had been coated with silicone by exposure to a 5% emulsion of Dow Corning Z4141 silicone. 100 ml of 40% K^{30} PVP (average molecular weight 40,000, General Analine and Film Corp.) were then added. The blood was rapidly frozen in liquid nitrogen in a Linde Blood Processing Unit followed by immediate thawing in the same instrument. 3. The assays used in this laboratory for coagulation Factors II (prothrombin)(7), V (proaccelerin)(7), VII and X (proconvertin and Stuart)(7), X (Stuart)(7), VIII (antihemophilic factor)(8) and fibrinogen(9), and the technics for the partial thromboplastin time (7) and thrombin time(7) have been described. Assay for Factor XI (PTA) was by the method of Horowitz(10), and the thromboplastin screening test followed Hicks and Pitney(11). Siliconed clotting times were done at 37°C with 1 ml blood in a 12 × 75 mm glass tube treated with Z4141 silicone. In experiments where centrifugation had to be avoided, Factor VIII was assayed without the usual prior absorption of the sample with aluminum hydroxide. With the assay method used, omission of this step makes little difference.

Results of coagulation tests on plasma stored in the siliconed aluminum containers mentioned above and in plastic bags (Fenwal TA-2) were identical.

Results. When the thawed units of whole blood were centrifuged, a dense white layer 2-3 mm thick was visible in the region of the buffy coat. Addition of PVP to normal plasma in the concentrations and PVP : plasma volume ratios employed with the units of whole blood resulted in gross turbidity which was easily sedimentable at 1000 *g* for 10 minutes. Such turbidity was evident at 0°C, room temperature or at 37°C.

TABLE I. Effect of 40% K^{30} PVP on Plasma Coagulation Factors.

	(1) Whole plasma			(2) Supernatant plasma		
ml PVP/ml plasma	.2	.5	1.0	.2	.5	1.0
Fibrinogen	85	82	80	19	0	2
Factor II (prothrombin)	105	99	98	80	44	0
" V	110	127	100	105	114	92
" VII & X	90	103	100	89	82	52
" X	110	98	120	66	80	65
" VIII (AHF)	91	95	58	4	2	0
" XI	101	100	95	83	61	29

All values expressed as % of those obtained when saline was substituted for PVP.
(1) Assayed with precipitate suspended in plasma.
(2) Precipitate removed by centrifugation and discarded.

The units of whole blood were aseptically sampled after gentle mixing at various points during the freeze-thaw process. There was a drop in coagulation factor activities after addition of PVP, due in part to dilution of the plasma with the additive; but the disappearance of Factor VIII and fibrinogen was almost complete and requires another explanation. No further changes occurred with freezing and thawing.

The PVP solution was then added to small aliquots of normal plasma so that tests could be repeated with plasma from a number of normal donors and so that the effect of various volume ratios of PVP to plasma could be evaluated. The mean values obtained in these experiments are listed in Table I. When assays were carried out on the PVP-plasma mixtures with the resulting precipitate remaining in suspension, the only marked loss of clotting factor activity apparent was a drop in Factor VIII in the presence of an equal volume of 40% K^{30} PVP. Removal of the precipitate by centrifugation before assay, however, resulted in much more dramatic changes. Loss of Factor VIII and fibrinogen was almost complete. Progressively lesser effects were seen on prothrombin, Factor XI, and Factors VII and X. Factor V was not lost; in fact, activity in the presence of one-half volume of PVP was consistently greater

TABLE II. Effect of 40% K^{10} PVP on Plasma Coagulation Factors.

	(1) Whole plasma			(2) Supernatant plasma		
ml PVP/ml plasma	.2	.5	1.0	.2	.5	1.0
Fibrinogen	100	111	70	108	113	17
Factor II (prothrombin)	117	94	90	67	63	46
" V	116	109	90	101	135	93
" VII & X	95	96	90	97	99	67
" X	104	94	97	91	80	66
" VIII (AHF)	124	95	93	14	5	2
" XI	44	47	43	35	35	34

All values expressed as % of those obtained when saline was substituted for PVP.
(1) Assayed with precipitate suspended in plasma.
(2) Precipitate removed by centrifugation and discarded.

than expected. Whenever clotting activity was reduced, the effect was greater the larger the volume of PVP used.

In Table II are shown the results of similar experiments with K^{10} PVP. This has a mean molecular weight of 17,000. Its ability to protect red cells during frozen storage is currently under exploration. Because of its smaller molecular size, it is more readily excreted by the kidney, and this should minimize reticuloendothelial storage of the polymer. Its effects on plasma coagulation factors (Table II) are quite similar to those of the K^{30} PVP, but are lesser in degree. A unique difference is seen in the results with the Factor XI (PTA) assay. This factor is depressed without removal of the precipitate and the degree of change does not vary with the volume of PVP used.

Since Factor VIII and fibrinogen were almost totally removed in the precipitate formed by PVP, an attempt was made to elute the factors from the sediment into citrated saline. Elution was incomplete, and a considerable proportion of the activities could not be accounted for in the sum of all of the fractions.

Discussion. The effective concentration of PVP for protection of red blood cells in the frozen state is extremely high and the solution as used (40%) is quite viscous. It has been noted previously that proteins are precipitated from plasma under these conditions(12).

The present studies demonstrate that certain coagulation factors are among the proteins which precipitate, especially Factor VIII (antihemophilic factor) and fibrinogen. Formation of the precipitate has relatively little effect on the activity of the clotting factors, but their binding in the precipitate is sufficiently firm that they are removed with the precipitate by centrifugation. It seems reasonable to expect that, *in vivo,* transfused precipitate would be removed by the reticuloendothelial system. This could be the basis on which reticuloendothelial storage begins, and the bound clotting factors might thus be permanently eliminated. The occurrence of a gross precipitate which is sedimented on top of the red cells by centrifugation means that less PVP is removed with the supernatant plasma than volume considerations alone would indicate.

Previous study with another synthetic plasma substitute, dextran, with a similar mean molecular weight (40,000) demonstrated findings quite similar to those outlined in the present report(13). Factor VIII and fibrinogen were the clotting factors primarily involved, and the effect on their activity was most evident after the precipitate had been removed by centrifugation. The nature of the bond between these artificial polymers and coagulation proteins is not known, but in both instances the effect was greater the larger the molecular weight of the additive. It is, perhaps, relevant to note that Factor VIII and fibrinogen are the most insoluble of the coagulation proteins, both appearing in the first precipitate (Fraction I) of the Cohn process for separation of plasma proteins(14).

Summary. Addition of polyvinyl pyrrolidone to plasma in concentrations optimal for preservation of red blood cells in the frozen state results in formation of a gross precipitate. Removal of this precipitate by centrifugation takes with it almost all of the plasma fibrinogen and antihemophilic factor and variable amounts of other coagulation factors. Prior to centrifugation, the precipitate demonstrates far less interference with coagulation factor activity.

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Activity Patterns of Several Enzymes of Liver, Adipose Tissue, and Mammary Gland of Virgin, Pregnant, and Lactating Mice.* (31573)

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The specific activities of many enzymes of mammary gland are higher in the lactating than in the non-lactating gland(1-8). Superficially, the response of the glucose-ATP phosphotransferases‖ appeared to be analogous to that of other mammary gland enzymes(3,6-8). Closer examination, however, revealed that the increase in glucose-ATP phosphotransferase activity in the mammary gland during lactation resulted from an increase in the proportion of hexokinase of high specific activity associated with the particulate fraction(9). It was suggested that this change in intracellular distribution of hexokinase during lactation is probably due neither to a change in intracellular site of the enzyme nor to an increase in enzyme synthesis, but to an increase in the proportion of secretory tissue in which hexokinase is particle-bound and a decrease in the proportion of adipose tissue in the lactating gland(9). If this is the true explanation, the intracellular distribution of hexokinase in adipose tissue should be unaffected by lactation.

The present study was undertaken, in part, to compare the intracellular distribution of glucokinase and hexokinase activities in adipose tissue devoid of mammary gland parenchyma with that in mammary glands of the same virgin, pregnant, and lactating mice.

It is clear that the activities of hepatic glucokinase(9), glucose-6-phosphate dehydrogenase (glucose-6-PO_4 dehydrogenase), and 6-phosphogluconate dehydrogenase(3,4) are also higher in lactating mice than in virgin and pregnant mice. The response of these hepatic enzymes to the transition from preg-

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§ This study was in progress at the time of Dr. Chaikoff's death, 25 January 1966.

‖ The total activity of the enzymes catalyzing the phosphorylation of glucose at the 6 position is referred to as glucose-ATP phosphotransferase activity. Under the conditions of the assay of these enzymes as described by Viñuela *et al*(23), enzymatic activity that has a high Michaelis constant (K_m) for glucose and is inhibited by N-acetylglucosamine is referred to as glucokinase, and that with a low K_m and unaffected by N-acetylglucosamine is referred to as hexokinase.